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Intrinsic disorder in proteins associated with neurodegenerative diseases

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1. ABSTRACT

Neurodegenerative diseases constitute a set of pathological conditions originating from the slow, irreversible and systematic cell loss within the various regions of the brain and/or the spinal cord. Neurodegenerative diseases are proteinopathies associated with misbehavior and disarrangement of a specific protein, affecting its processing, functioning, and/or folding. Many proteins associated with human neurodegenerative diseases are intrinsically disordered; i.e., they lack stable tertiary and/or secondary structure under physiological conditions *in vitro*. Intrinsically disordered proteins (IDPs) have broad presentation in nature. Functionally, they complement ordered proteins, being typically involved in regulation, signaling and control. Structures and functions of IDPs are intensively modulated by alternative splicing and posttranslational modifications. It is recognized now that nanoimaging offers a set of tools to analyze protein misfolding and self-assembly via monitoring the aggregation process, to visualize protein aggregates, and to analyze properties of these aggregates. The major goals of this review are to show the interconnections between intrinsic disorder and human neurodegenerative diseases and to overview a recent progress in development of novel nanoimaging tools to follow protein aggregation.

2. INTRODUCTION

2.1. Neurodegenerative diseases as proteinopathies

The large class of human neurodegenerative disorders includes many acquired neurological diseases with distinct phenotypic and pathologic expressions, all characterized by the pathological conditions in which cells of the brain and spinal cord are lost. The name for these diseases is derived from a Greek word νευρο-, *néuro-*, "nerval" and a Latin verb *dēgenerāre*, "to decline" or "to worsen". As neurons are not readily regenerated, their deterioration leads over time to dysfunction and disabilities. Neurodegenerative diseases can be divided into two groups according to their phenotypic effects: (i) Conditions causing problems with movements; and (ii) Conditions affecting memory and leading to dementia. Neurodegeneration is a slow process, which begins long before the patient experiences any symptoms. It can take months or even years before visible outcomes of this degeneration are felt and diagnosed. Symptoms are usually noticed when many cells die or fail to function and a part of the brain begins to cease functioning properly. For example, the symptoms of Parkinson's disease (PD) become apparent after more than ~70% dopaminergic neurons die in *substantia nigra* (a small area of cells in the mid-brain affected by PD.

Until recently, a link between Alzheimer's disease (AD), prion diseases, PD, Huntington's disease (HD), and several other neurodegenerative disorders was elusive. However, recent fascinating advances in molecular biology, immunopathology and genetics indicated that these diseases might share a common pathophysiologic mechanism, where disarrangement of a specific protein processing, functioning, and/or folding takes place. Therefore, neurodegenerative disorders represent a set of proteinopathies, which can be classified and grouped based on the causative proteins. In fact, from this viewpoint neurodegenerative disorders represent a subset of a broader class of human diseases known as protein conformational or protein misfolding diseases. These disorders arise from the failure of a specific peptide or protein to adopt its native functional conformational state. The obvious consequences of misfolding are protein aggregation (and/or fibril formation), loss of function, and gain of toxic function. Some proteins have an intrinsic propensity to assume a pathologic conformation, which becomes evident with aging or at persistently high concentrations. It is now believed that the ability of polypeptide chains to form amyloid structures is not restricted to the relatively small number of proteins associated with recognized clinical disorders, but represents a generic property of a polypeptide chain (1). Intriguingly, even such proteins as green fluorescent protein, GFP (2), and molecular co-chaperonin GroES (3) can be induced to aggregate. Furthermore, macromolecular crowding was shown to have a significant effect on protein aggregation (4-9).

Interactions (or impaired interactions) with some endogenous factors (e.g., chaperones, intracellular or extracellular matrixes, other proteins, small molecules) can change conformation of a pathogenic protein and increase its propensity to misfold. Misfolding can originate from point mutation(s) or result from an exposure to internal or external toxins, impaired posttranslational modifications (phosphorylation, advanced glycation, deamidation, racemization, etc.), an increased probability of degradation, impaired trafficking, lost binding partners or oxidative damage. All these factors can act independently or in association with one another. Table 1 lists some of the IDPs involved in various neurodegenerative diseases. As the major focus of this chapter is the neurodegenerative mechanisms of IDPs, subsequent paragraphs are devoted to the brief introduction of these interesting members of the protein kingdom.

2.2. Neurodegenerative diseases as amyloidoses

Many of the diseases listed in Table 1 are in fact protein deposition diseases associated with the formation of extracellular amyloid fibrils or intracellular inclusions with amyloid-like characteristics. Protein deposition diseases can be sporadic (85%), hereditary (10%) or even transmissible, as in the case of prion diseases (5%) (10). Although these diseases, being are very different clinically, they share similar molecular mechanisms where a specific protein or protein fragment changes from its natural soluble form into insoluble fibrils. It has been pointed out that prior to fibrillation, amyloidogenic polypeptides may be rich in beta-sheet, alpha-helix, beta-helix, or contain both alpha-helices and beta-sheets. They may be globular proteins with rigid 3D-structure or belong to the class of natively unfolded (or intrinsically unstructured) proteins (11). Molecular mechanisms of fibrillation of IDPs and ordered proteins are different (11): in ordered proteins, the first critical step in fibrillogenesis is the partial unfolding (12, 13) (11, 14-21), whereas the earliest stage of fibrillation of IDPs is their partial folding (11). Intriguingly, the

Table 1. IDPs and associated neurodegenerative diseases

Protein (number of residues)	Disease(s)	Disorder by prediction (%)	Disorder by experiment
A-beta (42)	Alzheimer's disease Dutch hereditary cerebral hemorrhage with amyloidosis Congophilic angiopathy	16.9 (28.6)	NMR and far-UV CD analyses revealed that the monomeric peptide is highly unfolded
Tau (758)	Tauopathies Alzheimer's disease Corticobasal degeneration Pick's disease Progressive supranuclear palsy	77.6 (99.1)	Tau protein was shown to be in a random coil-like conformation according to far-UV CD, FTIR, X-ray scattering and biochemical assays
Prion protein (231)	Prion diseases Creutzfeld-Jacob disease Gerstmann- Sträussler -Schneiker syndrome Fatal familial insomnia Kuru Bovine spongiform encephalopathy Scrapie Chronic wasting disease	55.8 (61.0)	According to NMR and far-UV CD, the N-terminal region (from amino acid 23 to 126) is largely unstructured in the isolated molecule in solution
Alpha-synuclein (140)	Synucleinopathies Parkinson's disease Lewy body variant of Alzheimer's disease Diffuse Lewy body disease Dementia with Lewy bodies Multiple system atrophy Neurodegeneration with brain iron accumulation type I	90.7 (37.1)	Highly unfolded structure of entire protein is confirmed by NMR, FTIR, SAXS, far-UV CD, gel filtration, dynamic light scattering, FRET, limited proteolysis, aberrant mobility in SDS-PAGE
Beta-synuclein (134)	Parkinson's disease Diffuse Lewy body disease	87.3 (52.2)	Highly unfolded conformation is confirmed by NMR, FTIR, SAXS, far-UV CD and gel filtration
Gamma-synuclein (127)	Parkinson's disease Diffuse Lewy body disease	100 (56.8)	Highly unfolded conformation is confirmed by NMR, FTIR, SAXS, far-UV CD and gel filtration
Huntingtin (3144; polyQ tract: 16-37 glutamines in norm; >38 glutamines in pathology)	Huntington's disease	35.5 (30.4)	The far-UV CD spectra of poly(Gln) peptides with repeat lengths of 5, 15, 28 and 44 residues were shown to be nearly identical and were consistent with a high degree of random coil structure
DRPLA protein (1185; polyQ tract: 7-23 glutamines in norm; 49-75 glutamines in pathology)	Hereditary dentatorubral-pallidoluysian atrophy	89.5 (84.2)	Aberrant electrophoretic mobility. Apparent molecular mass estimated by SDS-PAGE is ~1.6-fold higher than the predicted molecular mass
Androgen receptor (919; polyQ tract: 15-31 glutamines in norm; 40-62 glutamines in pathology)	Kennedy's disease or X-linked spinal and bulbar muscular atrophy	53.9 (46.7)	Far-UV CD, gel-filtration, limited proteolysis, ANS binding and urea-induced unfolding studies revealed that the AF1 transactivation domain is in the molten globule state
Ataxin-1 (816; polyQ tract: 6-39 glutamines in norm; 41-81 glutamines in pathology)	Spinocerebellar ataxia 1 Neuronal intranuclear inclusion disease	76.8 (73.4)	
Ataxin-2 (1312; polyQ tract: 22-31 glutamines in norm; >32 glutamines in pathology)	Spinocerebellar ataxia 2	93.8 (76.9)	Ataxin-2 contains two globular domains, Lsm and LsmAD, in an acidic region (amino acid 254–475). The rest of ataxin-2 outside of the Lsm and LsmAD domains is predicted to be intrinsically disordered
Ataxin-3 (376; polyQ tract: 12-40 glutamines in norm; 55-84 glutamines in pathology)	Spinocerebellar ataxia 3	52.1 (47.1)	Far-UV CD and NMR spectroscopies suggest that ataxin-3 is only partially folded. The far-UV CD signal of the full-length protein is dominated by the Josephin motif (N-terminal domain 1-198), with the C-terminal portion of the protein making a smaller contribution, consistent with its largely unstructured conformation.
P/Q-type calcium channel alpha1A subunit (2505; polyQ tract: 4-16 glutamines in norm; 21-28 glutamines in pathology)	Spinocerebellar ataxia 6	53.0 (49.3)	Aberrant electrophoretic mobility
Ataxin-7 (892; polyQ tract: 4-35 glutamines in norm; 36-306 glutamines in pathology)	Spinocerebellar ataxia 7	89.5 (70.2)	Aberrant electrophoretic mobility. Apparent molecular mass estimated by SDS-PAGE is 1.15-fold higher than that calculated from amino acid sequence
TATA-box-binding protein (339; polyQ tract: 25-42 glutamines in norm; >42 glutamines in pathology)	Spinocerebellar ataxia 17	53.9 (52.5)	Aberrant electrophoretic mobility. Apparent molecular mass estimated by SDS-PAGE is 1.3-fold higher than that calculated from amino acid sequence
ABri (34)	Familial British dementia	29.4 (23.5)	Far-UV CD and NMR spectroscopy revealed that ABri is in the random coil-like conformation at slightly acidic pH
ADan (34)	Familial Danish dementia	29.4 (23.5)	Far-UV CD revealed that ADan showed mostly

			random coil structure
Glial fibrillary acidic protein (432)	Alexander's disease	82.4 (68.5)	Extremely high susceptibility to proteolysis
Mitochondrial DNA polymerase gamma (1239)	Alpers disease	37.1 (36.7)	Aberrant electrophoretic mobility
DNA excision repair protein ERCC-6 (1493)	Cockayne syndrome	56.8 (47.8)	Aberrant electrophoretic mobility
Survival motor neuron protein (294)	Spinal muscular atrophy	69.7 (60.2)	Aberrant electrophoretic mobility

Disorder was predicted by two predictors, PONDR® VSL2 and VLXT (given in parenthesis), respectively. PONDR® VSL2 was chosen because of its high accuracy, whereas PONDR® VLXT was chosen because this predictor was shown to be very sensitive for the presence of molecular recognition features, which are disordered polypeptide segments that are predicted to acquire secondary structure upon forming complexes with binding partners.

fibrillation of ordered proteins is frequently associated with the pathogenesis of systemic amyloidoses (e.g., mutated lysozyme in hereditary systemic amyloidosis, mutated gelsolin in Finish-type familial amyloidosis, beta$_2$-microglobulin in amyloidosis associated with hemodialysis, immunoglobulin light chain variable domains in light chain associated amyloidosis and light chain deposition disease, etc.), but these proteins are not too common in neurodegenerative diseases (11). Cystatin C represents an illustrative example of ordered proteins, fibrillation of which is indirectly related to neurodegeneration. In fact, in hereditary cystatin C amyloid angiopathy, mutated cystatin C forms amyloid, predominantly in brain arteries and arterioles. This amyloid deposition in the vessel walls causes thickening of the walls leading to occlusion or rupture and resulting in brain hemorrhage (22).

Despite significant structural differences of fibrillating proteins, the fibrils from different pathologies display many common properties, including a core cross-beta-sheet structure in which continuous beta-sheets are formed with beta-strands running perpendicular to the long axis of the fibrils (23). This beta-pleated sheet structure of fibrils constitutes the basis of the unusual resistance of all kinds of amyloid to degradation and, therefore, the progressive deposition of the material (24). Furthermore, all fibrils have similar twisted, rope-like structures that are typically 7–13 nm wide (25, 26) and consist of a number of protofilaments (typically 2–6), each about 2–5 nm in diameter (25). Alternatively, protofilaments may associate laterally to form long ribbons that are 2–5 nm thick and up to 30 nm wide (27-29).

2.3. Relationships between amino acid sequence and fibrilogenesis

Although all proteins can form amyloid fibrils under the appropriate conditions (1), the list of currently known trouble makers (i.e., proteins aggregation of which is directly responsible for the development of various pathologies) is rather short, including 30-40 proteins and protein fragments. Why do some proteins form fibrils under the physiological conditions, whereas others do not? It has been pointed out that the propensity of a protein to aggregate can be reliably correlated with a set of simple physico-chemical parameters (30-32). In fact, it is believed that aggregation occurs when protein segments with a high hydrophobicity, a good beta-sheet propensity and a low net charge are solvent-exposed so that they can associate, act as nuclei for beta-aggregation, and therefore initiate the formation of an intermolecular beta-sheet (33-38). In the folded state, such aggregation-prone segments are buried, not exposed to the solvent, and therefore protein does not aggregate. On the other hand, aggregation of many globular proteins occurs during refolding or under conditions in which denatured or partially folded states are significantly populated, i.e. at high concentration or as a result of destabilizing conditions or mutations (11, 20). Based on these findings, the algorithm TANGO was developed to predict beta-aggregating stretches in proteins, based on a statistical mechanics algorithm that considers the physico-chemical parameters described above and also takes into account competition between different structural conformations: beta-turn, alpha-helix, beta-sheet aggregates and the folded state (39). This algorithm accurately predicted the aggregation propensity of ~250 peptides, including those derived from human disease-related proteins, such as prion protein, lysozyme and beta$_2$-microglobulin. It was even able to correctly predict pathogenic as well as protective mutations of the A-beta, human lysozyme and transthyretin, and discriminates between beta-sheet propensity and aggregation. Therefore, these data clearly confirmed the model of intermolecular beta-sheet formation as a widespread underlying mechanism of protein aggregation (39). Importantly, the application of TANGO also showed that the beta-aggregation propensity of all-alpha, all-beta and mixed alpha/beta globular proteins as well as membrane-associated proteins was fairly similar, suggesting that beta-aggregation was not determined by hydrophobicity and beta-sheet propensity alone (38). Importantly, it has been established that globular globular proteins contained almost three times as much aggregation nucleating regions as IDPs and that the formation of highly structured globular proteins comes at the cost of a higher beta-aggregation propensity because both structure formation and aggregation follow very similar physico-chemical rules (38).

In line with these observations, an absolute rate equation was derived from both first principles (i.e., from the detailed analysis of the physicochemical properties of amino acids that are essential for ordered aggregation) and analysis of aggregating sequences designed by a computational approach (40). This model based on physicochemical properties and computational design of beta-aggregating peptide sequences was shown to be able to predict the aggregation rate over a large set of natural polypeptide sequences. In addition to evaluating the aggregation rate, the proposed model gave the "amyloid spectrum" of any protein by identifying segments

potentially involved in beta-aggregation and was even able to predict the parallel or anti-parallel beta-sheet organization in fibrils. An important illustration of the strength of this model was it ability to recognize different beta-aggregating segments in mammalian and non-mammalian prion proteins, providing insights into the species barrier for the transmission of the prion disease (40).

Finally, a recent study on the sequence shuffling in the yeast prion proteins Ure2 and Sup35p should be briefly discussed (41). [URE3] and [PSI (+)] being the amyloid-based prions (infectious proteins), are self-propagating amyloid forms of Ure2p and Sup35p proteins of *Saccharomyces cerevisiae*, respectively. The prion forming ability of Ure2p and Sup35p has been largely localized to short N-terminal domains (so-called Q/N-rich domains). Surprisingly, it has been shown that the sequence of the prion domains is not critical to prion formation, since scrambling sequences of the Ure2p and Sup35p prion domains does not abrogate the ability of the proteins to become prions (41). This observation clearly showed that the amino acid composition of a polypeptide, rather than its specific amino acid sequence, determines the capability of a yeast protein to form amyloid fibrils and thus to become infectious (41).

2.4. Blocking fibrillogenesis

Since protein aggregation is associated with the pathogenesis of several neurodegenerative diseases, it is believed that the prevention of this process may represent an effective therapeutic approach for the treatment of AD, PD, HD, prion diseases, and other neurodegenerative maladies. Therefore, the search for small molecules-inhibitors of protein aggregation represents a very promising area of research. Significant progress is achieved in this field. For example, a number of small molecules have been reported to inhibit A-beta fibrillogenesis, including multiple modulators of A-beta fibrillation (42-59), some of which were able to inhibit A-beta-mediated cellular toxicity (42, 43, 51, 60, 61). In a recent study (62), the anti-oligomer antibody A11 (63) was utilized to find small molecules capable of inhibiting the A-beta aggregation and to characterize the mechanism of action of these A-beta aggregation inhibitors in terms of oligomer and fibril formation. The authors identified a number of small molecules capable of inhibiting oligomer formation, which were grouped into three distinct classes: 17 compounds that inhibited oligomerization but not fibrillation, 5 compounds that inhibited fibrillation but not oligomerization, and 13 compounds that inhibited both oligomerization and fibrillation (62). This study clearly showed that (*i*) selective inhibition of either A-beta oligomerization or fibrillation is possible, which allows the separate targeting of either species; (*ii*) the search for fibril inhibitors will only identify a subset of potential oligomer inhibitors since oligomer and fibril formation can be inhibited independently. Finally, it has been pointed out that selective inhibition of specific aggregated species is feasible and useful both for unraveling mechanisms underlying protein fibrillation and for therapeutic testing in models of neurodegeneration (62).

Neuritic plaques, neurofibrillary tangles, and neuropil threads are hallmark lesions of AD that contain filamentous intraneuronal inclusions of tau protein (64). It is believed that tau filament formation indicates the onset of cytoskeletal disorganization that is characteristic of degenerating neurons and may represent a fundamental pathobiological response of neurons to various insults. Therefore, suppression of tau fibrillation represents an attractive target for the drug development, as selective pharmacological inhibitors of the process may have utility in slowing neurodegeneration associated with the aggregation and fibrillation of this protein. Several small molecules were shown to inhibit tau fibrillation. This includes N744, or 3-(2-hydroxyethyl)-2-[2-[[3-(2-hydroxyethyl)-5-methoxy-2-benzothiazolylidene]methyl]-1-butenyl]-5-meth-oxybenzothiazolium (65), phenothiazines (66), anthraquinones (67), polyphenols, porphyrins (68). Recently, a library of ~51,000 compounds was analyzed by high throughput screening to find small molecules inhibitors of heparin-induced tau fibrillation (69). This analysis revealed a set of 11 compounds from eight compound classes: sulfonated dyes, phenothiazines, anthraquinones, benzofurans, porhyrins, quinoxalines, pyrimidotriazines, and a depsidone (69).

