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PERSPECTIVES

OPINION

What is the role of protein aggregation in neurodegeneration?

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Abstract | Neurodegenerative diseases typically involve deposits of inclusion bodies that contain abnormal aggregated proteins. Therefore, it has been suggested that protein aggregation is pathogenic. However, several lines of evidence indicate that inclusion bodies are not the main cause of toxicity, and probably represent a cellular protective response. Aggregation is a complex multi-step process of protein conformational change and accretion. The early species in this process might be most toxic, perhaps through the exposure of buried moieties such as main chain NH and CO groups that could serve as hydrogen bond donors or acceptors in abnormal interactions with other cellular proteins. This model implies that the pathogenesis of diverse neurodegenerative diseases arises by common mechanisms, and might yield common therapeutic targets.

Neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and the polyglutamine diseases that include Huntington's disease (HD), arise from abnormal protein interactions in the central nervous system. In all of these diseases, there are characteristic deposits of protein aggregates[1–11] in the brain, which can be cytoplasmic, nuclear or extracellular (FIG. 1). Protein aggregation can result from a mutation in the sequence of the disease-related protein, a genetic alteration that causes an elevation in the amounts of a normal protein, or can occur in the absence of genetic alterations, perhaps triggered by environmental stress or aging[12]. Even proteins that do not cause aggregation diseases can form aggregates and can cause toxicity[7,8]. In aggregation diseases, large intracellular or extracellular accumulations of aggregated protein, known as inclusion bodies, are often formed. The inclusion bodies frequently contain the disease protein in a fibrillar aggregated form called amyloid[11,13,14], and also may contain other material.

Although inclusion bodies are frequent pathological features of neurodegenerative disorders, there has been great controversy about the role of aggregation in the disease process. Abnormal protein conformation and protein aggregation are emerging as common features of the diseases, and many indirect lines of evidence link aggregation to toxicity. However, other studies indicate that inclusion body formation can be dissociated from toxicity, and might represent a cellular protective response. How can this apparent paradox be resolved: that the process of aggregation might be related to toxicity, but that the inclusion bodies might be protective? There is increasing, although still indirect, evidence that inclusion bodies might represent an end-stage manifestation of a multistep aggregation process. Early events before the formation of inclusion bodies might cause toxicity. Possible culprits include abnormal monomers of the disease proteins, or small assemblies of abnormal aggregated protein, which are often termed oligomers or protofibrils. A better understanding of the biochemistry and biophysics of the processes of conformational change and aggregation will be crucial to unraveling both cellular toxic events and cellular protective mechanisms.

Are inclusion bodies protective?

Inclusion bodies and other visible aggregates are distinctive features of neurodegenerative diseases (BOX 1). However, the presence of inclusion bodies correlates poorly with other markers of neurodegeneration, or with ante-mortem clinical features. In AD, for example, there is only a weak correlation between the density of amyloid-β (Aβ) peptide-derived amyloid plaques in human post-mortem material and the clinical severity of AD[15]. Similarly, in PD, there is little correlation between the presence of intracytoplasmic aggregates (known as Lewy bodies) and cell death in the substantia nigra of the brain[16]. This lack of correlation is most apparent in the polyglutamine diseases. For example, in HD, inclusion bodies are present in the cells of the striatum, which undergoes massive degeneration, but are more dense in the cerebral cortex, which undergoes only moderate degeneration[17]. Moreover, striatal inclusion bodies are most prevalent in large interneurons, which are spared in HD, rather than in medium spiny neurons, which are selectively lost in the disease[18].

Cell culture models provide some of the most direct experimental evidence regarding the role of protein aggregation in neuronal cell death. Expression of proteins that contain expanded polyglutamine stretches in neuronal cells grown in culture leads to robust cell death, making this a good model for mutant polyglutamine toxicity. Even in initial studies, the presence of inclusion bodies showed little correlation with neuronal toxicity[19], although the conditions might not have been representative of those occurring *in vivo*.

