

## NEW EMBO MEMBER'S REVIEW

# From Alzheimer to Huntington: why is a structural understanding so difficult?

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**An increasing family of neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, prion encephalopathies and cystic fibrosis is associated with aggregation of misfolded polypeptide chains which are toxic to the cell. Knowledge of the three-dimensional structure of the proteins implicated is essential for understanding why and how endogenous proteins may adopt a non-native fold. Yet, structural work has been hampered by the difficulty of handling proteins insoluble or prone to aggregation, and at the same time that is why it is interesting to study these molecules. In this review, we compare the structural knowledge accumulated for two paradigmatic misfolding disorders, Alzheimer's disease (AD) and the family of poly-glutamine diseases (poly-Q) and discuss some of the hypotheses suggested for explaining aggregate formation. While a common mechanism between these pathologies remains to be proven, a direct comparison may help in designing new strategies for approaching their study.**

**Keywords:** aggregation/amyloid/misfolding/poly-glutamine/structure

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### Misfolded proteins: the cause of a new family of diseases

We are accustomed to regarding genetic diseases as linked to the loss of function of one or more gene products. However, the 'one gene, one protein, one function' hypothesis has by now been contradicted more often than confirmed. One of the most interesting examples in which this concept seems to fail is represented by an increasing family of neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, prion encephalopathies and cystic fibrosis (Taylor *et al.*, 2002). The hallmark of these otherwise unrelated diseases is the formation of aggregates containing misfolded proteins, i.e. proteins in a non-native folding state, with a concomitant gain of function which eventually leads to neuronal death. Gain of function rather than loss of function is supported by the observation that the aggregates are toxic *per se* (Spillantini *et al.*, 1998; Hardy and Selkoe, 2002). The aggregates have various supramolecular organizations and, in most cases, form structurally well-defined

insoluble fibrillar deposits called amyloids (Glennner and Wong, 1984). In one case (prion diseases) aggregates are even believed to be responsible for disease transmission (Prusiner, 1995). What are the structural mechanisms that lead to aggregate formation of endogenous proteins? What makes the aggregates toxic? A great deal of literature has appeared in the last decade to address these questions. Since all members of the family of diseases are linked to a mechanism of aberrant protein folding, knowledge of the three-dimensional structure of the proteins implicated, both in their 'healthy' and in their pathological forms, is the prerequisite for understanding the mechanism of aggregate formation and, eventually, preventing it. Yet, only relatively limited structural information is currently available. Some of the structural hypotheses formulated to explain aggregate formation and toxicity have migrated from one field to the other, suggesting, at least apparently, the possibility of a somewhat similar mechanism. It is however important to question closely whether such a common mechanism exists or if the analogies are only apparent. Here we review in parallel some of the structural hypotheses currently suggested for two disease families, Alzheimer's disease (AD) (and related tauopathies) and the family of poly-glutamine (poly-Q) diseases, selected as paradigms of misfolding disorders (Table I). A direct comparison which analyses commonalities and differences among them may be helpful for increasing our understanding of misfolding diseases.

### A supramolecular view: fibres and aggregates

The first observation of brain aggregates was carried out by Alois Alzheimer, a German physician who, in 1906, presented a case history of a patient affected by a then unknown brain disorder (Kraepelin, 1910). The autopsy of the patient's brain showed the presence of two distinct types of lesions: extracellular deposits forming plaques and intracellular bundles of neurofibrillary tangles (NFTs). These lesions are now considered the invariant pathological feature of AD (for a recent review see Hardy and Selkoe, 2002). The current dominant view is that the plaques deposited outside and around the neurons are intrinsically toxic (Hardy and Selkoe, 2002), although more recent findings suggest the possibility that the intraneuronal deposits are the direct cause of toxicity (Mochizuki *et al.*, 2000; Kienlen-Campard *et al.*, 2002). According to the so-called amyloid hypothesis, tangles are considered only a secondary event as far as the time sequence in the disease progression is concerned, being a consequence of the formation of amyloid  $\beta$  (A $\beta$ ) plaques (Hardy and Selkoe, 2002). When brain lesions are made up only of intracellular bundles of self-assembled tau proteins, without accumulation of A $\beta$  in plaques, the