Similarly, various small molecules, such as rifampicin (70), dopamine and L-DOPA (71), dopamine-related catecholamines (72), flavanoid baicalein (73, 74), and nicotine (75) were shown to inhibit fibrillation of alpha-synuclein and to disaggregated the preformed fibrils. Recently, the fibrillation of this protein was successfully inhibited by dendrimers of polyamidoamine (PAMAM) (76). Dendrimers are highly-branched polymers of a well-defined spherical geometry and, thus, are monodisperse in solutions. Their tree-like architecture is synthesized by symmetrical branching from a multifunctional core towards the periphery, via a stepwise and repetitive reaction sequence. Each step of the reaction creates an additional "shell" on the dendrimer surface, called a "generation". Each generation doubles the molecular weight, as well as both the number of branching points and end-groups at the surface (76). The efficiency of PAMAM dendrimers to inhibit alpha-synuclein fibrillation and to disassociate fibrils was shown to increase both with generation number and PAMAM concentration (76).

Based on the analysis of the literature data, it has been pointed out that various polyphenols could serve as potential therapeutic agents for the treatment of amyloid-associated diseases, as several small polyphenol molecules were shown to remarkably inhibit the formation of fibrillar assemblies of various proteins *in vitro* and to suppress their associated cytotoxicity (77). The protective effect of polyphenols against amyloid cytotoxicity in cell culture and primary culture systems, as well as their fibrillation inhibitory activity are frequently attributed to the antioxidative features of polyphenols (78). However, based on structural similarities between various highly efficient polyphenol inhibitors (all of them are composed of at least two phenolic rings with two to six atom linkers, and a minimum number of three OH groups on the aromatic rings), and well-known amyloidogenic dye Congo red, an

additional inhibitory mechanism of polyphenol was proposed (77). The mentioned above structural similarities suggest that highly efficient polyphenol inhibitors possess specific three-dimensional conformations that are essential for the non-covalent interaction with beta-sheet structures of amyloid fibrils and for the stabilization of the inhibition–protein complex. Additionally, it was proposed that specific interactions between the phenolic compounds in the inhibitor molecules and aromatic residues in the amyloidogenic protein may direct the inhibitor to the amyloidogenic core and facilitate interaction, but interfere with fibril assembly (77).

2.5. Nanoimaging in amyloidoses

It is believed that amyloidogenic aggregation, being often a pathogenic state of proteins, is a generic phase of any polypeptide chain, representing a specific 3-D organization dominated by the backbone-backbone interactions. It is assumed to be mostly oblivious of the information encoded in the primary sequence. It has been also noted that the formation of amyloid fibrils does not represent the only pathological hallmark of conformational or protein deposition diseases. In several neurodegenerative disorders (as well as in numerous *in vitro* experiments) the protein depositions are composed of the amorphous aggregates, cloud-like inclusions without defined structure. Similarly, soluble oligomers represent another alternative final product of the aggregation process. The choice between three aggregation pathways, fibrillation, amorphous aggregate formation or oligomerization, is determined by the amino acid sequence (which could be modified by mutation) and by the peculiarities of the protein environment.

Obviously, the progress in understanding the pathology of protein misfolding diseases and in rational design of drugs to inhibit or reverse protein aggregation depends on our ability to study the details of the misfolding process, to follow the aggregation process, and to see and analyze the structure of the aggregated particles. Nanoimaging represents a set of unique tools that allow visualization and analysis of aggregated structures and aggregation process.

2.6. Intrinsically disordered proteins, IDPs

2.6.1. Concept

Evidence is rapidly accumulating that many protein regions and even entire proteins lack stable tertiary and/or secondary structure in solution, existing instead as dynamic ensembles of interconverting structures. These naturally flexible proteins are known by different names, including intrinsically disordered (79), natively denatured (80), natively unfolded (81), intrinsically unstructured (82), and natively disordered proteins (83). These proteins are called “intrinsically disordered” from now on. By “intrinsic disorder” it is meant that the protein exists as a structural ensemble, either at the secondary or at the tertiary level. In other words, in contrast to ordered proteins whose 3-D structure is relatively stable and Ramachandran angles vary slightly around their equilibrium positions with occasional cooperative conformational switches, IDPs or intrinsically disordered regions (IDRs) exist as dynamic ensembles in which the atom positions and backbone Ramachandran angles vary significantly over time with no specific equilibrium values and typically undergo non-cooperative conformational changes. To some extent conformational behavior and structural features of IDPs and IDRs resemble those of non-native states of “normal” globular proteins, which may exist in at least four different conformations: ordered, molten globule, pre-molten globule, and coil-like (84-87). Using this analogy, IDPs and IDRs might contain collapsed-disorder (i.e., where intrinsic disorder is present in a molten globular form) and extended-disorder (i.e., regions where intrinsic disorder is present in a form of random coil or pre-molten globule) under physiological conditions *in vitro* (79, 83, 85).

2.6.2. Experimental techniques for IDP detection

The disorder in IDPs has been detected by several physicochemical methods elaborated to characterize protein self-organization. The list includes but is not limited to X-ray crystallography (88), NMR spectroscopy (83, 89-93), near-UV circular dichroism (CD) (94), far-UV CD (95-98), optical rotatory dispersion (ORD) (95, 98), FTIR (98), Raman spectroscopy and Raman optical activity (99), different fluorescence techniques (100, 101), numerous hydrodynamic techniques (including gel-filtration, viscometry, small angle X-ray scattering (SAXS), small angle neutron scattering (SANS), sedimentation, and dynamic and static light scattering) (100, 101), rate of proteolytic degradation (102-106), aberrant mobility in SDS-gel electrophoresis (107, 108), low conformational stability (100, 109-112), H/D exchange (101), immunochemical methods (113, 114), interaction with molecular chaperones (100), electron microscopy or atomic force microscopy (100, 101), the charge state analysis of electrospray ionization mass-spectrometry (115). For more detailed reviews on methods used to detect intrinsic disorder see (83, 90, 101, 116). The aberrant mobility on SDS-gel electrophoresis will be frequently used in this review as an evidence for the IDP nature of a given protein, as the anomalous electrophoretic mobility was shown to be one of the characteristic features of IDPs (101, 107, 108). In fact, the apparent molecular masses of IDPs determined by this technique are often 1.2-1.8 times higher than real one calculated from sequence data or measured by mass spectrometry (101, 107, 108). Our analysis revealed that the abnormality degree of the electrophoretic mobility of an IDP is directly proportional to the amount of intrinsic disorder present in its sequence (Uversky, personal communication). It has been suggested that IDPs bind less SDS than “normal” proteins. This explains their abnormal mobility in SDS polyacrylamide gel electrophoresis experiments, resulting in the observed increase in the apparent molecular masses.

2.6.3. Sequence peculiarities of IDPs and predictors of intrinsic disorder

IDPs and IDRs differ from structured globular proteins and domains with regard to many attributes, including amino acid composition, sequence complexity, hydrophobicity, charge, flexibility, and type and rate of amino acid substitutions over evolutionary time. For example, IDPs are significantly depleted in a number of so-

called order-promoting residues, including bulky hydrophobic (I, L, and V) and aromatic amino acids (W, F, Y), which would normally form the hydrophobic core of a folded globular protein, and also possess low content of C and N residues. On the other hands, IDPs were shown to be substantially enriched in so called disorder-promoting amino acids: A, R, G, Q, S, P, E, and K (79, 117-119). Many of the mentioned differences were utilized to develop numerous disorder predictors, including PONDR® (117, 120), CH-plot (98), NORSp (121), GlobPlot (122, 123), FoldIndex© (124), IUPred (125), DisoPred (126-128) to name a few. It is important to remember that comparing several predictors on an individual protein of interest or on a protein dataset can provide additional insight regarding the predicted disorder if any exists (129-134).

2.6.4. Natural abundance of IDPs and their biological functions

Application of various disorder predictors to different proteomes revealed that intrinsic disorder is highly abundant in nature and the overall amount of disorder in proteins increases from bacteria to archaea to eukaryota, with over a half of the eukaryotic proteins containing long predicted IDRs (128, 129, 135). One explanation for this trend is a change in the cellular requirements for certain protein functions, particularly cellular signaling. In support of this hypothesis, an analysis of a eukaryotic signal protein database indicated that the majority of known signal transduction proteins were predicted to contain significant regions of disorder (136).

Although IDPs fail to form unique 3D-structures under physiological conditions, they might carry out important biological functions, the fact which was recently confirmed by several comprehensive studies (79, 82, 83, 85, 92, 98, 108, 116, 136-148). Furthermore, sites of posttranslational modifications (acetylation, hydroxylation, ubiquitination, methylation, phosphorylation, etc.) and proteolytic attack are frequently associated with regions of intrinsic disorder (147). The functional diversity provided by IDRs was suggested to complement functions of ordered protein regions (145-147).

IDPs have specific functions that can be grouped into four broad classes: (*i*) molecular recognition; (*ii*) molecular assembly; (*iii*) protein modification; and (*iv*) entropic chain activities (136). Despite (or may be due to) their high flexibility, IDPs are involved in regulation, signaling and control pathways in which interactions with multiple partners and high-specificity/low-affinity interactions are often requisite (138, 148). In a living organism, proteins participate in complex interactions, which represent the mechanistic foundation of the organism's physiology and function. Regulation, recognition and cell signaling involve the coordinated actions of many players. To achieve this coordination, each participant must have a valid identification ("ID") that is easily recognized by the others. For proteins, these "IDs" are often within IDRs (138, 148).

Another very important feature of the IDPs is their unique capability to fold under the variety of conditions (82, 84, 89, 92, 98, 108, 116, 136, 138, 140, 144, 148, 149). In fact, the folding of these proteins can be brought about by interaction with other proteins, nucleic acids, membranes or small molecules. It also can be driven by changes in the protein environment. The resulting conformations could be either relatively non-compact (i.e., remain substantially disordered) or be tightly folded.

3. ABUNDANCE OF IDPs IN NEURODEGENERATIVE DISEASES: EVIDENCE FROM THE BIOINFORMATICS ANALYSES

Because of the fact that IDPs play a number of crucial roles in numerous biological processes, it was not too surprising to find that some of them are involved in human diseases. An incomplete list of human neurodegenerative diseases associated with IDPs includes AD (deposition of amyloid-beta, tau-protein, alpha-synuclein fragment NAC (150, 151); Niemann-Pick disease type C, subacute sclerosing panencephalitis, argyrophilic grain disease, myotonic dystrophy, and motor neuron disease with neurofibrillary tangles (NFTs) (accumulation of tau-protein in form of NFTs (152)); Down's syndrome (nonfilamentous amyloid-beta deposits (153)); PD, dementia with Lewy body (LB), diffuse LB disease, LB variant of AD, multiple system atrophy (MSA) and Hallervorden-Spatz disease (deposition of alpha-synuclein in a form of LB, or Lewy neurites (LNs) (154)); prion diseases (deposition of PrP^{SC} (155)); and a family of polyQ diseases, a group of neurodegenerative disorders caused by expansion of GAC trinucleotide repeats coding for polyQ in the gene products (156).

Table 1 and Figure 1 illustrates that some individual proteins involved in human neurodegenerative diseases are either completely disordered or contain long disordered regions. Figure 1 represents the results of the comparison of the compositions of proteins from Table 1 with the composition of ordered proteins from PDB. The corresponding data for the DisProt (157) are shown for comparison. Calculations were done using a normalization procedure elaborated for analysis of IDPs (79, 158). In brief, compositional profiling is based on the evaluation of the $(C_{s1} - C_{s2})/C_{s2}$ values, where C_{s1} is a content of a given residue in a set of interest (proteins associated with neurodegenerative diseases or typical IDPs from DisProt), whereas C_{s2} is the corresponding value for the set of ordered proteins. In this presentation, negative values correspond to residues which are depleted in a given dataset in comparison with a set of ordered proteins, whereas the positive values correspond to the residues which are over-represented in the set.

Figure 1 shows that in general all proteins in Table 1 are highly different from typical ordered proteins and generally follow the trend for IDPs (with some exceptions). Proteins associated with neurodegenerative diseases are in general depleted in major order-promoting residues. This includes C, W, I, Y, F, V and N. They are highly enriched in the major disorder-promoting residues (Q, S, R, and P). There are also some deviations from the behavior of "typical" disordered proteins. This includes the high abundance of L and H and the depletion in T, D, and

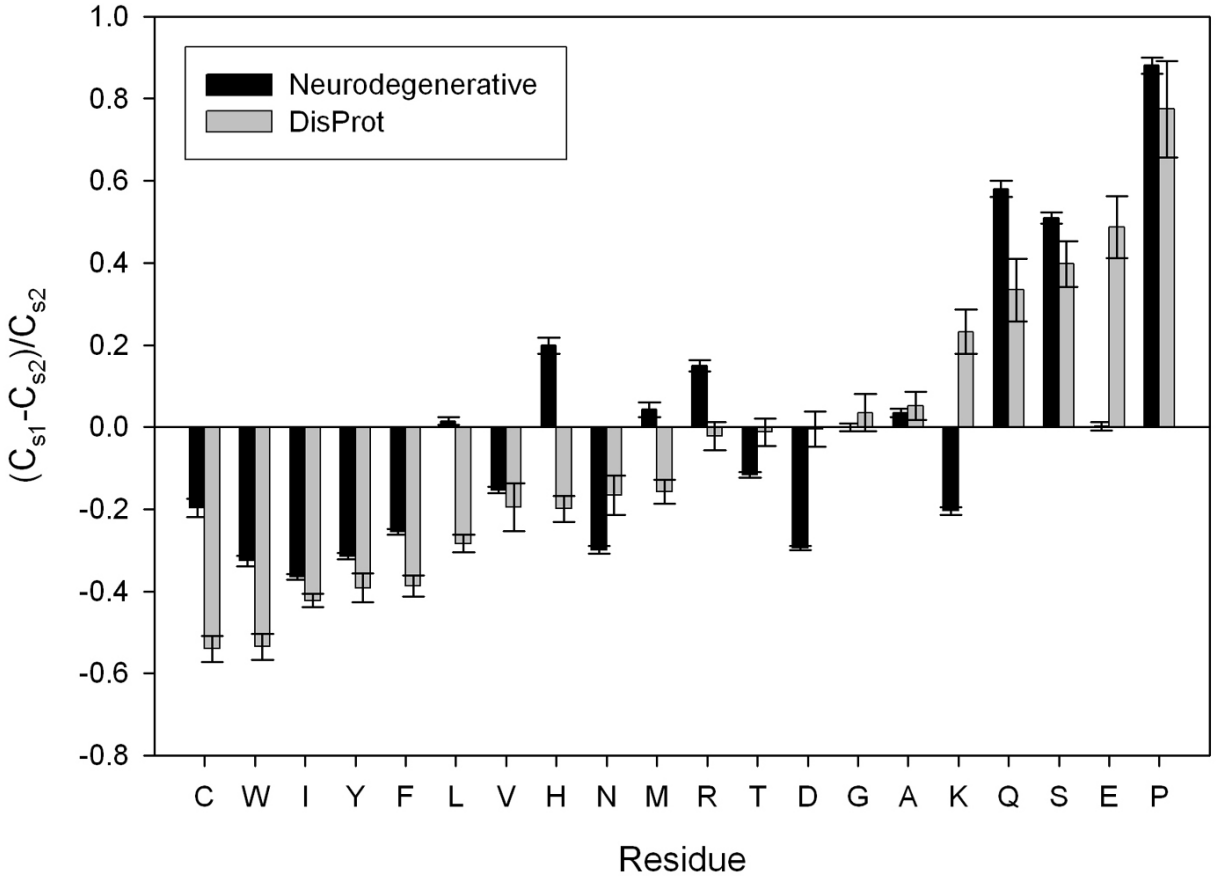

Figure 1. Compositional profiling of proteins involved in neurodegenerative disease. Analyzed proteins are listed in Table 1. Enrichment or depletion in each amino acid type appears as a positive or negative bar, respectively. Amino acids are indicated by the single-letter code and ordered according to the increasing disorder promoting strength. Error bars represent standard deviations from the mean. Corresponding data for well-characterized IDRs from DisProt are also shown.

K. This suggests that proteins listed in Table 1 are in general characterized by a high level of intrinsic disorder.

This fact raises the question of how abundant are the IDPs in various neurodegenerative conditions. To answer this question, a set of 689 proteins related to neurodegenerative diseases was collected and analyzed using an approach elaborated to analyze the abundance of intrinsic disorder in cancer-related proteins (149). In that study, 79% of cancer-associated and 66% of cell-signaling proteins were found to contain predicted regions of disorder of 30 residues or longer (149). In contrast, only 13% of proteins from a set of proteins with well-defined ordered structures contained such long regions predicted to be disordered by PONDR® VLXT. In agreement with these bioinformatics studies, the presence of intrinsic disorder has been directly observed in many cancer-associated proteins (149).