Using methods developed in the Finkbeiner laboratory for live cell imaging and tracking individual neurons in culture, the effect on cell viability of the expression

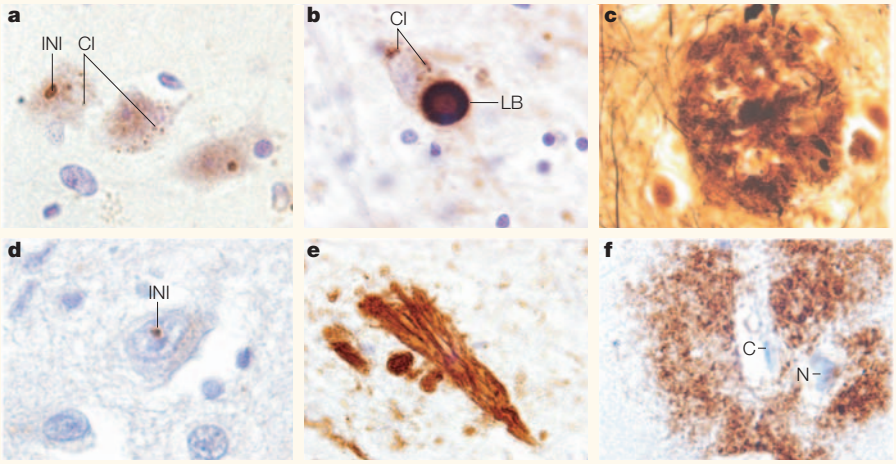

Figure 1 | **Intranuclear, cytoplasmic and extracellular aggregates and inclusion bodies visualized by light microscopy. a** | Intranuclear inclusions (INI) and cytoplasmic inclusions (CI) in the motor cortex of Huntington's disease brain recognized with 1C2 antibody to expanded polyglutamine. **b** | Lewy body (LB) and other cytoplasmic inclusions (CI) that contain α-synuclein within a neuron of the substantia nigra of Parkinson's disease brain recognized with an antibody specific for α-synuclein. **c** | Neuritic plaque of Alzheimer's disease in the cerebral cortex. Hirano silver stain identifies intracellular (neuritic) and extracellular protein aggregates. **d** | Intranuclear inclusion in the frontal cortex of Huntington's disease brain recognized with anti-ubiquitin antibody. **e** | Neurofibrillary tangles of Alzheimer's disease in the hippocampus immunostained with antibody specific for phosphorylated tau. **f** | Diffuse plaque of Alzheimer's disease in the cerebral cortex. Amyloid β (Aβ)-specific antibody recognizes extracellular deposits of Aβ (surrounding a neuron (N) and a capillary (C)). Figure provided by O. Pletnikova and J. C. Troncoso, John Hopkins University, Baltimore, USA.

of an N-terminal huntingtin fragment fused to green fluorescent protein could be followed over time. Cells with comparable expression levels of the huntingtin fragment were less likely to die if they formed inclusion bodies than if they did not form inclusion bodies[20]. Although this study does not prove that the inclusion bodies themselves are protective (cells which form inclusion bodies could be better protected in other ways), it provides strong evidence to support this hypothesis.

Comparable data from the study of cell models of PD are less definitive, but are consistent with these observations. Expression of mutant α-synuclein protein in cells grown in culture causes toxicity[21–23], but cells undergoing toxicity often have few, if any, α-synuclein-containing inclusion bodies. Also, co-transfection of cells with α-synuclein and the α-synuclein-interacting protein synphilin-1 enhances the formation of inclusion bodies[24], but also improves cell viability[25].

Taken together, these studies are consistent with the idea that inclusion body formation represents a cellular protective response, and indicate that neurons have developed both cellular and molecular mechanisms to deal with aggregated and misfolded proteins.

Cellular protective mechanisms

The inherent tendency of proteins to aggregate[7,8,10] has made it necessary for cells to evolve several defences against misfolded or abnormal proteins (FIG. 2). The first defence is provided by molecular chaperones, many of which have been identified as heat shock proteins. These molecules can refold abnormal proteins and render them non-toxic. Cell transfection studies have shown that molecular chaperones can increase the solubility of mutant polyglutamine proteins and suppress mutant polyglutamine-mediated neuronal toxicity *in vitro* and in invertebrate models *in vivo*[26,27], although the effects of molecular chaperone overexpression in mouse models have been variable[28,29].

A second important cellular defence against misfolded proteins involves degradation by the proteasome[30,31]. The proteasome is a complex molecular machine that can unfold proteins and process them to short peptide fragments in an interior space that contains proteolytic enzymes. Classically, proteins are targeted for proteasomal degradation by ubiquitylation, although other mechanisms of targeting are also possible, including targeting by the chaperone protein CHIP. Several of the gene products that are mutated in familial PD are intimately related to the ubiquitin–proteasome system, highlighting the relevance of this system for PD in particular.