**Table I.** Summary of main diseases discussed in the review

Disease family	Diseases	Responsible proteins	Aggregates (localization)	Reference
Alzheimer's (AD)		amyloid precursor protein (APP)	plaques of fibrils (extracellular)	Hardy and Selkoe, (2002)
Tauopathies <sup>a</sup>	Amyotrophic lateral sclerosis/Parkinsonism–dementia complex Argyrophilic grain dementia Corticobasal degeneration Dementia pugilistica Diffuse neurofibrillary tangles with calcification Frontotemporal dementia with Parkinsonism linked to chromosome 17 Pick's disease Progressive subcortical gliosis Progressive supranuclear palsy Tangle only dementia	tau protein	neurofibrillary tangles (intracellular)	Lee <i>et al.</i> , (2001)
Poly-Q	Huntington's (HD) Spinobulbar muscular atrophy (Kennedy disease or SBMA) Dentatorubral–pallidoluysian atrophy (Haw–River syndrome or DRPLA) Spinocerebellar ataxia 1 (SCA1) Spinocerebellar ataxia 2 (SCA2) Spinocerebellar ataxia 3 (Machado–Joseph or SCA3) Spinocerebellar ataxia 6 (SCA6) Spinocerebellar ataxia 7 (SCA7)	Huntingtin  androgen receptor  atrophin-1 ataxin-1 ataxin-2  ataxin-3 VDCC ataxin-7	amorphous or amyloid-like (intranuclear) <sup>b</sup>	Masino and Pastore, (2002)

<sup>a</sup>Pathologies, other than AD, in which tau-positive neurofibrils are the predominant neuropathologic feature.

<sup>b</sup>In SCA6 nuclear aggregates are accompanied by cytoplasmic aggregates which are predominant.

diseases are usually distinct from AD and called tauopathies (Lee *et al.*, 2001). NFTs are nevertheless as toxic as amyloid plaques and in tauopathies lead to clinical symptoms similar to those of AD (Spillantini *et al.*, 1998).

The poly-Q disorders [which include Huntington's chorea, spinobulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy and spinocerebellar ataxias (SCAs)] are also associated with the formation of neuronal nuclear inclusions (NI) (Mangiarini *et al.*, 1996). In several cases, NI are spherical aggregates, sometimes assembled by insoluble amyloid-like fibrils (Scherzinger *et al.*, 1999; McGowan *et al.*, 2000). Aggregates outside the nucleus have also been observed, but it has been shown recently that nuclear localization is required for toxicity (Yang *et al.*, 2002).

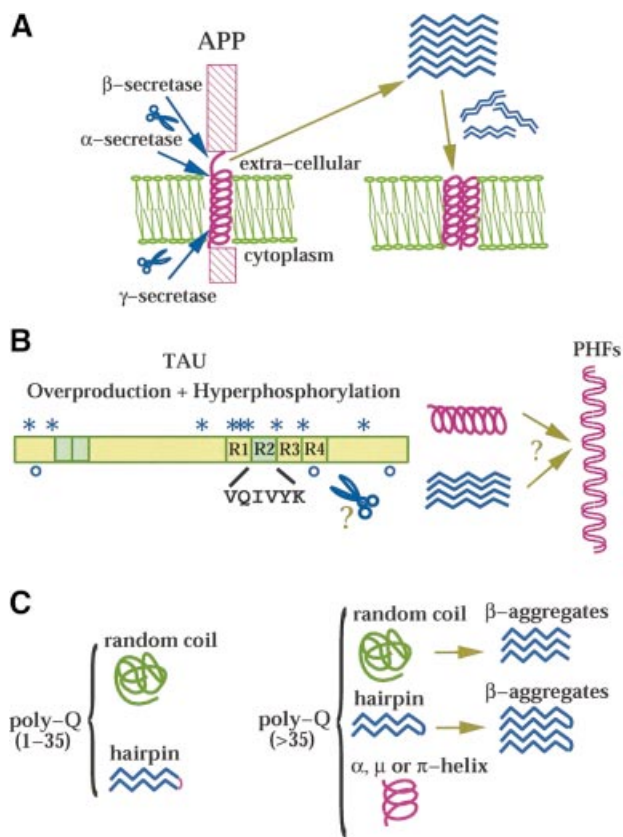
The pathologies of AD and poly-Q diseases are distinct, but both involve aggregate formation and degeneration of a specific subset of neuronal cells. The two disease families could therefore share a common mechanism of pathogenesis, in which the aggregates affect the cellular functions and eventually cause neuronal death.