The overall results of the analogous analysis for proteins associated with neurodegenerative disease are shown in Figure 2, which represents percentages of proteins with ≥30 consecutive residues predicted to be disordered by PONDR® VSL2 in various datasets, including cancer-related proteins, signaling proteins, ordered proteins from PDB, eukaryotic proteins and proteins involved in various neurodegenerative diseases. This figure illustrates that intrinsic disorder is highly prevalent in neurodegenerative disease-related proteins, being comparable with that of signaling and cancer-related proteins and significantly exceeds the level of intrinsic disorder in eukaryotic proteins from SWISS-PROT and in non-homologous, structured proteins from the PDB. Thus, intrinsic disorder is very common in neurodegeneration-associated proteins. To further illustrate this concept, Table 1 represents some of the IDPs and their corresponding neuropathological conditions. Many of these proteins were structurally characterized and experimental evidence on the presence of intrinsic disorder in some of these proteins is also listed. Table 1 shows that there is a great agreement between experimental and computational data. Finally, the results of the disorder prediction by two predictors, PONDR® VSL2 and VLXT, are shown. Figure 3 represents plots of the PONDR® VSL2 predicted disorder distribution within the sequences of 20 neurodegenerative disease-related IDPs. It clearly shows that these proteins are very divers: their length range from 34 to 3144 amino acids, the amount of predicted disorder range from 16.7 to 100%, and

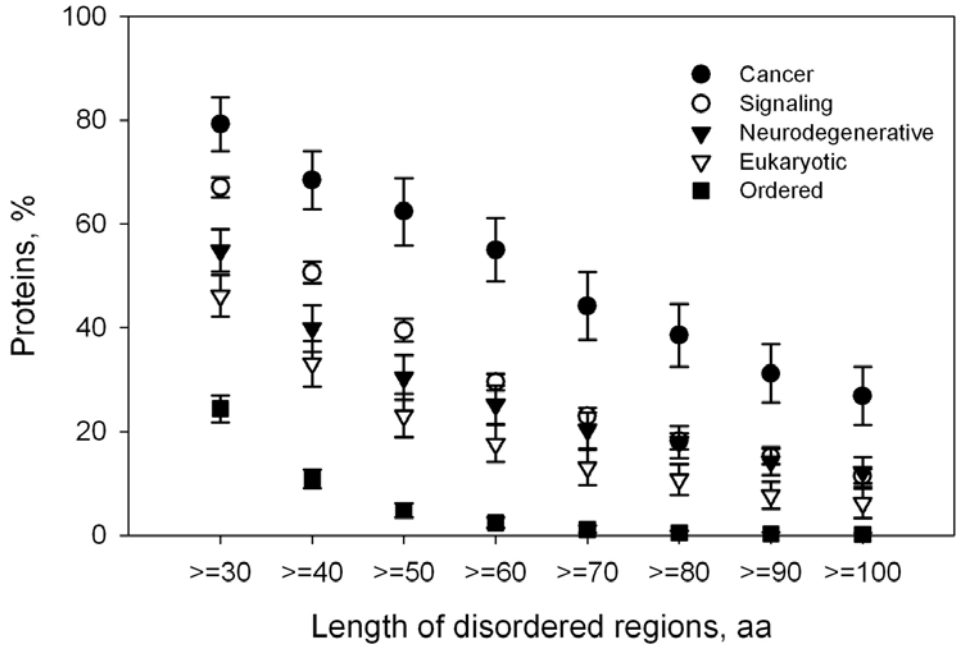

Figure 2. Abundance of intrinsic disorder in proteins associated with neurodegenerative diseases. Percentages of disease-associated proteins with ≥30 to ≥100 consecutive residues predicted to be disordered. The error bars represent 95% confidence intervals and were calculated using 1,000 bootstrap re-sampling. Corresponding data for signaling and ordered proteins are shown for the comparison. Analyzed protein sets included 1,786 proteins associated with cancer, 689 proteins involved in the neurodegenerative diseases, 2,329 proteins involved in cellular signaling, 1,138 non-homolohous ordered proteins from PDB Select 25 (this dataset contained only the ordered parts of the proteins), and 53,630 non-redundant eukaryotic proteins from SWISS-PROT.

the profiles of disorder distribution are very different. Therefore, computational analysis showed that the majority of the proteins involved into the pathogenesis of neurodegenerative disease are intrinsically disordered. Subsequent sections consider illustrative examples of some of the most important neurodegenerative IDPs and their corresponding diseases.

4. ALPHA-SYNUCLEIN AS AN ILLUSTRATIVE EXAMPLE OF INTRINSICALLY DISORDERED PROTEINS RELATED TO THE NEURODEGENERATION

Before considering various neurodegeneration-related IDPs and their related maladies, some characteristic structural features of these proteins should be introduced. This is done using an illustrative example of alpha-synuclein, which is the one of the most thoroughly studied IDPs. A brief description of structural properties of this protein is presented below. It is also pointed out that this protein is likely to maintain its intrinsically disordered structure even being located in a highly crowded environment of a living cell.

4.1. Structural properties of alpha-synuclein

Alpha-synuclein, a protein that links various synucleinopathies, is one of the most studied IDPs. It possesses little or no ordered structure under the "physiological" conditions *in vitro* (i.e., conditions of neutral pH and low to moderate ionic strength) (159). For example, at neutral pH alpha-synuclein is characterized by far-UV CD and FTIR spectra typical of a substantially unfolded polypeptide chain with a low content of ordered secondary structure (Figures 4A and 4B). This includes: the characteristic minimum in the vicinity of 196 nm and the absence of bands in the 210-230 nm region in far-UV CD spectrum and broad band at 1650 cm^{-1} in the FTIR spectrum. Deconvolution of the FTIR spectra followed by curve fitting revealed that the majority of the molecule (~70%) is disordered (159). The hydrodynamic properties of this protein are in a good agreement with the results of the far-UV CD and FTIR studies and show that alpha-synuclein, being essentially expanded, does not have a tightly packed globular structure, but is slightly more compact than expected for a random coil (159). This follows from the comparison of values of the measured Stokes radius, R_S, with those calculated for a completely unfolded polypeptide chain of the appropriate molecular mass (116, 144, 160, 161). It has been shown that the Stokes radius measured for alpha-synuclein was notably lower than the corresponding calculated value (31.8±0.4 vs. 34.3 Å, (162)). This conclusion was confirmed by measurement of the alpha-synuclein R_S in the presence of 8 M urea, where the protein behaved as a random coil (R_S=34.5±0.4 Å, (162)).

SAXS is a very useful method for the investigation of conformation, shape and dimensions of

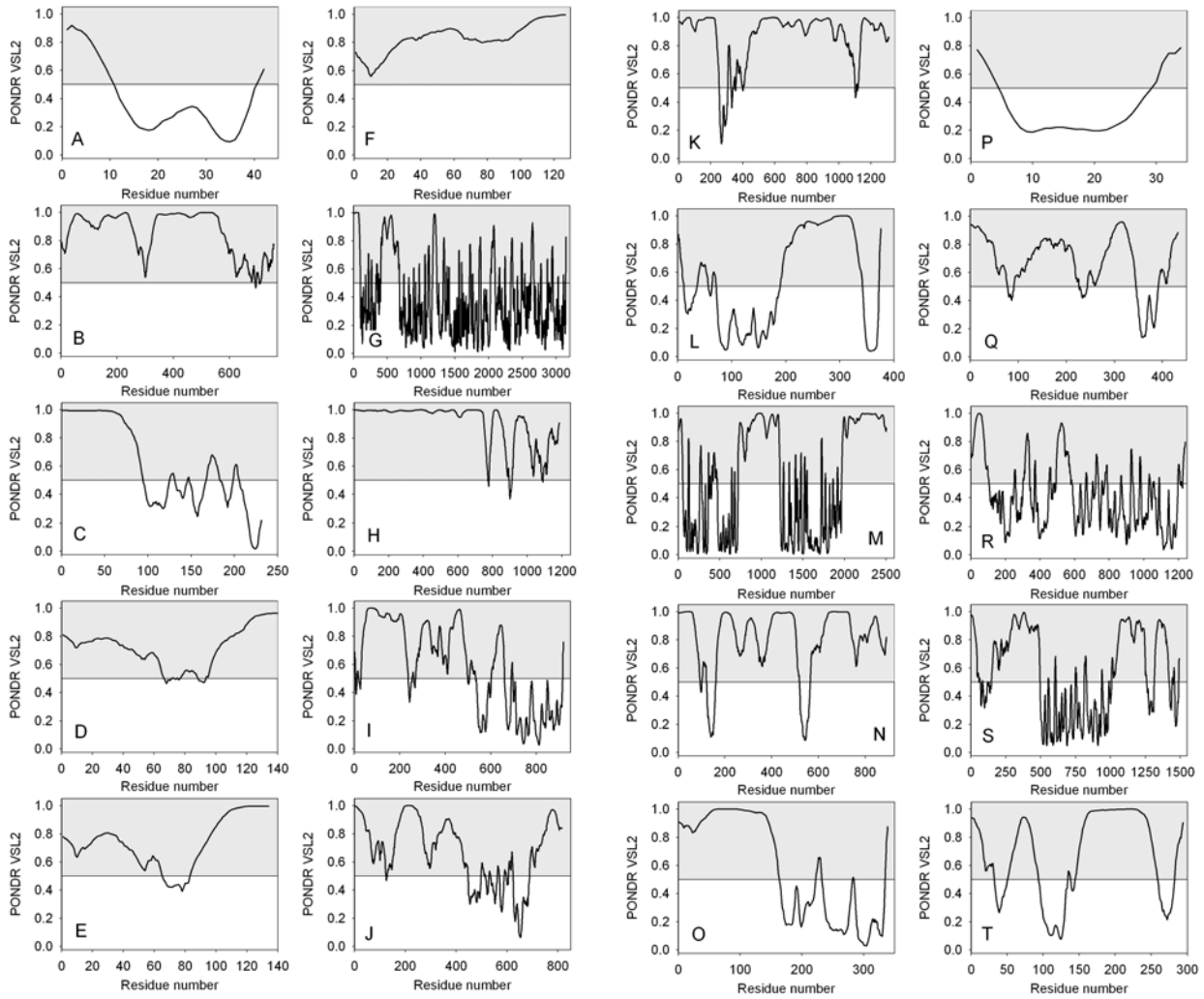

Figure 3. Distribution of intrinsic disorder in neurodegeneration-related proteins as predicted by PONDR VSL2. A, A-beta; B, Tau protein; C, Prion protein; D, Alpha-synuclein; E, Beta-synuclein; F, Gamma-synuclein; G, Huntingtin; H, DRPLA protein (atrophin-1); I, Androgen receptor; J, Ataxin-1; K, Ataxin-2; L, Ataxin-3; M, P/Q-type calcium channel alpha1A subunit; N, Ataxin-7; O, TATA-box-binding protein; P, ABri; Q, Glial fibrillary acidic protein; R, Mitochondrial DNA polymerase gamma; S, DNA excision repair protein ERCC-6 ; T, Survival motor neuron protein. Shaded areas in each plot correspond to the scores associated with intrinsic disorder. Details of the analyzed proteins are listed in Table 1.

biopolymers in solution. Analysis of the scattering curves using the Guinier approximation provides the radius of gyration, R_g. Scattering data in the form of Kratky plots provides information about the globularity (packing density) and conformation of the protein (163): for a native globular protein this plot has a characteristic maximum, whereas unfolded and partially folded polypeptides have significantly different-shaped Kratky plots. Figure 4C represents Kratky plots for a typical globular protein (Staphylococcal nuclease) and alpha-synuclein at variety of conditions. Figure 4C clearly shows that alpha-synuclein lacks a well-developed globular structure at both conditions studied (pH 7.5 and pH 3.0). In fact, the profile of the Kratky plot at neutral pH is typical for a random coil conformation, whereas that at pH 3 shows changes consistent with the development of the beginnings of a tightly packed core.

The radius of gyration of a completely unfolded polypeptide, R_g^U, may be estimated from the corresponding Stokes radius, R_S^U, using the relation R_g^U/R_S^U=1.51 (164). The observed R_g value for alpha-synuclein at neutral pH (40±1 Å) is smaller than that estimated for a random coil conformation for a protein of this size (52 Å), indicating that the natively unfolded conformation of this protein is more compact than that of a random coil (159, 162, 165, 166). Finally, the profile of the Kratky plot at neutral pH was typical for a random coil conformation (159, 162, 165, 166). Thus, at neutral pH alpha-synuclein was shown to be essentially disordered, but slightly more compact than a random coil. Based on the results of pulsed-field gradient

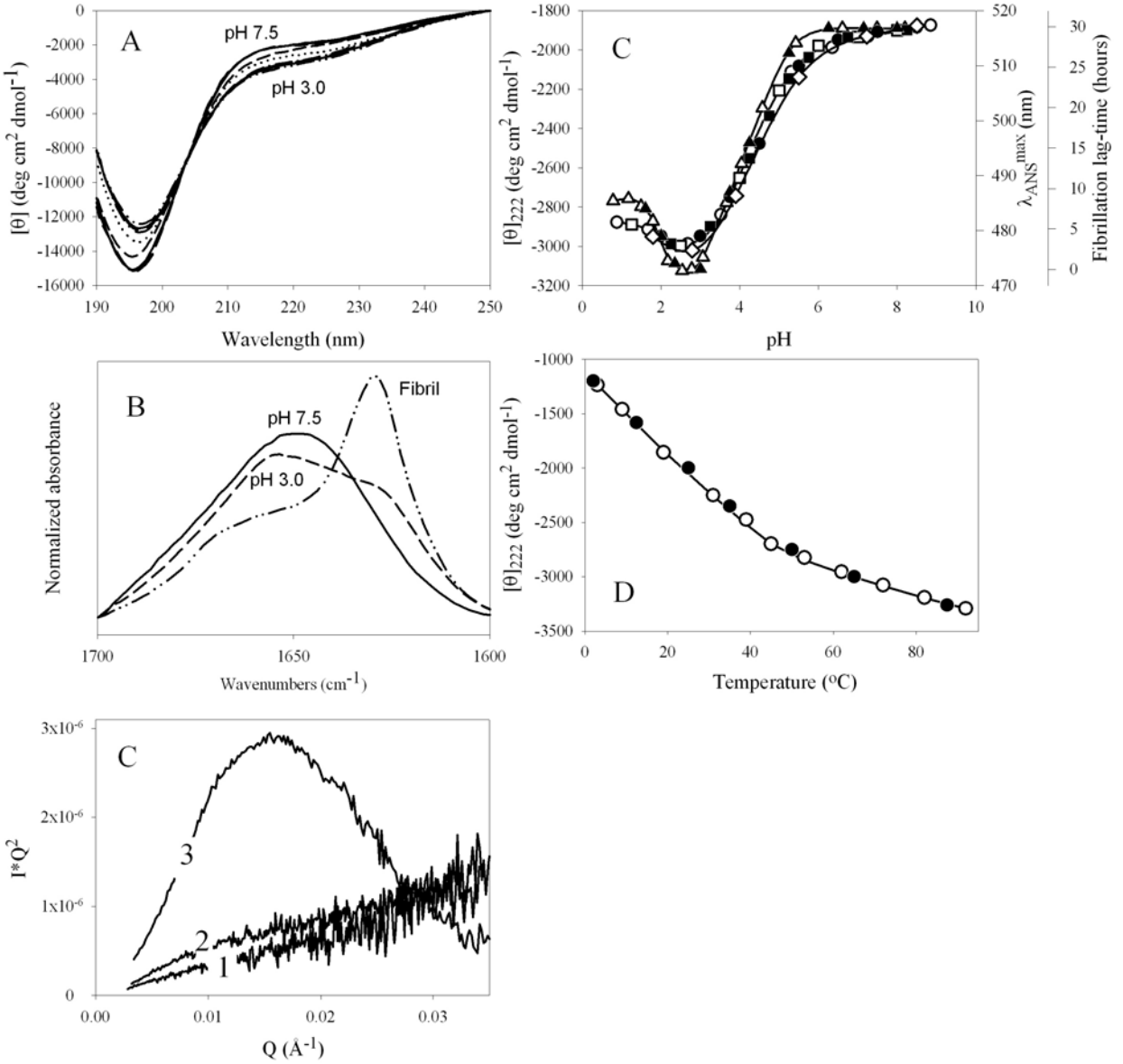

Figure 4. Structural properties and conformational behavior of alpha-synuclein. A. Far-UV CD spectra measured at different pH. B. FTIR spectra measured for natively unfolded, partially folded and fibrillar forms. C. Kratky plots for native unfolded at pH 7.5 (1) and partially folded alpha-synuclein at pH 3.0 (2) in comparison with the typical globular protein, staphylococcal nuclease (3). D. pH-Induced folding and fibrillation of alpha-synuclein. E. Temperature-induced folding of alpha-synuclein. Modified from (170).

NMR (which allows an estimation of the hydrodynamic radii), it has been concluded that alpha-synuclein is slightly collapsed (167). In agreement with this conclusion, a high resolution NMR analysis of the protein revealed that alpha-synuclein is largely unfolded in a solution, but exhibits a region between residues 6 and 37 with a preference for helical conformation (168). Interestingly, Raman optical activity spectra indicate that alpha-synuclein contains some helical poly-(L-proline) II-like conformation (169).

Alpha-synuclein, with its high propensity to aggregate, represents an ideal model for the amyloidogenic IDP and the molecular mechanisms underlying the amyloidogenesis of this protein were intensively studied. It has been shown that alpha-synuclein partially folds at acidic pH and high temperature; i.e., under conditions that enhanced dramatically the propensity of the protein to form amyloid-like fibrils (159). These behaviors are illustrated by Figure 4 which shows that alpha-synuclein adopts a partially folded conformation at acidic pH or at high temperatures (cf. (159, 162, 165, 166)). At neutral pH the protein possesses a far-UV CD spectrum typical of an unfolded polypeptide chain (Figure 4A). The spectrum has an intense minimum in the vicinity of 196 nm, with the

absence of characteristic bands in the 210-230 nm region. However, as the pH is decreased (or temperature increased) changes were observed in the shape of the spectrum. Figure 4A shows that the minimum at 196 nm becomes less intense, whereas the negative intensity of the spectrum around 222 nm increases, reflecting pH-induced formation of secondary structure. Figure 4B compares the FTIR spectra of alpha-synuclein measured at pH 7.5 and pH 3.0. The FTIR spectrum of alpha-synuclein at pH 7.5 is typical of a substantially unfolded polypeptide chain, whereas a decrease in pH leads to significant spectral changes, indicative of increased ordered structure. The most evident change is the appearance of a new band in the vicinity of 1626 cm^{-1}, which corresponds to beta-sheet. This means that at acidic pH natively unfolded alpha-synuclein is transformed into a partially folded conformation with a significant amount of beta-structure (159, 162, 165, 166). Furthermore, Figure 4D shows that a decrease in pH leads to a large blue shift of the 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence maximum (from ~515 to ~475 nm, open triangles in Figure 4D), reflecting the pH-induced transformation of the natively unfolded alpha-synuclein to the partially folded compact conformation. Figure 4D shows that the pH-induced structural transitions observed by ANS fluorescence and CD change simultaneously in a rather co-operative manner. This means that protonation of alpha-synuclein results in transformation of the natively unfolded protein into a conformation with a significant amount of ordered secondary structure and with affinity for ANS. The position of the transition (between pH 5.5 and 3.0) indicates that protonation of one or more carboxylates is responsible for the structural change. Finally, pH-induced transition from unfolded to partially folded conformation was shown to be completely reversible (Figure 4D, open and solid symbols, (159, 162, 165, 166)). Hydrodynamic methods revealed that pH-induced formation of partially folded conformation is accompanied by substantial decrease in hydrodynamic dimensions (R_S = 27.9±0.4 and R_g = 30±1 Å). Furthermore, changes in the profile of the Kratky plot at pH 3 were consistent with the development of the beginnings of a tightly packed core (See Figure 4C) (159, 162, 165, 166).

Figure 4E represents the temperature-dependence of $[\theta]_{222}$ and shows that increase in temperature induced formation of secondary structure in alpha-synuclein (170). The major spectral changes occurred over the range of 3 to 50°C. Further heating lead to a less pronounced effect. Interestingly, Figure 4E shows that the structural changes induced in alpha-synuclein by heating were completely reversible (cf. open and filled symbols). These data indicate that high temperatures induce a reversible transition of alpha-synuclein to a partially folded intermediate. This intermediate has a similar CD spectrum to that induced by low pH (170).

Conformational behavior of alpha-synuclein under the variety of environments revealed that structure of this protein is extremely sensitive to the environment. It adopts a variety of structurally unrelated conformations including the substantially unfolded state, an amyloidogenic partially folded conformation, and different alpha-helical or beta-structural species folded to a different degree, both monomeric and oligomeric (159). Furthermore, it might form several morphologically different types of aggregates, including oligomers (spheres or doughnuts), amorphous aggregates, and amyloid-like fibrils (159). Based on this astonishing conformational behavior the concept of a protein-chameleon was proposed, according to which the structure of alpha-synuclein to a dramatic degree depends on the environment and the choice between different conformations is determined by the peculiarities of protein surroundings (159).

4.2. Alpha-synuclein maintains disordered structure inside the living cell

The cell's interior is crowded with small and large molecules (171, 172). Recently, the effects of macromolecular crowding on α-synuclein were assessed by combining NMR data acquired in living *Escherichia coli* with *in vitro* NMR data (173). The technique of in-cell NMR spectroscopy has been developed and refined to investigate proteins in living *Escherichia coli* (174-176). Using this approach, it has been shown that crowded environment in the *E. coli* periplasm not only keeps alpha-synuclein disordered, but prevents a conformational change that is detected at 35 °C in dilute solution (173). Two disease-associated variants (A30P and A53T) behave in the same way in both dilute solution and in the *E. coli* periplasm. The authors reported the same stabilization *in vitro* upon crowding alpha-synuclein with 300 g/l of bovine serum albumin. Comparison of these *in vivo* and *in vitro* data suggests that crowding alone is sufficient to stabilize the intrinsically disordered, monomeric protein (173). This is a very important observation, which suggests that some IDPs, including alpha-synuclein, can maintain their disordered structure even in the highly crowded environment of a living cell.

5. NEURODEGENERATION-ASSOCIATED INTRINSICALLY DISORDERED PROTEINS AND CORRESPONDING MALADIES

5.1. Amyloid beta-protein and Alzheimer's disease

AD is the most prevalent age-dependent dementia, causing cognitive decline among people of age 65 and older. It currently affects 4.5 million Americans and is projected to afflict 13.2 million by the year 2050 in the US alone (177). AD ranks third in total health care cost after heart disease and cancer. The national direct and indirect annual cost of AD approaches 100 billion dollars per year (178).

AD was described for the first time in 1907 by a German physician Alois Alzheimer (179). AD is the most common aging-related neurological disorder, which constitutes about two thirds of cases of dementia overall (180, 181) and is characterized by slow, progressive memory loss and dementia due to a gradual neurodegeneration particularly in the cortex and hippocampus (182). The clinical hallmarks are progressive impairment in memory, judgment, decision making, orientation to physical surroundings, and language (183). From the initial symptoms, disease progression can last up

to 25 years, although typically the duration ranges from 8 to 10 years.

Sporadic AD is a disease of the elderly; most patients are diagnosed after 65 years of age. About 10 % of AD cases present under age 65 and have been referred to as having early onset AD. Three causative autosomal dominant mutations have been described – the amyloid beta-protein precursor (APP) gene mutation on chromosome 21, the presenilin 1 gene mutation on chromosome 14 and the presenilin 2 gene mutation on chromosome 1. These autosomal dominant forms comprise only about 2% of all AD (184). Having an extra copy of the APP gene, as in case of Down's patients (trisomy 21), also leads to early pathological and clinical changes of AD.

AD is characterized biochemically by the accumulation of two types of proteinaceous inclusions, extracellular amyloid deposits, senile plaques, in the cerebral cortex and vasculature and intracellular NFTs (paired helical filaments, PHFs) (185). Amyloid is a descriptive term for proteinaceous deposits that stain with Congo red and thioflavin S and demonstrate birefringence in polarized light. Amyloid deposits in AD contain the amyloid beta-protein (A-beta), which is a 40-42 residue peptide, produced by endoproteolytic cleavage of the APP. PHFs are assembled from a hyperphosphorylated form of the microtubular protein tau (see next section).

APP, the parent molecule of A-beta, plays a role in synaptic stabilization and plasticity, regulation of neuronal survival, neuritic outgrowth and cell adhesion (186, 187). Nexin-2, a secreted form of APP, inhibits coagulation factor XIa (188, 189). C-terminal fragment of APP originating after the gamma-secretase cleavage mediates nuclear signaling and modulate gene expression (190-193). The A-beta fragment of the APP protein is a byproduct of APP processing. The normally prevailing alpha-secretase-mediated APP processing splits the large APP molecule in the middle of the A-beta sequence and does not produce pathogenic A-beta species. However, alternative cleavage by the beta- and gamma-secretases results in generation of the pathogenic A-beta fragment. Depending on the exact site of action of gamma-secretase, several A-beta peptides with 39-43 amino acids are produced (194). The longer moieties are more amyloidogenic (63). Although beta- and gamma-secretase are active throughout the lifespan, plaques rarely form in young individuals, but after the age of 60 nearly all elderly develop some A-beta deposits (195, 196).

Many lines of evidence support the crucial role of A-beta in AD. Aggregated forms of the A-beta peptide with amyloid-like cross-beta structure are neurotoxic to cortical cell cultures (197-200). Some of the A-beta–derived diffusible ligands (small A-beta aggregates) kill mature neurons at nanomolar concentrations and cause neurological dysfunction in the hippocampus (201). The two major A-beta peptides are the 40-residue A-beta$_{1-40}$ and the 42-residue A-beta$_{1-42}$, which differ in the absence or presence of two extra C-terminal residues (Ile41-Ala42). The N-terminal (residues 1-28) residues comprise a hydrophilic domain with a high proportion of charged residues (46%), whereas the C-terminal domain (residues 29-40 or 29-42) is completely hydrophobic and is presumably associated with the cell membrane. Although the A-beta$_{1-40}$ and A-beta$_{1-42}$ peptides are ubiquitous in biological fluids of humans (at an approximate ratio of 9:1), it is thought that the longer A-beta$_{1-42}$ is more pathogenic, due to its higher quantities in the amyloid plaques of sporadic AD cases, its even higher quantities in patients afflicted with early onset AD (202, 203), and because of the greater *in vitro* tendency of the A-beta$_{1-42}$ to aggregate and precipitate as amyloid (204, 205). Fibrillation of A-beta is associated with the development of the cascade of neuropathogenic events, ending with the appearance of cognitive and behavioral features typical of AD.

A-beta appears to be unfolded at the beginning of the fibrillation under physiological conditions. NMR studies have shown that monomers of A-beta(1-40), or A-beta(1-42) possess no alpha-helical or beta-sheet structure (206); i.e., they exist predominantly as random coil-like highly extended chains. Partial refolding to the pre-molten globule-like conformation has been detected at the earliest stages of A-beta fibrillation (206).

Besides AD, A-beta aggregation was implicated in several other neurodegenerative diseases (see Table 1). For example, the E22Q mutation of A-beta is associated with the rare disorder, hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). HCHWA-D is characterized by severe cerebral amyloid angiopathy (CAA), which is characterized by extensive amyloid deposition in the small leptomeningeal arteries and cortical arterioles, leading to hemorrhagic strokes of mid-life onset, dementia and an early death of those afflicted in their fifth or sixth decade. Therefore, this disorder is an autosomal dominant form of vascular amyloidosis restricted to the leptomeninges and cerebral cortex. CAA severity tends to increase with age (207). In HCHWA-D, parenchymal A-beta deposition is enhanced, with non-fibrillar membrane-bound A-beta$_{42}$ deposits evolving into relatively fibrillar diffuse plaques variously associated with reactive astrocytes, activated microglia, and degenerating neurites (207). Although silver stain-positive, "senile plaque-like" structures found in the HCHWA-D brain were immunopositive for A-beta, yet these lesions lacked the dense amyloid cores present in typical AD plaques (208). No NFTs are present in this disorder. The total A-beta production is not affected by E22Q mutation. However, the proteolytic degradation of A-beta and its transport across the blood–brain barrier as well as the A-beta$_{42}$:A-beta$_{40}$ ratio are altered. A-beta E22Q aggregates faster and fibrils formed by this variant are more stable than amyloid-like fibrils produced by the wild-type A-beta (207).

5.2. Tau protein in Alzheimer's disease and other tauopathies

The tau gene is located on chromosome 17. It encodes for a protein with four 31-32 amino acid tandem repeats close to its C-terminus. Tau protein is a vital structural element of the microtubular transport system in the nervous system. Its aggregation is implicated in AD and

several other diseases collectively known as tauopathies (see Table 1). Tau protein represents a family of isoforms migrating as close bands of 55-62 kDa in SDS gel electrophoresis. Heterogeneity is due in part to alternative mRNA splicing. The tau primary transcript contains 16 exons. Exons 1, 4, 5, 7, 9, 11, 12 and 13 are constitutive exons, exon 14 is part of the 3′ untranslated region of tau mRNA, whereas exons 2, 3 and 10 are alternatively spliced (209). The alternative splicing of these three exons produces six combinations (2−3−10−; 2+3−10−; 2+3+10−; 2−3−10+; 2+3−10+; 2+3+10+), and in the human brain, the tau primary transcript gives rise to six mRNAs (210, 211). Thus, in the human brain, the tau proteins constitute a family of six isoforms with the range from 352-441 amino acids. They differ in either no, one or two inserts of 29 amino acids at the N-terminal part (exon 2 and 3), and three or four repeat-regions at the C-terminal part exon 10 missing. The longest isoform in the CNS (441 amino acids total) has four repeats (R1, R2, R3 and R4) and two inserts, while the shortest isoform (352 amino acids total) has three repeats (R1, R3 and R4) and no insert (212, 213). In normal cortex the three and four-repeat forms are equally expressed. In tauopathies the ratio of isoforms is changed. AD is the only dementia with both three- and four-repeat tau (214). Furthermore, it has been shown that pathological tau proteins in different tauopathies are characterized by different electrophoretic patterns, representing the bar code of tauopathies based on variation in distribution of the pathological tau bands at 60, 64, 69 and 74 kDa (215).

In vitro, tau binds to microtubules, promotes microtubule assembly, and affects the dynamic instability of individual microtubules (216-220). *In situ*, tau is highly enriched in the axons (221). In living cells and brain tissue, tau protein has been estimated as comprising 0.025-0.25% of total protein (222, 223). On the basis of its *in vitro* activity and its distribution, it is believed that tau regulates the organization of neuronal microtubules. Interest in tau dramatically increased with the discovery of its aggregation in neuronal cells in the progress of AD and various other neurodegenerative disorders, especially frontotemporal dementia (224, 225). In these cases specific tau-containing NFTs or PHFs are formed (225). Hyperphosphorylation was shown to be a common characteristic of pathological tau (226). Hyperphosphorylated tau isolated from patients with AD was shown to be unable to bind to microtubules and promote microtubule assembly. However, both of these activities were restored after enzymatic dephosphorylation of tau protein (227-230). Although tau inclusions can be stained with hematoxylin-eosin and amyloid stains, they are much easier visualized after silver impregnation. The most sensitive and specific method is tau immunohistochemistry. There are three types of tau deposits in AD – NFTs, neuropil threads, and dystrophic neurites.

NFTs are composed of 22 nm PHF and each PHF is composed of 8-14 tau monomers (231). They commonly affect the pyramidal cortical neurons and assume a flame-like shape. Extracellular NFTs are rare and are referred to as ghost tangles. They are presumed to be the remnants of dead neurons and are most commonly seen in the hippocampus. When surrounded by dystrophic neuritis, they are called tangle associated neuritic clusters (232). Although NFTs correlate better with dementia severity than amyloid plaques (195), they can be absent in the neocortex in 10% of patients with AD and in as many as the 50% of mild AD cases (233).

Neuropil threads are most commonly seen in AD and only rarely identified in other tauopathies such as corticobasal degeneration (234). They are short tortuous neuronal dendrites filled with abnormal tau (232). Dystrophic neurites are tau-containing dendritic structures that are seen in the periphery of the senile plaques.

Post-translational phosphorylation of tau is an additional source of microheterogeneity (235). During brain development, tau is phosphorylated at many residues with GSK-3-beta, cdk 5, and MAPK (236). *In vitro*, tau can be phosphorylated on multiple sites by several kinases, too (for a review, see (237)). Most of the *in vitro* phosphorylation sites are located within the microtubule interacting region (repeat domain) and sequences flanking the repeat domain. Many of these sites are also phosphorylated in PHF-tau (238, 239). In fact, 10 major phosphorylation sites have been identified in tau isolated from PHFs from patients with AD (238). Hyperphosphorylation was shown to be accompanied by the transformation from the unfolded state of tau into a partially folded conformation (240, 241), accelerating the self-assembly of this protein into paired helical filaments *in vitro* (228). To analyze the potential role of tau hyperphosphorylation in tauopathies, mutated tau proteins have been produced, in which all 10 serine/threonine residues known to be highly phosphorylated in PHF-tau were substituted for negatively charged residues, thus producing a model for a defined and permanent hyperphosphorylation-like state of tau protein (242). It has been demonstrated that, like hyperphosphorylation, glutamate substitutions induce compact structure elements and SDS-resistant conformational domains in tau protein, as well as lead to the dramatic acceleration of its fibrillation (242).

Prior the aggregation, tau protein was shown to be in a mostly random coil-like state. This conclusion followed from the conformational analysis of this protein by CD, Fourier transform infrared spectroscopy, small angle X-ray scattering and biochemical assays (243). Analysis of the primary structure reveals a very low content of hydrophobic amino acids and a high content of charged residues, which was sufficient to explain the lack of folding (243). Analysis of the hydrodynamic radii confirms a mostly disordered structure of various tau isoforms and tau domains. However, the protein was further unfolded in the presence of high concentrations of strong denaturant GdmCl, indicating the presence of some residual structure. This conclusion was supported by a FRET-based approach where the distances between different domains of tau were determined. The combined data show that tau is mostly disordered and flexible but tends to assume a hairpin-like overall fold which may be important in the transition to a pathological aggregate (243).

Intriguingly, purified recombinant tau isoforms do not detectably aggregate over days of incubation under physiological conditions. However, aggregation and fibrillization can be dramatically accelerated by the addition of anionic surfactants (244). Based on the detailed analysis of tau fibrillation in the presence of anionic inducers using a set of spectroscopic techniques (CD and reactivity with thioflavin S and ANS fluorescent probes) it has been established that the inducer stabilized a monomeric partially folded species with the structural characteristics of a pre-molten globule state (245). The stabilization of this intermediate was sufficient to trigger the fibrilliation of full-length tau protein (245).

5.3. Prion protein and prion diseases

Prion diseases are a group of incurable, fatal neurodegenerative maladies that afflict mammals. These diseases, collectively referred to as the transmissible spongiform encephalopathies (TSEs), are caused by the pathological deposition of the prion protein (PrP) in its aggregated form. TSEs include Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) disease, fatal familial insomnia (FFI) and kuru in humans, scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in mule deer and elk (246). The most important aspect is the transmission of PrP aggregates from one individual or species to another, causing prion diseases. Prion diseases are unique among all illnesses in that they can manifest as sporadic, genetic or infectious maladies. Similar to many other neurodegenerative diseases, the sporadic form of prion disease accounts for ~80-90% of cases whereas the genetic forms account for 10 to 20% (247, 248). Infection by exogenous prions seems to be responsible for <1% of all human cases of prion disease (249).

The characteristic pathological features of TSEs are spongiform degeneration of the brain and accumulation of the abnormal, protease-resistant PrP isoform in the central nervous system, which sometimes forms amyloid-like plaques. The prion concept was introduced in 1982 in order to explain a vast body of scientific data, much of which argued the pathogen causing scrapie is devoid of nucleic acid but contains a protein that is essential for infectivity (250). Prions are unprecedented infectious pathogens that give rise to invariably fatal neurodegenerative diseases via an entirely novel mechanism of disease.

Native prion protein (PrP^C) is attached to the extracellular plasma membrane surface by a glycosylphosphatidylinositol lipid anchor and undergoes endocytosis. The N-terminal region of about 100 amino acids in PrP^C (from amino acid 23 to 126) is largely unstructured in the isolated molecule in solution (251). The C-terminal domain is folded into a largely alpha-helical conformation (three alpha-helices and a short antiparallel beta-sheet) and stabilized by a single disulphide bond linking helices 2 and 3 (252). The central event in the pathogenesis of prion diseases is believed to be a major conformational change of the C-terminal region of the PrP from an alpha-helical (PrP^C) to a beta-sheet-rich isoform (PrP^{Sc}), and PrP^{Sc} propagates itself by causing the conversion of PrP^C to PrP^{Sc}. Although unstructured in the isolated molecule, the N-terminal region contains tight binding sites for Cu^{2+} ions and acquires structure following copper binding (253, 254).

Two pathological GSS-like mutations, Y145Stop and Q160Stop, result in C-terminal truncated isoforms. The truncation occurs just after the central region from amino acid 90 to 145, which was shown to be converted into beta-sheet as a result of the PrP^C to PrP^{Sc} conversion (255, 256). Structural properties and aggregation propensities of these variants *in vitro* were analyzed by a variety of biophysical techniques (257). It has been shown that although both proteins are substantially disordered, a continuous stretch of positive secondary chemical NMR shifts was found for residues 144-154 in Q160Stop protein, indicative of helical structure. This clearly demonstrated that although the vast majority of a polypeptide chain is substantially disordered, a significantly populated helix 1 is present in human Q160Stop protein (257). Q160Stop protein was shown to fibrillate faster than shorter Y145Stop variant. Intriguingly, helix 1 was not converted to the beta-sheet during the protein aggregation. Based on the results of this analysis it has been concluded that the highly charged helix 1 is involved in the aggregation of Q160Stop protein likely via the formation of intermolecular salt bridges (257).

Investigations of the steps required for prion propagation and neurodegeneration in transgenic mice expressing chimeric mouse–hamster–mouse or mouse–human–mouse PrP transgenics indicated that the last 50 residues in the disordered N-terminal region play a particularly important role in the interaction of PrP^C with PrP^{Sc} leading to the conversion of the former to the latter (258, 259). Those residues are largely unordered or weakly helical in the full-length PrP^C (260, 261), but are predicted to be beta-structure in PrP^{Sc} (250). These observations emphasize a crucial role of the disordered N-terminal region in the modulation of PrP aggregation. Several kinetics studies have revealed the existence of partially folded intermediates for the PrP (250, 262, 263), and it is reasonable to assume that fibrillation requires partial unfolding of the C-terminal domain prior to self-association.