A causative link between aggregation and disease is not however universally acknowledged. It has been conversely suggested that the aggregates could have a protective role for neuronal cells, being the result of the cell's attempts to proteolytically degrade or inactivate the toxic expanded protein (Zoghbi and Orr, 2000; Hardy and Selkoe, 2002). Pathogenesis could thus be initiated by other causes and only result in amyloid fibre formation.

## A molecular view: proteins or peptides?

While predisposition to AD has been linked to mutations of several genes, including those of presenilins and apolipoprotein E, mainly two protein components are present in the two types of AD aggregates (reviewed in Hardy and Selkoe, 2002). Plaques are generated by deposition of the amyloid peptides (A $\beta$ ), which are degradation products of the amyloid precursor protein (APP). APP is a transmembrane cell surface glycoprotein, expressed in five isoforms, with APP(695) being the dominant isoform in brain. APP can be cleaved by three different proteases, called  $\alpha$ ,  $\beta$  and  $\gamma$  secretases. When APP is concomitantly hydrolysed by  $\beta$ -secretase at the N-terminus of A $\beta$  and by the  $\gamma$ -secretase within the membrane (Lichtenthaler *et al.*, 2002), the two main products, A $\beta$ (1–40) and A $\beta$ (1–42), migrate outside the cell and give rise to fibrils. When APP is cut by  $\alpha$ -secretase, the resulting soluble peptides are generally considered non-toxic, although a recent report shows that the p3 peptide derived by  $\alpha$ - and  $\gamma$ -secretase cleavage of APP is also toxic (Wei *et al.*, 2002). A $\beta$ (1–42) readily aggregates and seeds the formation of fibrils that can then act as templates for plaque formation. Several shorter synthetic peptides corresponding to partial sequences of A $\beta$ (1–42) are able to form fibrils *in vitro* and have been studied along with 'natural' A $\beta$ .

NFTs are mostly composed of the tau protein (from which tauopathies are named) (Garcia and Cleveland,



**Fig. 1.** Hypothetical mechanisms of conformational transitions for A $\beta$  peptides (A), tau (B) and poly-Q containing proteins (C). (A) The peptides are proteolytically cleaved from APP, precipitate as  $\beta$ -structures in equilibrium with oligomers which may eventually redissolve into the membrane. The lipid double layer is shown schematically in green. (B) A schematic picture of the architecture of tau. The four repeats are indicated with R1–R4. Regions differentially spliced are coloured in green. The sites identified as calpain and caspase-3 cleavage are indicated with stars and open circles respectively (Canu *et al.*, 1998). The proteolytic products may assemble or nucleate aggregate formation (PHFs). (C) Summary of the current models of conformational transitions involving poly-Q tracts both in the pathological and non-pathological range. For a more detailed account see Masino and Pastore (2002).

2001; Hardy and Selkoe, 2002), a highly hydrophilic microtubule-associated protein whose main function is microtubule stabilization, but also covers membrane interactions and anchoring of enzymes (Brandt *et al.*, 1995; Sontag *et al.*, 1999). Tangle formation is accompanied by further increase of tau production, hyperphosphorylation and loss of microtubule binding in the affected neurons. Proteolysis has been proposed to be important also for tau since the C-terminus of tau, when cleaved by proteases, generates cytotoxic fragments (Canu *et al.*, 1998). These and other fragments are thought to nucleate tangle formation from full-length tau (von Bergen *et al.*, 2000). However, it remains much more uncertain than for the well-defined A $\beta$  which regions of tau are responsible for tangle formation.

Poly-Q diseases result from expansion of CAG nucleotide repeats that encode poly-Q tracts within the corresponding gene product (reviewed in Paulson, 1999; Zoghbi and Orr, 2000). The resulting proteins show no sequence homology outside the poly-Q, span different lengths, have

different cellular localization and, when known, different functions. The only common feature is the presence of poly-Q tracts, which are, however, located in different regions of the corresponding proteins. A direct role of poly-Q in neurotoxicity has been demonstrated (Ordway *et al.*, 1997; Yang *et al.*, 2002). Interestingly, pathological effects occur in patients only when the length of poly-Q exceeds a rather sharp threshold of 35–40 glutamines. The length of the poly-Q tract correlates directly with the age of onset and with the severity of the symptoms in the diseases. The molecular composition of NI is much more heterogeneous than that of the AD aggregates, since they contain the ubiquitinated affected protein, proteolytic fragments, chaperones and other poly-Q proteins. While for AD and related tauopathies the mechanism of aggregation is now widely accepted to be linked to proteolysis of APP (Hardy and Selkoe, 2002) and tau (von Bergen *et al.*, 2000), it is still unclear whether proteolysis is a universal event also for poly-Q proteins: proteolytic fragments and not the full-length proteins have been observed in NI for at least three poly-Q diseases (huntingtin, the androgen receptor and ataxin 1) (DiFiglia *et al.*, 1997; Wellington *et al.*, 1998; Welch and Diamond, 2001; Lunkes *et al.*, 2002). Huntingtin exon 1 with an expanded poly-Q is sufficient to cause a progressive neurological phenotype in transgenic mice (Mangiarini *et al.*, 1996). However, other reports suggest that the expanded proteins are more protease resistant and that release of N-terminal fragments of huntingtin occurs also in the non-toxic non-expanded protein (Dyer and McMurray, 2001; Kim *et al.*, 2001; Lunkes *et al.*, 2002).

Clarification of the precise nature of the aggregates obviously bears important consequences for structural/functional studies of tau and poly-Q proteins.

### Structural hypotheses on conformational transitions: how to solubilize the 'insoluble'

The prevailing hypothesis for explaining aggregation and amyloid formation involves a structural transition of a polypeptide chain from a native fold to an improperly folded or misfolded conformation. Much attention has been paid to understanding the molecular mechanisms producing this transition. The starting point for these studies implies the characterization of the structure of the non-aggregated protein or protein fragments. These are often referred to as the 'soluble form', as opposed to aggregates, but the actual situation is more complex since the starting conformation may not necessarily be soluble, especially at concentrations suitable for structural studies, as in the case of A $\beta$ . Structural studies are therefore strongly hampered by the low solubility in water and by the tendency of the proteins and peptides to aggregate, i.e. ironically for the very reason why it is interesting to study them. Several strategies have been devised to overcome these limitations.

In AD, A $\beta$  are believed to exist in an  $\alpha$ -helical conformation when it is part of the transmembrane APP (Ortega-Aznar *et al.*, 2000), with an amphipathic character distributed along the sequence: the hydrophilic N-terminus of the A $\beta$  is exposed to an aqueous environment whereas the highly hydrophobic C-terminus is embedded in the membrane lipids (Figure 1A). To overcome their lack of

solubility and to mimic the membrane environment, most solution studies of A $\beta$  have been performed either in mixtures of water and alcohols or in micellar solutions (Serpell, 2000; and references therein). In all these media, A $\beta$  assume helical conformations, characterized by two helical segments interrupted by a central tract that was termed the 'kink region' (Coles *et al.*, 1998; Shao *et al.*, 1999). However, the length of the helical segments and the conformation of the kink region varies according to the media employed. In the structure of A $\beta$ (1–40) in a trifluoroethanol (TFE)/water mixture (Sticht *et al.*, 1995) the two helices, spanning residues 15–23 and 31–35, are separated by a disordered region. In a mixture of hexafluoroisopropanol and water the kink region of A $\beta$ (1–42) has been found to adopt a regular type I  $\beta$ -turn, yielding a well-defined tertiary structure (Crescenzi *et al.*, 2002). The striking similarity of this structure with that of the fusion domain of haemagglutinin of influenza virus hints to a possible mechanism of toxicity of A $\beta$  (discussed later). A random coil conformation has instead been observed in aqueous solution for Met-oxidized A $\beta$ (1–40) and smaller fragments (Jarvet *et al.*, 2000; Zhang *et al.*, 2000; Riek *et al.*, 2001) and in dimethylsulfoxide and water mixtures (Crescenzi *et al.*, 2002).