5.4. Alpha-synuclein and synucleinopathies

Synucleinopathies (see Table 2) is a group of neurodegenerative disorders characterized by fibrillar aggregates of alpha-synuclein protein in the cytoplasm of selective populations of neurons and glia (264-267). Clinically, synucleinopathies are characterized by a chronic and progressive decline in motor, cognitive, behavioral, and autonomic functions, depending on the distribution of the lesions. Because of clinical overlap, differential diagnosis is sometimes very difficult (268). Depending on the type of pathology, alpha-synuclein inclusions are present in neurons (both dopaminergic and non-dopaminergic), where they can be deposited in perikarya or in axonal processes of neurons, and in glia. At least five morphologically different alpha-synuclein containing inclusions have been determined: LBs, LNs (dystrophic neurites), glial

Table 2. Human neurodegenerative disorders with alpha-synuclein deposits

Diseases with neuronal inclusions
❑ Normal aging
❑ Parkinson's disease
• Idiopathic
• Neurotoxicant-induced (incidental)
• Familial
o With alpha-synuclein point mutations
o With alpha-synuclein gene triplication
o With mutations in other proteins
• Pure autonomic failure
• Lewy body dysphagia
❑ Parkinsonism plus syndromes
• Sporadic
o Progressive supranuclear palsy
o Olivoponto cerebrellar atrophy (Shy-Dragger syndrome)
o Cortical-basal ganglionic degeneration
o Sporadic pallidal degeneration
o Bilateral striatopallido dentate calcinosis
o Parkinsonism with neuroacanthocytosis
• Familial
o Familial diffuse Lewy body disease
o Familial dementia with swollen achromatic neurons and cortico-basal inclusion bodies
o Frontotemporal dementia with parkinsonism linked to chromosome 17
o Associated with psychiatric disturbances
o Associated with respiratory disturbances
o Associated with dystonia
o Associated with myoclonus and seizures
o Familial progressive supranuclear palsy
❑ Alzheimer's disease
• Sporadic
• Familial with APP mutation
• Familial with PS-1 mutation
• Familial with other mutations
• Familial British dementia
❑ Lewy body diseases
o Dementia with Lewy bodies
o Pure form - transitional/limbic
o Pure form - neocortical
• Diffuse Lewy body disease
o Common form
o Pure form
• Lewy body variant of Alzheimer's disease
• Incidental Lewy body disease
• Lewy body dementia
• Senile dementia of Lewy body type
• Dementia associated with cortical Lewy bodies
❑ q Amyotrophic lateral sclerosis-parkinsonsim/dementia complex of Guam
❑ Neuroaxonal dystrophies
❑ Down's syndrome
❑ Amyotrophic lateral sclerosis-parkinsonsim/dementia complex of Guam
• Neurodegeneration with brain iron accumulation, type I (Hallervorden-Spatz syndrome or adult neuroaxonal dystrophy)
• Motor neuron disease
❑ Amyotrophic lateral sclerosis
• Familial
• Sporadic
❑ Tauopathies
• Frontotemporal degeneration/dementia
• Pick's disease
• Post-encephalitic parkinsonism
• Dementia pugilistica
• Argyrophilic grain disease
• Corticobasal degeneration
❑ Prion diseases
• Transmissible spongiform encephalopathies
o Sporadic
▪ o Creutzfeldt-Jakob disease
• Familial
o Familial Creutzfeldt-Jakob disease
o Gertsmann-Straussler-Scheinker syndrome
• Infectious
o Iatrogenic Creutzfeldt-Jakob disease

o Variant Creutzfeldt-Jakob disease
o Kuru
• Fatal familial insomnia
❑ Ataxia telangiectatica
❑ Meige's syndrome
Diseases with neuronal and glial inclusions
❑ Multiple system atrophy
• Shy-Drager syndrome
• Striatonigral degeneration (MSA-P)
• Olivopontocerebellar atrophy (MSA-C)

cytoplasmic inclusions (GCIs), neuronal cytoplasmic inclusions and axonal spheroids. Some of the disorders associated with the alpha-synuclein depositions are discussed below to illustrate a wide range of pathological manifestations in synucleinopathies.

5.4.1. Alpha-synuclein and Parkinson's disease

PD is the most common aging-related movement disorder and second most common neurodegenerative disorder after AD. It is estimated that ~1.5 million Americans are affected by PD. Since only a small percentage of patients are diagnosed before the age of 50, PD is generally considered as an aging-related disease, and approximately one of every 100 persons over the age of 55 in the US suffers from this disorder (269). PD is a slowly progressive disease that affects neurons of the *substantia nigra*, a small area of cells in the mid-brain. Gradual degeneration of the dopaminergic neurons causes a reduction in the dopamine content. This, in turn, can produce one or more of the classic signs of PD: resting tremor on one (or both) side(s) of the body; generalized slowness of movement (bradykinesia); stiffness of limbs (rigidity); and gait or balance problems (postural dysfunction). The *substantia nigra* consists of ~400,000 nerve cells, which begin to pigment after birth and are fully pigmented at age 18. The symptoms of PD become apparent after more than ~70% dopaminergic neurons die. This neurodegeneration is characterized by the dramatic depigmentation of the *substantia nigra*, indicating that there is a relationship between pigmentation and function of the *substantia nigra*. The "normal" rate of nigral cell loss is ~2,400 per a year. Thus, if an unaffected person lives to be 100 years old he (she) will probably develop PD. In PD, the neuron loss is accelerated. Although, it is unknown why nerve cells loss accelerates, it appears to be due to a combination of genetic susceptibility and environmental factors. Some surviving nigral dopaminergic neurons contain cytosolic filamentous inclusions known as LBs when found in the neuronal cell body, or LNs when found in axons (270, 271).

Several observations implicate alpha-synuclein in the pathogenesis of PD. Autosomal dominant early-onset PD was shown to be induced in a small number of kindreds as a result of three different missense mutations in the alpha-synuclein gene, corresponding to A30P, E46K, and A53T substitutions in alpha-synuclein (272-274) or as a result of the hyper-expression of the wild type alpha-synuclein protein due to gene triplication (275-277). Antibodies to alpha-synuclein detect this protein in LBs and LNs. A substantial portion of fibrillar material in these specific inclusions was shown to be composed of alpha-synuclein, and insoluble alpha-synuclein filaments were recovered from purified LBs (278, 279). The production of wild type alpha-synuclein in transgenic mice (280) or of WT, A30P, and A53T in transgenic flies (281), leads to motor deficits and neuronal inclusions reminiscent of PD. Under the particular conditions, cells transfected with alpha-synuclein might develop LB-like inclusions. Other important observations correlating alpha-synuclein and PD pathogenesis were reviewed in more detail elsewhere (154, 266, 282-285).

5.4.2. Alpha-synuclein in dementia with Lewy bodies and other Lewy body disorders

5.4.2.1. Dementia with Lewy bodies

Dementia with Lewy bodies (DLB), being the second most frequent neurodegenerative dementing disorder after AD, is a common form of late-onset dementia that exists in a pure form or overlaps with the neuropathological features of AD. This disease is characterized clinically by neuropsychiatric changes often with marked fluctuations in cognition and attention, hallucinations, and parkinsonism (286). Similar to PD, neuropathological hallmarks of DLB are numerous LBs and LNs in the substantia nigra, which are strongly immunoreactive for alpha-synuclein (278). However, unlike PD, DLB is characterized by large numbers of LBs and LNs in cortical brain areas (287). It has been noted that filaments from LBs in DLB are decorated by alpha-synuclein antibodies (279, 288, 289), and that their morphology closely resembles that of filaments extracted from the *substantia nigra* of PD brains (279, 289). DLB and PD with dementia, being different in the temporal course of the disease, share most of the same clinical and neuropathological features and are often considered as belonging to a spectrum of the same disease (290-292). It is well recognized now that the incidence of dementia in PD is higher than expected from aging alone (286), as dementia affects about 40% of PD patients (293), and the incidence of dementia in PD patients is up to six times greater than observed in normal aged matched control subjects (294).

5.4.2.2. Amyotrophic lateral sclerosis-parkinsonsim/dementia complex of Guam

Guam disease is another example of PD and dementia junction. Guam disease is a neurodegenerative disorder with unusually high incidence among the Chamorro people of Guam (295-297). The neurotoxic plant *Cycas circinalis*, a traditional source of food and medicine used by the Chamorro people, plays a role in the development of Guam ALS-parkinsonism-dementia (297). Intriguingly, recent studies revealed that in general three neurodegenerative disorders, ALS, dementia, and PD, co-

occur within families more often than expected by chance, suggesting that there may be a shared genetic susceptibility to these disorders (298).

5.4.2.3. Other Lewy body diseases

Several peripheral and central areas of the nervous system can be affected by the LB deposition. Besides already discussed *substantia nigra*, this includes *hypothalamic nuclei*, *nucleus basalis of Meynert*, *dorsal raphe*, *locus ceruleus*, *dorsal vagus nucleus*, and *intermediolateral nucleus* (299). A 'neuritic' form of LB was also described in the *dorsal vagus nucleus*, *sympathetic ganglia*, and in *intramural autonomic ganglia* of the gastrointestinal tract, as well cases were demonstrated with extensive cortical and basal ganglia involvement (287, 300). This broad spectrum of the nervous system regions potentially affected by LB formation produces great variability in the disease manifestation and LB pathology is also a characteristic feature of several rarer diseases, such as pure autonomic failure, LB dysphagia, incidental LB disease (283, 284). Pure autonomic failure (also known as Bradbury-Eggleston syndrome) (301) and LB dysphagia (302) are the results of the predominant involvement of the peripheral nervous system with minimal central nervous system involvement. In incidental LB disease, ~5%-10% of asymptomatic individuals have insignificant numbers of LBs bodies, usually located in *substantia nigra* (303).

5.4.3. Alpha-synuclein and Alzheimer's disease

Detailed analysis of the alpha-synuclein immunoreactivity in the brains from the patients with sporadic AD revealed the presence of alpha-synuclein-positive inclusions resembling LBs and LNs in ~50% cases studied (304). Alpha-synuclein-positive LB-like intra-cytoplasmic inclusions were found in the amygdale, the temporal cortex, the parahippocampal gyrus, and in the parietal cortex, whereas LN-like inclusions were abundant in the amygdala, the CA2/3 region of hippocampus formation, parahippocampal gyrus, the temporal cortex, substantia nigra, locus ceruleus, the frontal cortex, and in the parietal cortex (304).

5.4.4. Alpha-synuclein and Down's syndrome

Down's syndrome is a genetic disorder characterized by an extra chromosome 21 (trisomy 21, i.e., instead of having the normal 2 copies of chromosome 21, the Down's syndrome patient has 3 copies of this chromosome). The person with Down's syndrome has mild mental retardation, short stature, a flattened facial profile, a risk of multiple malformations (including heart malformations; duodenal atresia, where part of the small intestines is not developed and leukemia), and susceptibility to early-onset AD. Incidence of this disorder among the newborn is estimated at 0.03%, whereas in the general population it is approximately 0.01%. The difference reflects the early mortality. The analysis of Down's syndrome with Alzheimer pathology revealed presence of numerous LBs and LNs in the neurons of the limbic areas, predominantly of the amygdala. Similar lesions were less common in other regions of these brains (305, 306). Importantly, in the vast majority of cases examined no LBs and LNs were detected in the *substantia nigra* and *locus ceruleus*, and there was no significant neuronal loss in the *substantia nigra*.

5.4.5. Alpha-synuclein and multiple system atrophy

Multiple system atrophy (MSA) is an adult-onset progressive neurodegenerative disorder of unknown etiology which is characterized clinically by any combination of parkinsonian, autonomic, cerebellar or pyramidal symptoms and signs, and pathologically by cell loss, gliosis and GCIs in several brain and spinal cord structures. Most patients affected by MSA deteriorate rapidly and survival beyond ten years after disease onset is unusual. It is believed that the motor impairment in MSA results from L-DOPA-unresponsive parkinsonism, cerebellar ataxia and pyramidal signs, with 80% of MSA cases showing predominant parkinsonism (MSA-P) due to underlying striatonigral degeneration, and the remaining 20% developing predominant cerebellar ataxia (MSA-C) associated with olivopontocerebellar atrophy (307). Autonomic dysfunction including urogenital failure and orthostatic hypotension is common in both motor presentations, MSA-P and MSA-C, reflecting degenerative lesions of central autonomic pathways (308). Distinguishing MSA-P from PD is problematic at early stages owing to PD-like features in MSA-P, including a transient L-dopa response in some patients (309). MSA is less common than PD as epidemiological studies suggested a prevalence of 1.9–4.9 people per 100,000 and an incidence of 3 patients per 100,000 people per year (310-312). Histologically, MSA is characterized by the variable neuron loss in the *striatum*, *substantia nigra pars compacta*, *cerebellum*, pons, inferior olives and intermediolateral column of the spinal cord (313). The histological hallmark of MSA is the presence of argyrophilic fibrillary inclusions in the oligodendrocytes, referred to as GCIs, which are also known as Papp-Lantos bodies (314). Fibrillar inclusions are also found in the neuronal somata, axons, and nucleus. Neuronal cytoplasmic inclusions are frequently found in the pontine and inferior olivary nuclei (315). It has been established that alpha-synuclein is a major component of glial and neuronal inclusions in MSA (289, 315). Although both LBs and GCIs contain alpha-synuclein, they are differently localized, with alpha-synuclein inclusions being neuronal in PD and DLB, and oligodendroglial in MSA. This suggests the existence of a unique pathogenic mechanism that ultimately leads to neuron loss via disturbance of axonal function (314). In MSA, besides formation of GCIs alpha-synuclein also aggregates in the cytoplasm, axons and nuclei of neurons, and the nuclei of oligodendroglia. The relationship between GCIs and these additional alpha-synuclein deposition sites is not understood (314).

5.4.6. Alpha-synuclein and neurodegeneration with brain iron accumulation type 1 (NBIA1)

Neurodegeneration with brain iron accumulation type 1 (NBIA1) (formerly know as Hallervorden-Spatz disease (HSD) or adult neuroaxonal dystrophy) represents a rare progressive neurodegenerative disorder that occurs in both sporadic as well as in familial forms. Clinically, NBIA1 is characterized by rigidity, dystonia, dyskinesia, and choreoathetosis (316-319), together with dysarthria,

dysphagia, ataxia, and dementia (319-321). Symptoms usually present in late adolescence or early adult life and this disease is persistently progressive (316, 320, 321). The histopathologic hallmarks of NBIA1 include neuronal loss, neuraxonal spheroids, and iron deposition in the *globus pallidus* and *substantia nigra pars compacta*, as well as by the presence of the LB-like and GCI-like inclusions and dystrophic neuritis (320). NBIA1 is characterized by an association of extrapyramidal movement disorders with neuroaxonal dystrophy (NAD) and iron accumulation in the basal ganglia. It represents a pantothenate kinase-associated neurodegeneration caused by the PNAK2 gene linked to chromosome 20p12.3-13 (322). It has been shown that the LB-like inclusions throughout the cortex and brainstem, axonal swellings, and rare GCI-like inclusions of the midbrain clearly possess alpha-synuclein immunoreactivity (323-325). Importantly, axonal spheroids were also shown to contain alpha-synuclein (325, 326).

5.5. Beta- and gamma-synucleins in Parkinson's disease and dementia with Lewy bodies

Synucleins are members of a family of closely related presynaptic proteins that arise from three distinct genes, described currently only in vertebrates (327). This family includes: alpha-synuclein, which also known as the non-amyloid component precursor protein (NACP) or synelfin (151, 328, 329); beta-synuclein, also referred to as phosphoneuro-protein 14 or PNP14 (329-331) and gamma-synuclein, also known as breast cancer-specific gene 1 or BCSG1 and persyn (332-335).

Human beta-synuclein is a 134-aa neuronal protein showing 78% identity to alpha-synuclein. The alpha- and beta-synucleins share a conserved C-terminus with three identically placed tyrosine residues. However, beta-synuclein is missing 11 residues within the specific non-amyloid component (NAC) region (336, 337). The activity of beta-synuclein may be regulated by phosphorylation (330). This protein, like alpha-synuclein, is expressed predominantly in the brain, however, in contrast to alpha-synuclein, beta-synuclein is distributed more uniformly throughout the brain (338, 339). Besides the central nervous system beta-synuclein was also found in Sertoli cells of the testis (340, 341), whereas alpha-synuclein was found in platelets (342).

The third member of the human synuclein family is the 127-aa gamma-synuclein, which shares 60% similarity with alpha-synuclein at the amino acid sequence level (336, 337). This protein is specifically lacks the tyrosine rich C-terminal signature of alpha- and beta-synucleins (337). Gamma-synuclein is abundant in spinal cord and sensory ganglia (334). Interestingly, this protein is more widely distributed within the neuronal cytoplasm than alpha-and beta-synucleins, being present throughout the cell body and axons (334). It was also found in metastatic breast cancer tissue (333) and epidermis (343).

It has recently been established that in addition to the traditional alpha-synuclein-containing LBs and LNs, the development of PD and DLB is accompanied by appearance of novel alpha-, beta- and gamma-synuclein-positive lesions at the axon terminals of hippocampus (344). These pathological vesicular-like lesions located at the presynaptic axon terminals in the hippocampal dentate, hilar, and CA2/3 regions have been co-stained by antibodies to alpha- and beta-synucleins, whereas antibodies to gamma-synuclein detect previously unrecognized axonal spheroid-like inclusions in the hippocampal dentate molecular layer (344). This broadens the concept of neurodegenerative "synucleinopathies" by implicating beta- and gamma-synucleins, in addition to alpha-synuclein, in the onset/progression of these two diseases.

Structural properties of the members of synuclein family have been compared using several physico-chemical methods (162). All three proteins showed far-UV CD spectra typical of an unfolded polypeptide chain. Interestingly, alpha- and gamma-synucleins possessed almost indistinguishable spectra, whereas the far UV-CD spectrum of beta-synuclein showed a slightly increased degree of disorder. The increased unfoldedness of beta-synuclein was further confirmed by hydrodynamic studies performed by size-exclusion chromatography and SAXS (162). This emphasized the importance of the NAC region to maintain the residual partially collapsed structure in alpha- and gamma-synucleins.

Conformational analysis revealed that alpha-, beta-, and gamma-synucleins are typical natively unfolded proteins that are able to adopt comparable partially folded conformations at acidic pH or at high temperature (162). Although both alpha- and gamma-synucleins were shown to form fibrils, beta-synuclein did not fibrillate, being incubated under the same conditions (162). However, even non-amyloidogenic beta-synuclein can be forced to fibrillate in the presence of some metals (Zn^{2+}, Pb^{2+}, and Cu^{2+}) (345).

Intriguingly, the addition of either beta- or gamma-synuclein in a 1:1 molar ratio to alpha-synuclein solution substantially increased the duration of the lag-time and dramatically reduced the elongation rate of alpha-synuclein fibrillation (162). Fibrillation was completely inhibited at a 4:1 molar excess of beta- or gamma-synuclein over alpha-synuclein (162). Beta-synuclein inhibited alpha-synuclein aggregation in an animal model, too (346). This suggests that beta- and gamma-synucleins may act as regulators of alpha-synuclein fibrillation *in vivo*, potentially acting as chaperones. Therefore, one possible factor in the etiology of PD would be a decrease in the levels of beta- or gamma-synucleins (162). The logical question is how the chaperone roles of beta- and gamma-synucleins are compatible with their ability to fibrillate on their own. The answer to this question is in the specific details of the fibrillation kinetics of three synucleins: conditions promoting beta-synuclein aggregation are very different from conditions favoring alpha-synuclein fibrillation (345), whereas gamma-synuclein fibrillats slower than alpha-synuclein (162). The ability to form amyloid fibrils was recently shown for a typical member of the molecular chaperone family, co-chaperonin GroES (3).