Sequence analysis of tau suggests that it is a natively unfolded protein with an even distribution of hydrophobic fragments along its sequence and up to four repeats of ~30 residues in its central region (Ruben *et al.*, 1991; von Bergen *et al.*, 2000) (Figure 1B). Experimental information derived from electron microscopy (EM), small angle X-ray scattering and NMR of tau confirms that the protein is essentially unfolded with little  $\alpha$ -helix and  $\beta$ -sheet content (Crowther and Wischik, 1985; Kirschner *et al.*, 1986; Schweers *et al.*, 1994). Peptides from tau are much more soluble than A $\beta$ , making solution studies less problematic. Short synthetic peptides spanning tau repeat sequences are mostly random in solution but were shown to be able to aggregate as  $\beta$ -structures (von Bergen *et al.*, 2000). A peptide from the C-terminus (residues 423–441), which forms an alternative nucleation region of NFTs, forms a regular helix in a TFE/water mixture with a helical stabilizing C-capping motif (Esposito *et al.*, 2000).

More debated is the nature of the conformation and the structural transition of poly-Q, for which experimental validation is even harder. In addition to the solubility problems, it is hard to produce this homopolymer both by conventional peptide synthesis and by recombinant methods. In 1993, Perutz proposed that poly-Q chains could form antiparallel  $\beta$ -strands held together by hydrogen bonds between side chain and main chain amides (Perutz *et al.*, 1993). These 'polar zippers' would pair either intramolecularly (leading to a  $\beta$ -hairpin) or intermolecularly (leading to aggregates) when the poly-Q length exceeded a threshold. Although since then this hypothesis has strongly dominated the field, other researchers have invoked different models which imply transitions from random and  $\alpha$ -to- $\beta$  conformations (Lathrop *et al.*, 1998; Starikov *et al.*, 1999) (Figure 1C). In addition, an entirely different conformation, a right-handed helix with 6.2 residues/turn called  $\mu$ -helix, was suggested by Monoi (1995). Such structure has a cylindrical pore along its helical axis larger than in an  $\alpha$ -helix (~6.6 Å) and might be favoured in poly-Q sequences

owing to the unique possibility of hydrogen bonds involving the amide groups of glutamine side chains. Experimental evidence is mostly based on model peptide studies of poly-Q tracts, which have been solubilized using either hydrophilic flanking sequences or various mixtures of organic solvents, and on poly-Q sequences fused to different protein carriers (reviewed in Masino and Pastore, 2002). Attempts to characterize poly-Q structure in protein contexts different from the natural ones are justified by the observation that poly-Q is toxic independently of the specific protein (Ordway *et al.*, 1997). Most peptide and protein studies seem to alternatively support one of these hypotheses: in one, soluble poly-Q is in random coil conformation, in the second, it forms  $\beta$ -structures. The apparent contradictions between these studies may be reconciled into a unifying picture by considering that the results are dominated by the strong aggregative tendency of poly-Q in water. Only when either the solvent composition, the flanking regions or the carrier protein are able to offset the insolubility of poly-Q, can this then adopt a random coil conformation. Since long poly-Q tracts have stronger tendency to aggregate, the competing effect of the environment will have a reduced effect.