5.6. Polyglutamine repeat diseases associated with aggregation of huntingtin, ataxins, androgen receptor, and atrophin-1

5.6.1. Polyglutamine repeat diseases

Currently there are at least nine known hereditary diseases in which the expansion of a CAG repeat in the gene leads to neurodegeneration (347, 348). Table 1 shows that these polyglutamine repeat diseases includes HD, Kennedy disease (also known as spinal and bulbar muscular atrophy, SBMA), spinocerebellar ataxia type 1 (SCA1), dentatorubral-pallidoluysian atrophy (DRPLA), spinocerebellar ataxia type 2 (SCA2), Machado-Joseph disease (MJD/SCA3), SCA6, SCA7 and SCA17. These diseases are accompanied by the progressive death of neurons, with insoluble, granular, and fibrous deposits being found in the cell nuclei of the affected neurons. The neurotoxicity in these diseases is due to the expansion of the $(CAG)_N$-encoded polyglutamine (polyQ) repeat, which leads to the formation of amyloid fibrils and neuronal death. In HD, the CAG repeat that encodes the polyQ region is part of exon 1 in the 3,140-residue huntingtin protein (349). The polyQ repeat varies between 16 and 37 residues in healthy individuals, and individuals who are afflicted by disease have repeats of >38 residues.

The age of onset and the severity of the progression of SCA1, an autosomal-dominant neurodegenerative disorder characterized by ataxia and progressive motor deterioration, are directly correlated with the length of the polyQ segment in ataxin-1, a nuclear protein of ~800 residues (350-352). When the number of glutamine residues in the polyQ tract exceeds a threshold (39–44 glutamine residues), ataxin-1 aggregates with granular or fibrillar morphologies accumulate intranuclearly and eventually lead to cell death (353, 354).

SBMA is linked to the expansion of a Q-rich segment in the androgen receptor (355); healthy individuals have a 15- to 31-residue polyQ segment, and individuals who are afflicted with the disease have 40-62 Q residues. Intriguingly, in the human androgen receptor there are three polyglutamine repeats ranging in size from five to 22 residues, stretches of seven prolines and five alanines, and a polyglycine repeat of 24 residues. Polymorphisms in the length of the largest polyglutamine and the polyglycine repeats of the androgen receptor have been associated with a number of clinical disorders, including prostate cancer, benign prostatic hyperplasia, male infertility and rheumatoid arthritis (356).

The onset of the DRPLA, another progressive neurodegenerative disorder characterized by a distinctive pathology in the cerebellar and pallidal outflow pathways, is inversely correlated with the polyQ repeat size in the corresponding DRPLA protein (also known as atrophin-1), a product of the gene on chromosome 12p (357). The repeat size varied from 7-23 in normal individuals and was expanded to 49-75 in DRPLA patients.

5.6.2. Huntingtin and structure of polyglutamine stretches

Huntingtin, a protein with an estimated molecular mass of 350 kDa, contains a polyglutamine tract near its N terminus that being expanded beyond 37 glutamines causes HD (349). The N terminus of wild-type huntingtin interacts with proteins involved in nuclear functions, including HYPA/FBP-11, which functions in pre-mRNA processing (splicesome function) (358), nuclear receptor co-repressor protein (NCoR) (359), which plays a role in the repression of gene activity, and p53 (360), a tumor suppressor involved in regulation of the cell cycle. Full-length huntingtin contains candidate binding sites for other proteins with nuclear functions. Huntingtin contains a P*X*DLS motif, a candidate-binding site for the transcriptional corepressor C-terminal binding protein (CtBP) (361), suggesting that huntingtin may play a role in transcriptional repression.

The localization and potential function of normal and mutated huntingtin in the nucleus was suggested to be important for understanding HD pathogenesis. For example, N-terminally mutated huntingtin was shown to be toxic when targeted to the nucleus of cultured striatal neurons (362). Mutated huntingtin has been implicated in abnormal transcriptional repression in HD. In cellular systems, short N-terminal mutated huntingtin fragments disrupt transcriptional regulation, which occurs through a mechanism involving sequestration of transcription factors including p53 (360), TATA-box-binding protein (TBP) (363), and CREB-binding protein (364) into huntingtin-positive aggregates. These results suggest that the N terminus of mutated huntingtin may disrupt neuronal function in HD by interfering with nuclear organization and transcriptional regulation. Full-length huntingtin was shown to co-immunoprecipitate with the transcriptional corepressor C-terminal binding protein, and polyglutamine expansion in huntingtin reduced this interaction (365). Interestingly, although full-length wild-type and mutated huntingtin both repressed transcription when targeted to DNA, N-terminally truncated huntingtin was shown to repress transcription, whereas the corresponding wild-type fragment did not (365).

Proteolytic cleavage of mutated huntingtin is suggested to play a key role in the pathogenesis of HD. Huntingtin was shown to be cleaved by caspases and calpains within a region between 460-600 amino acids from the N-terminus. Furthermore, two smaller N-terminal fragments produced by unknown protease have been described as cp-A and cp-B (366). In fact, based on the analysis of human HD patients, animal models and cell-based models of HD it has been suggested that truncated polyglutamine-containing fragments are more toxic than full-length huntingtin (367).

The mechanistic hypothesis linking CAG repeat expansion to toxicity involves the tendency of longer polyQ sequences, regardless of protein context, to form insoluble aggregates (156, 368-375). To help evaluate various possible mechanisms, the biophysical properties of a series of simple polyglutamine peptides have been analyzed. The far-UV CD spectra of polyQ peptides with repeat lengths of 5, 15, 28 and 44 residues were shown to be nearly identical and were consistent with a high degree of random coil structure, suggesting that the length-dependence of disease is not related to a conformational change in the monomeric states of expanded polyQ sequences (373). In contrast,

there was a dramatic acceleration in the spontaneous formation of ordered, amyloid-like aggregates for polyQ peptides with repeat lengths of greater than 37 residues. Several studies established the role of partially folded intermediates of polyglutamine-repeat proteins as key species in fibrillation (374, 376, 377).

Huntingtin was shown to interact with more than 200 proteins (378). One of these huntingtin interactors, huntingtin yeast-two hybrid protein K (HYPK) was recently identified as a typical IDP using a set of biophysical and biochemical techniques (378). Among the experimental data supporting this conclusion there were aberrant electrophoretic mobility [the molecular weight of HYPK determined by gel electrophoresis was found to be about 1.3-folds (~22 kDa) higher than that obtained from mass spectrometric analysis (16.9 kDa)]; increased hydrodynamic dimensions [in size exclusion chromatography experiment, HYPK was eluted as a protein with the hydrodynamic radius which was ~1.5-folds (23 Å) higher than that expected for globular proteins of equivalent mass (17.3 Å)]; random coil characteristics of far-UV CD spectra; and highly sensitive to limited proteolysis by trypsin and papain (378). Subsequent analysis of HYPK revealed that this huntingtin interacting protein was able to reduce aggregates and apoptosis induced by N-terminal huntingtin with 40 glutamines in Neuro2a cells and exhibited chaperone-like activity (379).

5.6.3. Dentatorubral-pallidoluysian atrophy protein (atrophin-1)

Investigations of the DRPLA gene (encoding for atrophin-1) indicate that it is widely expressed in brain and other tissues as a 4.5-kb transcript with an open reading frame encoding 1184 amino acids (380-382). The rat atrophin-1 coding sequence is 88% identical to the coding sequence of human atrophin-1 at the level of DNA and 94% identical at the protein level, but encodes a shorter glutamine repeat that is followed by a series of alternating glutamine and proline residues (383, 384). The predicted molecular mass of the atrophin-1 gene product is 124 kDa, yet atrophin-1 appeared to migrate at about 200 kDa (385).

5.6.4. Androgen receptor

CD analysis of a region of the androgen receptor N-terminal domain lacking the largest polyglutamine stretch, but containing the remaining repeats, showed that it lacked stable tertiary structure in aqueous solutions (356). Detailed conformational studies using a combination of experimental and computational techniques revealed that the AF1 transactivation domain is in the molten globule-like conformation (386). In fact, this region of the receptor was predicted to contain long disordered regions, when analyzed by amino acid composition, PONDR®, RONN, and GlobPlot. However, this domain was predicted to have compact globular structure when analyzed by CH-plot (98). This discrepancy between the CH-plot and PONDR®-based predictions for the androgen receptor AF1 suggests that this domain possesses properties consistent with a dynamic conformation and to fall into a "collapsed disorder class" of proteins, typical of the molten globule folding intermediate (98, 129). This conclusion was confirmed by the analysis of a hydrophobic fluorescence probe, ANS, binding and by size-exclusion chromatography (386). The results of this analysis suggest that native androgen receptor AF1 exists in a collapsed disordered conformation, distinct from extended disordered (random coil) and a stable globular fold (386).

5.6.5. Ataxin-2

SCA2 is an autosomal-dominantly inherited, neurodegenerative disorder, caused by the expansion of an unstable CAG/polyQ repeat located at the N-terminus of ataxin-2 protein. The age of onset of SCA2 is in the third to fourth decade. The characteristic phenotypic features of SCA2 are the degeneration of specific vulnerable neuron populations and the presence of intracellular aggregations of the mutated protein in affected neurons. Ataxin-2 has 1312 residues (including 22 glutamines of the polyQ stretch) and a molecular mass of ~140 kDa. Ataxin-2 is a highly basic protein except for one acidic region (amino acid 254-475) containing 46 acidic amino acids (387). This region consists of two predicted globular domains, Lsm (Like Sm, amino acid 254-345) and LsmAD (Lsm-associated domain, amino acid 353-475). The LsmAD domain contains a clathrin-mediated *trans*-Golgi signal (YDS, amino acid 414-416) and an endoplasmic reticulum (ER) exit signal (ERD, amino acid 426-428). This domain is composed mainly of alpha-helices according to the results from secondary structure prediction servers. The rest of ataxin-2 outside of the Lsm and LsmAD domains is only weakly conserved in eukaryotic ataxin-2 homologues and is predicted to be intrinsically disordered (387).

5.6.6. Ataxin-3

Human ataxin-3, the protein related to SCA3/MJD, is a ubiquitously expressed 41 kDa protein whose polyQ tract contains 12-40 glutamines in normal individuals and 55-84 glutamines in the pathogenic form (348). Ataxin-3 is present in the genomes of several species, from nematodes to human, including plants (388). Alignment of the ataxin-3 family shows a conserved N-terminal block that corresponds to the sequence motif named Josephin (residues 1-198 in the human protein) (388). The C-terminus is non-conserved throughout different species and contains long stretches of low complexity regions which include the polyQ tract, preceded by a highly charged region (388).

Human ataxin-3 was analyzed by a range of biophysical and biochemical techniques, including limited proteolysis, CD and NMR spectroscopies (389). The deconvolution of the far-UV CD spectra indicated that ataxin-3 contained 32% α-helix, 17% β-sheet, 20% β-turn, and 31% random coil. Based on these results, it has been concluded that the high percentage of random coil conformation estimated by this analysis suggests the presence of unstructured portions of the molecule alongside one or more folded regions (389). This conclusion was further supported by the 2D ^{15}N NMR spectra (HSQC), which were shown to contain two main resonance types: well dispersed resonances typical of a folded conformation and sharp highly overlapped peaks typical of a random coil conformation. Furthermore, limited proteolysis revealed

that the intact protein was almost completely digested after 1 min of incubation with a series of proteases and a protease-resistant N-terminal domain was generated (389). These data indicated that ataxin-3 is composed of a structured N-terminal domain, followed by a flexible tail.

5.6.7. P/Q-type calcium channel alpha1A subunit (CACNA1A)

The underlying mutation in SCA6, a dominantly inherited neurodegenerative disease characterized by progressive ataxia and dyasrthria caused by cerebellar atrophy, is an expansion of the trinucleotide CAG repeat in exon 47 of the *CACNA1A* gene which encodes the α1A subunit of the P/Q type voltage-dependent calcium channel (390). Unlike many other polyglutamine diseases the expanded SCA6 alleles unusually have small expansions (21-30 repeats compared to generally >40 repeats in other polyglutamine diseases) (390). The product of the *CACNA1A* gene, P/Q-type Calcium Channel alpha1A Subunit (CACNA1A), is a protein with 2505 residues and a calculated molecular mass of 282.4 kDa. It has been found that the CACNA1A is processed in such a way that a C-terminal polyglutamine-containing fragment which is less soluble and more toxic than the truncated polyglutamine stretch itself is produced (391). In one set of transcript variants, the $(CAG)_n$-repeats occur in the 3' UTR, and are not associated with any disease. But in another set of variants, an insertion extends the coding region to include the (CAG)n-repeats which encode a polyglutamine tract. Expansion of the $(CAG)_n$-repeats from the normal 4-16 to 21-28 in the coding region is associated with spinocerebellar ataxia 6 (392). This protein was predicted to have several long IDRs.

5.6.8. Ataxin-7

Spinocerebellar ataxia type 7 (SCA7) is characterized by cone-rod dystrophy retinal degeneration and is caused by a polyglutamine expansion within ataxin-7. It has been recently reported that ataxin-7 is a component of the mammalian STAGA (SPT3-TAF9-ADA-GCN5 acetyltransferase) transcription coactivator complex (393). In this complex, ataxin-7 interacts directly with the GCN5 histone acetyltransferase component of STAGA, and mediates a direct interaction of STAGA with the CRX (cone-rod homeobox) transactivator of photoreceptor genes. Furthermore, polyQ-expanded ataxin-7 was incorporated into STAGA and inhibited the nucleosomal histone acetylation function of STAGA GCN5. Based on these results it has been suggested that the normal function of ataxin-7 may intersect with its pathogenic mechanism (393). Normal SCA7 alleles contain 4–35 CAG repeats, whereas pathological alleles contain from 36 to 306 CAG repeats (394). Ataxin-7 has 892 amino acids and a molecular mass of 95.4 kDa. However, at the SDS-PAGE this protein migrates at about 110 kDa (393). In other words, the apparent molecular mass of ataxin-7 determined by gel electrophoresis was found to be about 1.15-folds higher than that expected from amino acid sequence. This suggests that ataxin-7 possesses significant amount of intrinsic disorder.

5.6.9. TATA-box-binding protein

SCA17 is characterized by the heterogeneous clinical phenotype, including ataxia, dementia, psychiatric symptoms, and, in some cases, epilepsy. Neurodegeneration in SCA17 is frequently widespread (atrophy of the striatum, thalamus, cerebral cortex, inferior olive, and nucleus accumbens have been reported), being most prominent in the cerebellum (395). Ubiquinated intranuclear inclusions were found in postmortem brain tissue from SCA17 patients as a result of immunohistochemical examination (395). SCA17 originates from the polyQ expansion of the TBP, which normally contains the polyQ tract of 25–42 glutamine residues, but is expanded >42 glutamines in SCA17 (395). TBP is required for transcriptional initiation by the three major RNA polymerases (RNAP I, II, and III) in eukaryotic nuclei. Being a component of distinct multi-subunit transcriptional complexes, TBP is involved in the expression of most eukaryotic genes (396). TBP is a 339 amino acids-long protein, which can be divided on two functional domains. The C-terminal domain is highly conserved among eukaryotes and mediates virtually all of the transcriptionally relevant interactions involving TBP (397), whereas the N-terminal domain is evolutionarily divergent and shows sequence conservation only in vertebrates. It has been demonstrated that polyQ expansion caused abnormal interaction of TBP with the general transcription factor TFIIB and induced neurodegeneration in transgenic SCA17 mice (398). Furthermore, polyQ expansion was shown to reduce the *in vitro* binding of TBP to DNA. The mutated TBP fragments lacking an intact C-terminal DNA-binding domain were shown to be present in transgenic SCA17 mouse brains. PolyQ-expanded TBP with a deletion spanning part of the DNA-binding domain did not bind DNA *in vitro* but formed nuclear aggregates and inhibited TATA-dependent transcription activity in cultured cells (399). SDS-PAGE analysis of the murine TBP revealed that this protein is characterized by the apparent molecular mass of ~37 kDa, which exceeds the predicted molecular mass of 34.7 kDa (399). The difference between observed and calculated molecular masses was even higher for a truncated TBP fragment that lacks an intact C-terminal domain (399). Similarly, human TBP, a protein with the calculated molecular mass of 37.7 kDa, was shown to possess an apparent molecular mass of ~49 kDa (400).

5.7. ABri peptide and familial British dementia

The ABri is a 34 residue peptide that is the major component of amyloid deposits in familial British dementia (FBD), which is an autosomal dominant disorder with onset at around the fifth decade of life and full penetrance by age 60 characterized by the presence of amyloid deposits in cerebral blood vessels and brain parenchyma that coexist with NFTs in limbic areas (401). FBD patients develop progressive dementia, spasticity, and cerebellar ataxia. The protein subunit (termed ABri) is an example of an amyloid molecule created *de novo* by the abolishment of the stop codon in its precursor, a 266-amino acids-long type 2 transmembrane protein of unknown function (BRI-266) that is encoded by a single gene, *BRI2*, located on the long arm of chromosome 13 (402, 403). The FBD has a single nucleotide change (TGA→AGA, codon 267) that results in an arginine residue substitution for the stop codon in the wild-type precursor molecule and a longer open reading

frame of 277 amino acids in a disease-related protein (BRI-277 instead of BRI-266). The ABri amyloid peptide is formed by the 34 C-terminal amino acids of the mutated precursor protein BRI-277, presumably generated from furin-like processing (404). Thus, the point mutation at the stop codon of BRI results in the generation of the 34 residue ABri peptide (instead of the shorter 23 residue wild type peptide), which is deposited as amyloid fibrils causing neuronal dysfunction and dementia (405). It has been emphasized that although FBD and AD share almost identical neurofibrillar pathology and neuronal loss that co-localize with amyloid deposits, the primary sequences of the amyloid proteins (ABri and Aβ) differ. Therefore, ABri and Aβ amyloid deposition in the brain can trigger similar neuropathological changes (neuronal loss and dementia) and thus may be a key event in the initiation of neurodegeneration (405).

Using far-UV CD and NMR spectroscopy it has been recently established that ABri is in the random coil-like conformation at slightly acidic pH (405). The solution pH was shown to play an important role in promoting the amyloid-like beta-sheet structure and the characteristic fibril morphology of ABri and this protein forms amyloid fibrils at pH 4.9 with no distinct fibril morphology being observed at neutral and slightly basic pH (pH 7.1–8.3), except for smaller spherical aggregates that gradually disappeared and assembled into larger amorphous aggregates (405). It has been also pointed out that at pH 4.9 the ABri undergoes relatively slow beta-aggregation, where it is possible for fibril formation to occur, similar to the behavior of the amyloid A-beta peptide (405).

5.8. ADan in familial Danish dementia

Familial Danish dementia (FDD) is a neurodegenerative disorder linked to a genetic defect in the *BRI2* gene. Similar to FBD, FDD results form the genetic alterations in this gene and the deposited amyloid protein, ADan, is the C-terminal proteolytic fragment of a genetically altered BRI2 precursor molecule (406). The amyloid peptides ABri and ADan originate as a result of two different genetic defects at, or immediately before, the BRI2 stop codon with a common final outcome in both diseases: regardless of the nucleotide changes, the ordinarily occurring stop codon is either non-existent (in FBD) or out of frame (in FDD) causing the genesis of an extended precursor featuring a C-terminal piece that does not exist in normal conditions (reviewed in (407)). ABri and ADan are released by a furin-like proteolytic processing. Both these peptides are 34-residues-long, which share 100% identity on the first 22 residues, a completely different 12 amino acid C-terminus and have no sequence identity to any other known amyloid protein. Despite the structural differences among the corresponding amyloid subunits FDD and FBD show striking clinical and neuropathological similarities with AD, including the presence of NFTs, parenchymal amyloid and pre-amyloid deposits and CAA co-localizing with inflammatory markers, reactive microglia and activation products of the complement system (reviewed in (407)). Structural analysis revealed that similar to A-beta and ABri, ADan is a typical natively unfolded protein, which is characterized by a random coil structure in a wide pH range and is prone to form fibrils in a pH-dependent manner (408).

5.9. Glial fibrillary acidic protein and Alexander disease

Alexander disease is a specific astrocytic disease caused by a dominant heterozygous mutation in glial fibrillary acidic protein (GFAP) (409, 410). A major pathological hallmark of Alexander disease is a presence of specific inclusion bodies called Rosenthal fibers (RFs) in astrocytes that are formed by the mutated GFAP (411). Besides mutated GFAP, these inclusions contains small heat shock proteins, including alphaB-crystallin and HSP27(412). Clinically, the phenotype of Alexander disease depends on the age of onset. The infantile form severely affects the entire central nervous system, with rapid progression and is characterized by megalencephaly, epilepsy, motor impairment, cognitive decline, and extensive loss of white matter with frontal predominance. However, the adult form progresses slowly and is characterized by predominant rhombencephalic degeneration without epilepsy, cognitive impairment, and little, if any, leukodystrophy. The juvenile form is intermediate in severity (413, 414). It has been shown that GFAP is characterized by an extremely high susceptibility to proteolysis (415). Electrophoretic analysis of GFAP produced an apparent molecular mass of 54 kDa, which exceeds the calculated molecular mass of 49.9 kDa (416). This abberant electrophoretic mobility suggests that GFAP contains regions on intrinsic disorder.

5.10. Mitochondrial DNA polymerase gamma and Alpers disease

Alpers disease, also known as progressive neuronal degeneration of childhood, is characterized by developmental regression, intractable epilepsy, progressive neurological deterioration, liver disease, and death usually before 10 years of age (417-419). Neuropathological changes include patchy neuronal loss and gliosis, particularly in the striate cortex (420), whereas the liver shows steatosis, cellular necrosis, focal inflammation, and fibrosis (421). Alpers disease is attributed to mutations in the catalytic subunit of the mitochondrial DNA (mtDNA) polymerase encoded by the polymerase gamma gene (*POLG1*) (422). POLG is the only known DNA polymerase in the mitochondrion, which is responsible for ~1% of the total cellular DNA polymerase activity. The human POLG holoenzyme comprises a 140 kDa catalytic subunit (POLG-alpha) and a 55 kDa accessory subunit (POLG-beta). POLG-alpha is a member of a DNA polymerase family with separate polymerase and 3'-5' exonuclease domains thus exhibiting both DNA polymerase and 3'–5' exonuclease activities. POLG-beta increases DNA-binding affinity, stimulates the catalytic activities and enhances the processivity of the holoenzyme (423). The region of POLG-alpha (444-820 fragment) that lies between the exonuclease and polymerase is known as spacer. Its size and sequence in POLG-alpha are substantially different from those of other members of the DNA polymerase family. In POLG-alpha, this large interdomain region is likely to participate in DNA-template binding and guidance, as well as in subunit interactions. Importantly, spacer mutations were found frequently in the infantile

Alpers syndrome, affecting most severely the brain and the liver (422, 424). These reports emphasize the exceptional variability of POLG-alpha-associated neurological phenotypes and the specific role for spacer mutations in the most severe neurological manifestations (425). POLG-alpha was shown to possess an apparent molecular mass of 145-147 kDa (based on the aberrant mobility in SDS-gel electrophoresis (426)), whereas its theoretical molecular mass is 139.5 kDa suggesting that POLG-alpha contains a number of disordered regions.

5.11. DNA excision repair protein ERCC-6 and Cockayne syndrome

Cockayne syndrome (CS) (also known as Weber-Cockayne syndrome, or Neill-Dingwall Syndrome) is a rare, autosomal recessive disorder. Affected individuals suffer from postnatal growth failure resulting in cachectic dwarfism, photosensitivity, skeletal abnormalities, mental retardation and progressive neurological degeneration, retinopathy, cataracts and sensorineural hearing loss (427-429). Two complementation groups of CS (CS-A and CS-B) have been identified, the corresponding genes, *CSA* and *CSB*, have been cloned (430, 431) and their products biochemically characterized. The majority of CS cases are caused by defects in the CS complementation group B protein. CSA is a 44 kDa protein and belongs to the 'WD repeat' family of proteins (431), which exhibit structural and regulatory roles but no enzymatic activity. The *CSB* gene product is a 168 kDa protein (430), also known as DNA excision repair protein ERCC-6, belongs to the SWI/SNF family of proteins, which all contain seven sequence motifs conserved between two superfamilies of DNA and RNA helicases and which have roles in transcription regulation, chromosome stability, and DNA repair. The involvement of CSB in transcription, transcription-coupled repair of DNA, and base-excision repair might be simultaneous. However, it is suggested that some interregulation, depending on cellular status, takes place. This regulation is done via posttranslational modifications of CSB and changes in function and localization of its interaction partners. These many roles of CSB explain the multisystem manifestations of the CS phenotype (429).

In vitro studies demonstrate that CSB exists in a quaternary complex composed of RNA pol II, CSB, DNA and the RNA transcript. The CSB protein contains an acidic amino acid stretch (~60% of the residues in a 39-amino-acid stretch are acidic), a glycine-rich region and two putative nuclear localization signal (NLS) sequences (432). The cellular and molecular phenotypes of CS include increased sensitivity to oxidative and UV-induced DNA lesions. The CSB protein plays a crucial role in transcription-coupled repair. The corresponding CS-B cells are defective in the repair of the transcribed strand of active genes, both after exposure to UV and in the presence of oxidative DNA lesions (432). According to SDS-PAGE analysis, the CSB protein has an apparent molecular mass of ~200 kDa (432), whereas its theoretical molecular mass calculated from amino acid sequence is 168.4 kDa. This abberant electrophoretic mobility suggests that CSB contains significant intrinsic disorder.

5.12. Survival of motor neurons protein and spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive disease with a carrier frequency of about 1 in 50. SMA is the most common genetic cause of childhood mortality and leads to muscle weakness and atrophy due to the degeneration of motor neurons from the spinal cord (433). The gene responsible for disease is mapped to the survival of motor neurons (SMN) 1 (*smn1*) gene, which carries mutations in over 98% of all SMA patients (434). SMN variants (SMNDelta7 and SMN-Y272C) found in patients with SMA not only lack antiapoptotic activity but also are potently proapoptotic, causing increased neuronal apoptosis and animal mortality. The SMN protein is a part of a larger protein complex that is present both in the nucleus and the cytoplasm. In the nucleus, SMN protein localizes to spots that are rich in small nuclear ribonucleoprotein particles (snRNPs). In the cytoplasm, the SMN protein plays an important role in the assembly of these snRNPs (435). The SMN protein interacts with core components of the snRNPs, Sm proteins. SMA-causing mutations in a C-terminal region and in the central Tudor domain of the SMN protein have been shown to affect the Sm interaction. Mutations in the C-terminal region may interfere with the Sm interaction indirectly, since this region is also required for SMN protein oligomerization (436). The SMN protein Tudor domain has been shown to directly bind to the arginine-glycine (RG) rich tails of the Sm proteins *in vitro* (437, 438). Furthermore, the type I SMA causing point mutation E134K in the SMN protein Tudor domain abolishes Sm binding *in vitro* and interferes with snRNP assembly *in vivo* (438).

In vivo the RG-rich tails of the SmB, SmD1 and SmD3 proteins are post-translationally modified and contain symmetrically dimethylated arginine residues (439, 440). This modification strongly enhances the affinity of the SMN/Sm interaction and has been implicated in the regulation of uridine-rich snRNP assembly (440, 441). Many other proteins, including coilin, RNA helicase A, fibrillarin and heterogeneous nuclear ribonucleoproteins, interact with the SMN complex and contain RG-rich domains that can potentially be methylated (442, 443), suggesting that the SMN protein Tudor domain could have an additional function in the regulation of these interactions. The crystal structure of the SMN protein Tudor domain comprising residues 82-147 was solved to high (1.8 Å) resolution (444, 445). The crystal structure consists of a five-stranded beta-sheet that forms a beta-barrel. Comparison of the crystal structure and an NMR structure revealed that the backbone conformation of both structures is very similar. However, differences were observed for the cluster of conserved aromatic side-chains in the symmetrically dimethylated arginine residues (sDMA) binding pocket, suggesting that the SMN protein Tudor domain adopts two different conformations in the sDMA binding pocket (444). Using the SDS-PAGE analysis, full-length SMN protein (calculated molecular mass 31.8 Kda) was shown to possess an apparent molecular mass of 39 kDa (446), suggesting that a noticeable fraction of this protein is intrinsically disordered.

5.13. Septins in neuropathology

Septins are ubiquitous, evolutionarily conserved GTP-binding proteins that can form homo- and heteromeric complexes to assemble into filaments that may serve as structural scaffolds for the assembly of other proteins (447, 448). Septins are the members of the family of cell division cycle (Cdc) genes. They are evolutionary conserved across the eukaryotic phylogeny from unicellular yeast to complex metazoa including *Homo sapiens* (449), and constitute a group of GTP-binding and filament-forming proteins that belong to the large superclass of P-loop GTPases (450). In humans, there are at least 12 septin genes encoding septins SEPT1-12. Most of them undergo complex alternative splicing with some degree of tissue specificity (450). Analysis of septin amino acid sequences revealed potential motifs and regions, some of which are conserved among the currently known orthologues. For example, the septin family of genes possesses a conserved GTP-binding domain, and they fall into the large superclass of P-loop GTPases (451). This central GTP-binding domain is highly conserved in all human septins, with 58% identity or similarity. All septins have a P-loop (452, 453) that is defined by the Walker A motif (GxxGxGKST), Walker B motif (DxxG), and the GTP-specificity motif (xKxD). Septins lie in the TRAFAC subclass of P-loop GTPases defined by a conserved threonine required for hydrolysis of the triphospate moiety (451). Contrarily to this highly conserved GTP-binding domain, the N- and C-terminal domains vary greatly both within and between species. In fact, the N-terminal domain of septins contains a polybasic region, which is somewhat conserved through eukaryotic phylogeny. The C-termini of the known human septins are more diverse. The diversity in the N- and C-terminal regions flanking the core of the 12 known human septins is combined with the extensive alternate splicing allowing septin genes to encode multiple isoforms (450).

Originally, septins were identified based on the cell division cycle mutants with defects in cytokinesis (454). Now, it is established that septins are heavily involved in various biological processes inside the cell. They were shown to have diverse biological roles including cytokinesis (455-457), cell polarity determination and maintenance (458-460), cell movement and membrane associations (461), vesicle trafficking (462), exocytosis (463, 464), and apoptosis (465). Septins can interact with both microtubules and actin (455, 466-468), potentially playing a role as adaptors between the two cytoskeletons and as regulators of processes in which both actin and microtubules are involved (469). Septins are proposed to be involved in several microtubule-dependent processes, including karyokinesis, exocytosis, and maintenance of cell shape (469). Various septins have been shown to colocalize or interact with the microtubule cytoskeleton. Therefore they might play an important role in regulation of the microtubule dynamics via specific interaction with microtubule-associated proteins modulating microtubule stability (469).

Recently, the structural properties of the SEPT4 were analyzed by a number of biophysical techniques, including native gel electrophoresis, CD spectroscopy, fluorescence spectroscopy, DLS, and SAXS as well as with bioinformatics tools (470). To this end, the full-length form of human SEPT4 and its individual N-terminal, GTPase, and C-terminal domains were expressed in *E. coli* and purified. Biophysical analysis revealed that the N-terminal domain behaves as a typical IDP, containing little regular secondary structure. The central GTPase domain was catalytically active and represented a mixed alpha/beta structure, probably based on an open beta-sheet. The C-terminal domain was shown to form homodimers and can be divided into two regions, the second of which is alpha-helical and consistent with a coiled-coil structure (470).

Septins are involved in several neurological disorders. This conclusion follows from several observations including the brain-specific expression of some septins, the differential regulation of septins in neural development, and the association of septins with some disease states or pathological hallmarks (450). For example, septins were found in neurofibrillary tangles in Alzheimer's disease (471). In fact, SEPT1, 2, and 4 were shown to be associated with tau-based helical filaments. It has been even proposed, that septins contribute to the formation of tangles and therefore are pathogenetically significant. Although several human septins are expressed exclusively or predominantly in the nervous system, their expression is under the strict spatial (472) and temporal (473) regulation. Furthermore, since the septin expression was linked with exocytosis (463, 464) and since septins were shown to form complexes with syntaxin and other secretion-associated molecules, it has been proposed that septins can be associated with the critical function of secretion via the exocyst complex and can be involved in vesicle trafficking (450). SEPT2 is included into the exocyst complex (462) and modification of its GTP binding activity was shown to be accompanied by the altered neurite sprouting (474). This septin has been also reported to be overexpressed in brain tumors (475).

Based on the results of the proteomic analysis the abnormalities in the septin expression were found in Down's syndrome (476). SEPT5-v2 has been reported to be a parkin-binding protein and parkin can function as an E2-dependent ubiquitin ligase capable of promoting the degradation of SEPT5 (477). SEPT5 was shown to accumulate in the brains of individuals with autosomal recessive juvenile Parkinsonism (478), whereas SEPT4 has been found in neurofibrillary tangles of Alzheimer disease brains and in alpha-synuclein-positive cytoplasmic inclusions in Parkinson disease brains (479).

5.14. Neurotrophin NGF and neurodegeneration

The neurotrophin family of growth factors consists of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophins-3 and -4 (NT-3, NT-4) and regulates neuronal survival and synaptic plasticity (480). Neurotrophins are synthesized as precursors (proneurotrophins) that are proteolytically cleaved to mature, biologically active neurotrophins (481). For example, the neurotrophin NGF is translated as a pre-pro-protein [pre-proNGF (precursor of NGF with signal peptide)] of 27 kDa, containing a signal peptide for protein

secretion (pre-peptide) and the precursor protein (proNGF, NGF precursor, without signal peptide). ProNGF is cleaved by the convertase furin to produce the mature NGF. Mature neurotrophins selectively bind to members of the Trk family of receptor tyrosine kinases, but they also interact with a structurally distinct receptor, pan-neurotrophin receptor p75NTR. Although p75NTR can increase the affinity and specificity of Trk-neurotrophin interactions, p75NTR can also induce apoptosis in oligodendrocytes, neurons, and vascular smooth muscle cells when Trk activation is reduced or absent (482).

NGF is involved in the maintenance and growth of neurons (483). ProNGF was found to be a high-affinity ligand for p75NTR and was shown to induce p75NTR-dependent apoptosis (484). The specific receptor for the proNGF is sortilin (485). ProNGF has also been found to bind to the high-affinity NGF receptor TrkA (tropomyosin receptor kinase A) and to induce the survival-signaling pathway, although it is less efficient than the mature NGF (486). ProNGF was shown to be the predominant form of NGF in mouse, rat, and human brain tissue whereas little or no mature NGF was detected (487).

Interestingly, a high-throughput crystallization screen of proNGF failed to produce crystals, suggesting that the high flexibility of the pro-part of proNGF might influence its crystallization propensity (488). In agreement with this hypothesis, solution small angle X-ray scattering measurements revealed that proNGF was dimeric and appears to have two equally populated structures (see Figure 5), a globular crab-like form and an elongated rod-like form, pointing to an intrinsically disordered pro-region of NGF (488). It has been also emphasized that these two models provide grounds for the interpretation of the available biological data for proNGF.

In AD, neuronal dysfunction and degeneration occur in the basal forebrain cholinergic neurons (BFCN), with reduction in neocortical cholineacetyltransferase (ChAT) activity (489-491). This reduced ChAT activity correlates with the degree of dementia and thus has been regarded as a principal factor associated with the memory loss characteristic of AD (492). NGF, which is synthesized within BFCN target regions, such as the hippocampus and cerebral cortex (493), maintains survival of the BFCN after injury and regulates cholinergic neurotransmitter levels (494-497). Blocking NGF availability to BFCN results in memory deficits (498, 499). This led to the theory that BFCN degeneration in AD is the result of a deficit in NGF (500). Decreased NGF immunoreactivity in the basal forebrain of AD patients and increased NGF protein in the cerebral cortex and hippocampus have been demonstrated using bioassay and ELISA (501-505). These changes and the decreased trkA expression in AD (506-510) were consistent with a defect in the NGF transport in AD brain. Alternatively, the availability of NGF protein to BFCN can be reduced by defects in posttranslational modification of NGF. This, in turn, might result in the decrease in the ChAT and trkA levels. In agreement with this hypothesis, a twofold increase in proNGF in AD parietal cortex compared to controls was found, indicating that this precursor form preferentially accumulates in AD (487).

6. RECENT ADVANCES IN NANOIMAGING: THE DYNAMICS AND MISFOLDING OF INTRINSICALLY DISORDERED PROTEIN

It has been pointed out that the progress in understanding the protein misfolding pathologies (including discussed above neurodegenerative diseases) and successful attempts of rational drug design for inhibition or reversal of protein aggregation depends on the ability to study the details of the misfolding process, to follow the aggregation process, and to see and analyze the structure and mechanical properties of the aggregated particles. Nanoimaging represents a set of unique tools specifically designed for these purposes. It allows the direct visualization of the processes of protein misfolding and aggregation. Therefore, it potentially helps researchers to avoid the situation of “six blind men and an elephant” (511), which typically takes place when various researchers are interpreting the results retrieved by different experimental techniques. Nanoimaging provides a possibility to see the whole “elephant” rather than its “tail”, “trunk”, “legs” and “ears”. Current set of nanoimaging tools includes different visualization approaches (atomic force microscopy (AFM), scanning tunneling microscopy (STM), electron microscopy (EM), environmental scanning electron microscopy (ESEM), optical microscopy (OM), confocal laser scanning microscopy (CLSM), internal reflection fluorescence microscopy (TIRFM), and other variants of fluorescent microscopy utilizing different histopathologic dyes, immunohistochemical approaches, and quantum dots). An advantage of nanoimaging methods is that different aggregate morphologies can be recognized and processed separately (512). Nanoimaging gives a chance to physically see the processes of aggregation and fibril formation in real time. It also offers unique means for evaluating the fibril mechanical properties and measuring the strength of interprotein interactions. The applications of the nanoimaging tools and techniques range from simple visualization of aggregated materials to sophisticated morphological analyses, direct measurements of mechanical properties, diagnostics, kinetic analysis, and even potential clinical use. The current state of the art in this field was a subject of several recent reviews (512-515). However, this branch of science is experiencing an explosive development. Therefore, a brief overview of the most recent developments in the nanoimaging of IDPs is presented below.