### Structural models of aggregates: how to study the 'insoluble'

While the insolubility of the non-aggregated proteins hampers solution studies, a structural characterization of the aggregates is, if possible, even harder since it has to deal with their amorphous nature. These circumstances have so far prevented the determination of a consensus structure at atomic detail of any of the major aggregates. The consensus hypothesis is that amyloid fibres are constituted by  $\beta$ -sheet conformations. Increasing experimental evidence shows in fact that it is possible to induce  $\beta$ -sheet fibril formation in many proteins *in vitro* (reviewed in Dobson, 1999). Figure 2 shows a comparison of current models of amyloid fibres (Serpell, 2000; and references therein), NFTs (Moreno-Herrero *et al.*, 2001) and poly-Q aggregates (Perutz *et al.*, 2002). Direct evidence from solid state techniques, such as X-ray fibre diffraction, solid-state NMR, Fourier transform-infrared and EM shows unequivocally that amyloid AD plaques are composed of long straight fibres of 6–12 nm in diameter with a basic  $\beta$ -sheet conformation in the fibrils (Sunde *et al.*, 1997). The main controversy is whether in amyloid fibrils the  $\beta$ -strands are parallel, as indicated by work with solid-state NMR and electron paramagnetic resonance (Balbach *et al.*, 2002; Torok *et al.*, 2002) or antiparallel, as suggested by several X-ray diffraction studies (Serpell, 2000).

A water-filled nanotube structure formed by a single cylindrical  $\beta$ -sheet stabilized by backbone and side chain hydrogen bonds was suggested as a possible model for poly-Q fibres (Perutz *et al.*, 2002). No atomic model is yet available for NFTs. Morphological studies based on EM and atomic force microscopy show tangles as formed by intra-cellular paired helical filaments (PHF), which are assembled into two twisted ribbons with a helical pitch of ~75–80 nm (Crowther and Wischik, 1985; Pollanen *et al.*, 1997; Moreno-Herrero *et al.*, 2001). Conflicting evidence exists about the structural nature of PHF. A hexapeptide

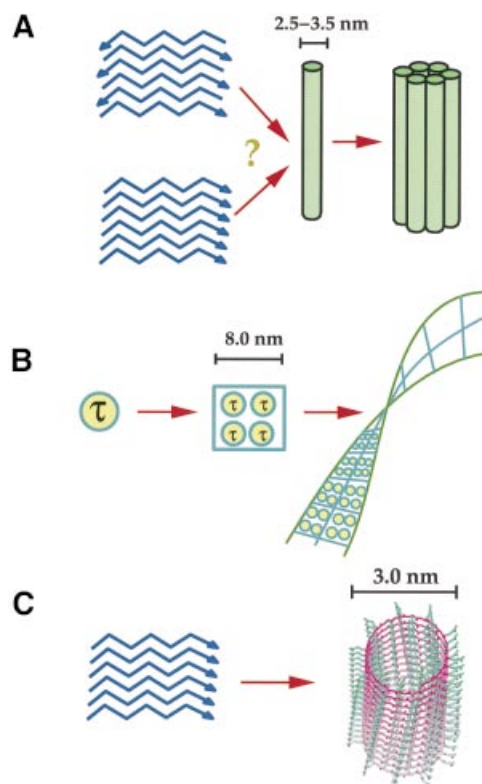
$^{306}\text{VQIVYK}^{311}$  from the beginning of the third repeat of tau has been shown to assemble into thin  $\beta$ -filaments (although without PHF appearance) and to nucleate PHFs from full-length tau (von Bergen *et al.*, 2000). Such results are, however, at variance with a preliminary report claiming a helical structure for unpurified PHFs isolated from brain samples of AD patients (Sadqi *et al.*, 2002).

### Why should aggregates be toxic?

Even when we understand the mechanism leading to aggregate formation, we shall still need to explain how and why these are toxic for the cell. It is hard to imagine how a common mechanism could be consistent with the very different localizations of the polypeptide chains involved in AD and poly-Q diseases. Toxicity is particularly obscure for AD where, if toxicity were due to the plaques external to the neurons, aggregates could not interfere directly with the cell metabolism. It is natural to think that plaques adjacent to the cellular membrane can directly damage it. Whereas a trivial explanation might suggest the mechanical blockage by plaques of all cellular exchanges with the outer environment, a widely invoked cause of membrane damage has been free radical oxidative stress (Varadarajan *et al.*, 2000). On a more general level, the idea that amyloid plaques in AD act as large reservoirs of species in equilibrium with smaller neurotoxic oligomers is gaining more credit (Walsh *et al.*, 2002).