The AFM-based single-molecule mechanical unfolding methodology was recently applied to study the conformational equilibrium of human wild-type and mutated alpha-synuclein (516). The utilized single-molecule force spectroscopy (SMFS) approach was based on the analysis of the mechanical stretching of an individual alpha-synuclein molecule by AFM, where the proteinaceous handles were used to connect one end of the target protein to the tip and the other to the substrate (517). To this end, a chimeric polyprotein composed of a single alpha-synuclein module flanked on either side by three

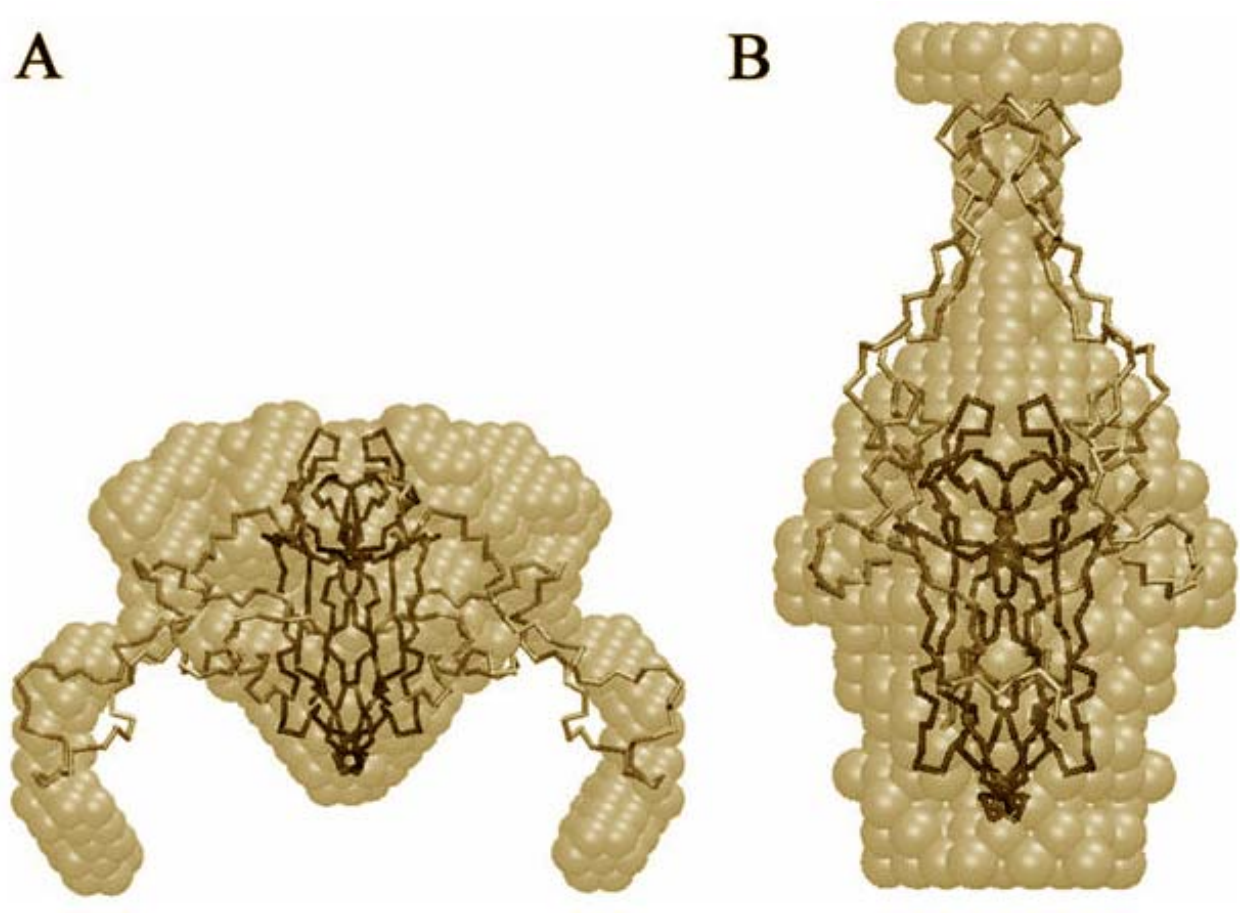

Figure 5. *Ab initio* models for the shape determination of proNGF. The models obtained by DAMMIN are shown by grey spheres. These models are superimposed on the C_a traces obtained with BUNCH for the pro-peptide region (light grey); in black, the C_a traces of the crystallographic structure of mouse NGF (PDB code 1BET) are shown. (A) Crab-like shape; (B) rod-like shape. Reproduced with permission from (488).

tandem I27 domains was utilized. The I27 domains acted as molecular handles holding a single alpha-synuclein molecule. They also introduced well-characterized fingerprint signals into the recorded force curves (see Figure 6). In fact, the experiment was designed in such a way that when the number of unfolding signals coming from the polyprotein was larger than six, this was used as a reflection of the mechanical stretching of the six I27 domains and the alpha-synuclein module in the middle (516). Such an experimental setup made it possible to identify different alpha-synuclein conformations based on the profiles of the selected force curves, which were grouped into three main classes. Two of the classes were unambiguously assigned to well-defined classes of conformers: one with the typical mechanical behavior of random-coil chains and the other of β-like structures. The third class was proposed to correspond to fairly compact architectures, likely to be stabilized by mechanically weak interactions (MWI). Therefore, the application of this technique allowed the authors to characterize the conformational heterogeneity of monomeric alpha-synuclein at the single-molecule level. Importantly, the populations of the beta-like structures were significantly increased under the conditions favoring the alpha-synuclein aggregation. These aggregation-promoting conditions included the pathogenic A30P mutation, as well as the use of Cu^{2+} or high ionic strength (see Figure 7). Based on the obtained data it was concluded that the proposed methodology is able to explore the full conformational space of a protein molecule at the single-molecule level, detecting even poorly populated conformers and measuring their distribution at the variety of biologically important conditions. This study clearly produced the first time evidence of a conformational equilibrium that controls the population of a specific class of monomeric alpha-synuclein species positively correlated with conditions known to promote the formation of aggregates. This new tool is therefore suitable for the direct testing of the influence of mutations and pharmacological strategies on the conformational equilibrium of monomeric alpha-synuclein (516). More recently this approach was used to characterize the conformational heterogeneity of pathological alpha-synuclein variants A30P, A53T, and E46K (518). It has been shown that all the variants have a high propensity to adopt a monomeric aggregation-prone conformation compatible with the acquiring of beta-structure (518).

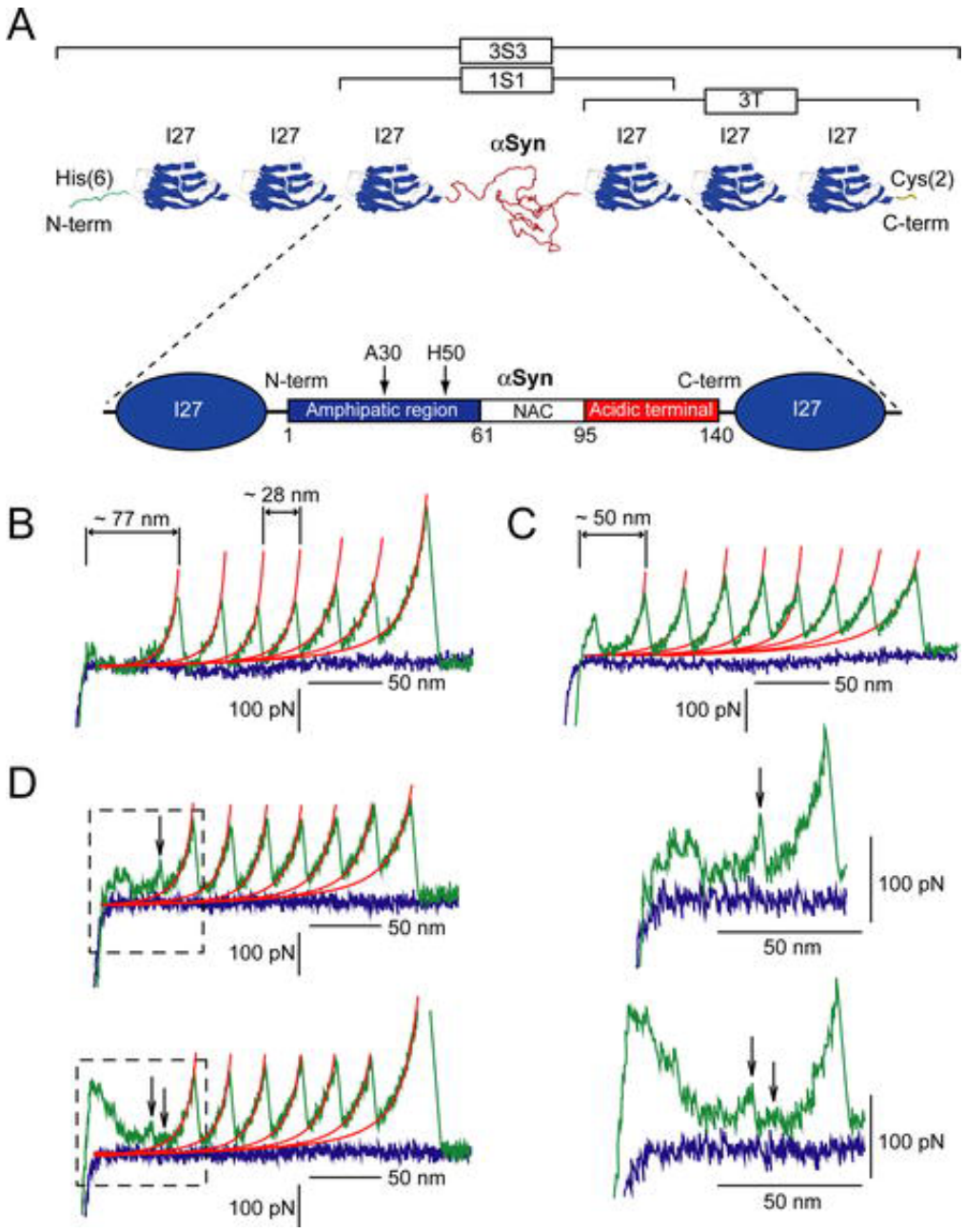

Figure 6. The mechanical signatures of alpha-synuclein conformational classes as recorded by SMFS.A. Schematic representation of the polyprotein constructs: 3S3 contains the alpha-synuclein sequence (red) flanked on either side by three titin I27 modules (blue), the N-terminal His-tag needed for purification purposes (green), and the C-terminal Cys-Cys tail needed for covalent attachment to the gold surface (yellow). In 1S1, the alpha-synuclein moiety is flanked only by one I27 on both sides; the 3T is made up by three I27s. In the αSyn moiety (enlarged), three regions are shown: (i) the amphipathic region, prone to fold in α-helical structures when in contact with phospholipid membranes; (ii) the fibrillogenic NAC region, characteristic of the fibril core of alpha-synuclein amyloid; and (iii) the acidic C-terminal tail, strongly charged and not prone to fold. The positions of alanine 30, site of the A30P mutation and histidine 50, which is crucial for the binding of Cu^{2+}, are marked.B. Example of curve characterized by a featureless region assigned to the stretching of αSyn moiety having, in this case, the mechanical properties of a random coil. This region is followed (from left to right) by six unfolding peaks of about 200 pN, with about 28-nm gaps between each, assigned to the unfolding of I27 domains. C. Example of the curves featuring the β-like signature of alpha-synuclein, showing seven practically indistinguishable unfolding events of similar magnitude and spacing. D. Curves featuring the signature of mechanically weak interactions, showing single or multiple small peaks (arrows) superimposed on the purely entropic worm-like chain behavior of the trace preceding the six saw-tooth–like peaks. The right panels show a zoom of the region enclosed by the dashed squares. Reproduced with permission from (516).

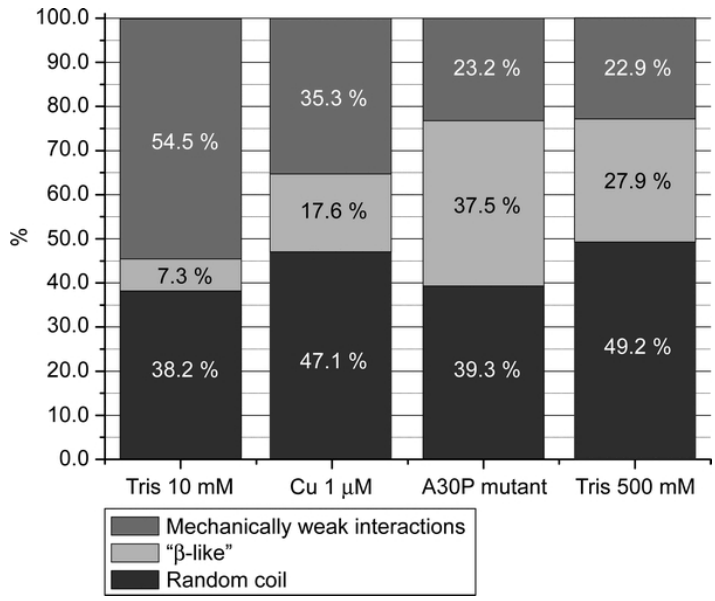

Figure 7. Population shift of alpha-synuclein conformers at different conditions. Population of alpha-synuclein conformers in the four different conditions tested. Percentages observed for each curve type (see Figure 6) at 10 mM Tris/HCl (n = 55), 10 mM Tris/HCl with 1 μM Cu^{2+} (n = 34), the A30P variant in 10 mM Tris/HCl (n = 56), and 500 mM Tris/HCl (n = 61). Reproduced with permission from (516).

Recently, the high-speed AFM was utilized to observe the mobility of the heterodimeric FACT (facilitates chromatin transcription) protein (519). FACT is a highly conserved eukaryotic protein involved in transcription. It displaces histone H2A/H2B dimers from nucleosomes thus facilitating RNA polymerase II transcription (520, 521) and chromatin remodeling (522). This protein is a heterodimer consisting of structure-specific recognition protein-1 (SSRP1) and SPT16; the latter has a higher molecular mass than the former. The charge-enriched termini of SSRP1 and SPT16 were predicted to contain long intrinsically disordered regions. The predicted ID region of SPT16 is essential for mRNA transcriptional elongation by FACT (520). The presence of these disordered regions was recently confirmed via the high-speed AFM. In fact, successive AFM images of FACT taken at imaging rates of 5-17 frames per second clearly showed two tail-like segments protruding from the main body of FACT. These tails were highly flexible and their mechanical properties estimated from the AFM images suggested that they have more relaxed structures than random coils (519). This is the first study utilizing high-speed AFM for direct observation of ID regions. The obtained results clearly show that this state-of-the-art microscopy method can be used to characterize unstructured protein segments that are difficult to visualize with other experimental techniques.

7. CONCLUSIONS

Intrinsic disorder is highly abundant among proteins associated with human neurodegenerative diseases. This provides a strong factual support to a D^2 (disorder in disorders) concept (523). The validity of this concept in neurodegeneration is illustrated at several levels, starting from the results of the bioinformatics analysis of an extended set of proteins associated with various neurodegenerative conditions and ending with the extensive data for a number of well-characterized neurodegeneration-related proteins. High degree of association between intrinsic disorder and neurodegenerative diseases is due to the unique structural and functional peculiarities of IDPs and IDRs. IDPs/IDRs are among major cellular regulators, recognizers and signal transducers. Their functionality and misbehavior are modulated via a number of posttranslational modifications (i.e., tau protein). Many IDPs/IDRs can fold (completely or partially) upon interaction with corresponding binding partners. They possess multiple binding specificity and they are able to participate in one-to-many and many-to-one interactions.

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Abbreviations: AD, Alzheimer's disease, FM, atomic force microscopy, LS, amyotrophic lateral sclerosis, NS, 8-anilino-1-naphthalenesulfonic acid, APP, amyloid b-protein precursor, BDNF, brain derived neurotrophic factor, BSE, bovine spongiform encephalopathy, CAA, cerebral amyloid angiopathy, CACNA1A, P/Q-type calcium channel a1A subunit, CD, circular dichroism, Cdc, cell division cycle, Cdk 5, cyclin dependent kinase 5, ChAT, cholineacetyltransferase, CH-plot, charge-hydropathy plot, CJD, Creutzfeldt-Jakob disease, CLSM, confocal laser scanning microscopy, CS, Cockayne syndrome, CWD, chronic wasting disease, DLB, dementia with Lewy bodies, DLS, dynamic light scattering, DRPLA, dentatorubral-pallidoluysian atrophy, EM, electron microscopy, ER, endoplasmic reticulum, ERCC-6, excision repair cross-complementing rodent repair deficiency, complementation group 6, ERD, ER exit signal, ESEM, environmental scanning electron microscopy, FACT, facilitates chromatin transcription, FBD, familial British dementia, FDD, familial Danish dementia, FFI, fatal familial insomnia, FRET, fluorescence resonance energy transfer, FTIR, Fourier transform infra red spectroscopy, GCIs, glial cytoplasmic inclusions, GdmCl, guanidinium chloride, GFAP, glial fibrillary acidic protein, GSK-3b, glycogen synthase kinase 3 beta, GSS, Gerstmann-Sträussler-Scheinker, HCHWA-D, hereditary cerebral hemorrhage with amyloidosis-Dutch type, HD, Huntington's disease, HSD, Hallervorden-Spatz disease, HYPK, huntingtin yeast-two hybrid protein K, IDP, intrinsically disordered protein, IDR, intrinsically disordered region, LB, Lewy body, LN, Lewy neurite, Lsm, like Sm, LsmAD, Lsm-associated domain, MAPK, microtubule associated protein kinase, MJD, Machado-Joseph disease, MSA, multiple system atrophy, MWI, mechanically weak interactions, NAC, non-amyloid component, NACP, non-amyloid component precursor protein, NBIA1, neurodegeneration with brain iron accumulation type 1, NGF, nerve growth factor, NLS, nuclear localization signal, NFT, neurofibrillary tangle, ODR, optical rotatory dispersion, OM, optical microscopy, PD, Parkinson's disease, PHF, paired helical filament; POLG, polymerase g, polyQ, polyglutamine repeat, PONDR, predictor of naturally disordered regions, proNGF, NGF precursor, without signal peptide, PrP, prion protein, p75NTR, pan-neurotrophin receptor, RF, Rosenthal fiber, SAXS, small angle X-ray scattering, SANS, small angle neutron scattering, SBMA, spinal and bulbar muscular atrophy, SCA1, spinocerebellar ataxia type 1, SCA2, spinocerebellar ataxia type 2, sDMA, symmetrically dimethylated arginine residues, SEPT, gene encoding septin, SMA, spinal muscular atrophy, SMN, survival of motor neurons, SMFS, single-molecule force spectroscopy, STAGA, SPT3-TAF9-ADA-GCN5 acetyltransferase, STM, scanning tunneling microscopy, SWI/SNF, SWItch/Sucrose NonFermentable, TBP, TATA-box-binding protein, TIRFM, total internal reflection fluorescence microscopy, TrkA, tropomyosin-related kinase A, TSE, transmissible spongiform encephalopathy

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