A specific mechanism for the toxicity of oligomeric assemblies seems to be suggested by solution studies of isolated  $\text{A}\beta$ : the surprising similarity of the structure of  $\text{A}\beta(1-42)$  to the fusion domain of influenza haemagglutinin in an apolar microenvironment (Crescenzi *et al.*, 2002) points to membrane poration as the key event for the peptide neurotoxicity. The  $\alpha$ -helical peptide would induce formation of membrane channels, allowing the penetration of substances (such as metal ions) that can cause neuronal death (Rhee *et al.*, 1998; Lin *et al.*, 2001). The ability of  $\text{A}\beta$  to induce vesicle fusion (Pillot *et al.*, 1996) and the recent observation that  $\text{A}\beta$  can enhance infection at the stage of attachment or entry into the cell of several viruses further support this hypothesis (Wojtowicz *et al.*, 2002).

A similar mechanism has been proposed to explain the toxicity of expanded poly-Q. Poly-Q stretches can induce large membrane-depolarizing ion channels in membranes which could damage the cell by altering the ionic balance (Hirakura *et al.*, 2000; Monoi *et al.*, 2000). The  $\mu$ -helix conformation (Monoi, 1995) would provide a justification for the puzzling threshold that separates pathological (>35–40) from non-pathological lengths of poly-Q tracts in poly-Q diseases. The minimum peptide length required for the  $\mu$ -helix to span the hydrophobic core of a lipid bilayer (estimated to be ~3 nm) is ~37 residues. Similarly, the restraints imposed by the helical nature of the model proposed by Perutz *et al.* (2002) impose a minimum of 40 residues for stabilizing the nanotube structure. The poration hypothesis is however not supported by a membrane localization of most of poly-Q proteins, in conflict with the possibility of a common mechanism related to poly-Q. Alternative albeit only theoretical models relate the threshold to an intrinsic random coil instability with respect to  $\beta$ -conformations in chains containing >40 glutamines (Starikov *et al.*, 1999). A



**Fig. 2.** Comparison of current models of amyloid fibres (A), tangles (B) and poly-Q aggregates (C). (A) The amyloid protofilament of 2.5–3.5 nm diameter would originate from  $\beta$ -strands running perpendicular to the fibre axes, although it is still disputed whether they run parallel or anti-parallel (Serpell, 2000). Several protofilaments are thought to further assemble to form mature fibrils. (B) Although no details are available yet about the molecular arrangement of tau in tangles, ample evidence of their morphology suggests an arrangement of paired helical filaments (Moreno-Herrero *et al.*, 2001). (C) Model of water-filled nanotube in which the poly-Q forms a helical fibre with 20 residues per turn (Perutz *et al.*, 2002). The main chains are shown in purple. The side chains (shown in green) protrude inside and outside the cylinder surface, according to  $\beta$ -sheet periodicity. Hydrogen bonds are formed both between the main chains and the side chains and would stabilize the structure.

structural difference could promote interactions with cellular components which do not recognize short poly-Q proteins and/or lead more readily to aggregates. In both cases, the normal cellular functions would be altered.

### Towards a unifying picture?

Although AD and poly-Q proteins were identified 10–15 years ago, still relatively few structural studies describe the conformation of these proteins both in their pathological and non-pathological forms. This is at least partially due to the specific challenges posed by this unusual class of polypeptides which respond differently to different environments. In all cases, significant information can be obtained only by using a multidisciplinary approach in which the properties in solution and in a solid-state environment are compared. While a common mechanism for misfolding diseases still remains to be proven, a unifying picture emerging from the current studies is that all peptides generated by proteolysis of the parent proteins

are water insoluble and aggregate *in vitro*. However, protein fusion, complexation or a solvent medium can offset aggregation. It is clear that unambiguous identification of the entity prone to aggregation and a more detailed understanding of the structural bases of the conformational transition is a key step for designing possible therapies.

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