A third defence mechanism involves autophagy, which has several variants, including macroautophagy, microautophagy and chaperone-mediated autophagy. Soluble cytoplasmic proteins, especially those that do not turn over rapidly, can be degraded by these lysosomal pathways. Chaperone-mediated autophagy involves the delivery of proteins that contain a consensus motif to transporters in the lysosomal membrane by a chaperone and co-chaperone complex[32,33]. Chaperone-mediated autophagy can be activated by oxidative stress[34] or nutrient stress, and is involved in the degradation of α-synuclein through a consensus recognition site within α-synuclein[35]. Autophagy can also be involved in the degradation of proteins with mutant polyglutamine repeats[36]. Proteasomes and autophagy are also involved in the normal turnover of proteins.

When abnormal and aggregated proteins cannot be refolded or degraded by proteasomal degradation and chaperone-mediated autophagy, the cell has an alternative line of defence: cells can sequester aggregates by microtubule-mediated transport and collect them at a single cytoplasmic site near the centriole. This process, described by Ron Kopito[37], generates a large inclusion body, visible by light microscopy, called an aggresome. The Lewy bodies of PD are strikingly similar to aggresomes[38,39] and contain the centrosome markers γ-tubulin and pericentrin, ubiquitin-activating enzymes and proteasome activators, as well as markers of autophagy. Furthermore, Lewy bodies show characteristic alterations of intermediate filaments and other cytoskeletal elements. Therefore, Lewy bodies, and presumably aggresomes in general, probably represent the end product of an active cellular process of accommodating misfolded protein.

After abnormally folded proteins are collected in an aggresome, they can potentially be disposed of by macroautophagy, a process by which a cell can sequester a large portion of its cytoplasm and package it into a membrane-bound structure, know as an autophagosome, which can later be extruded by the cell[40]. Macroautophagy can eliminate aggresomes in cultured cells[39,41,42]. Transfected huntingtin protein accumulates, in part, in structures that have many of the characteristics of autophagosomes[43].

Cellular localization of inclusions

The role of active cellular protective processes in dealing with aggregated protein is clearest for cytoplasmic aggresomes, and therefore presumably for the Lewy bodies of PD. However, mutant tau protein, which accumulates in neurofibrillary tangles in AD, does not seem to be packaged into aggresomes, perhaps because tau is already anchored to the cytoskeleton and is therefore less displaceable. Mutant huntingtin often accumulates in dendritic locations (as does α-synuclein in PD), without being transported to the perinuclear location of aggresomes. Why aggresomes are formed under some circumstances but not others is unclear.

There do not seem to be comparable mechanisms for extracellular aggregates, such as Aβ-derived amyloid plaques. These aggregates can be targeted and partially engulfed by activated microglia, inducing an inflammatory response, and probably exacerbating, rather than ameliorating, amyloid-associated neurodegeneration in AD brain[44]. Intranuclear inclusion bodies are a pathological hallmark of the polyglutamine diseases, and might contain proteolytic fragments of the mutant proteins. However, an active transport mechanism comparable with aggresome formation has not yet been described in the nucleus. If production of mutant huntingtin is stopped, neurons can clear nuclear inclusions effectively[45,46], possibly through proteasomes because nuclei do not seem to have an autophagic system[39].

Nature of the toxic species

An important question in the study of mutant proteins in all of the neurodegenerative diseases remains — what are the toxic species? A key question is whether the abnormally folded monomers are toxic, or whether toxicity requires some aspect of the aggregation process. Protein aggregation is probably a complex multi-step process with several possible intermediate species, including oligomeric forms and protofibrillar forms (see BOX 2 and FIG. 3). Particular molecular species could be responsible for toxicity — for example, monomers, oligomers, protofibrils or specific forms of fibrils.

Another possibility is that toxicity is not exclusively due to any one species *per se*, but to the dynamics of the aggregation process. There seem to be structures that are shared by various aggregating proteins[7,8,10,47,48]. For example, there might be a "…common toxic fold" that is "…maintained upon assembly into dimers, oligomers and fibrils" (L. Pieri, M. Bucciantini and M. Stefani, personal communication). Alternatively, toxicity might involve the exposure of moieties that are usually hidden in globular protein structures, such as hydrophobic side chains, or main chain NH and CO groups in an abnormal β-conformation that could lead to the formation of non-native hydrogen bonds. It has also been proposed that the abnormal fold of mutant polyglutamine might form a toxic surface for the binding of other molecules (E. Wanker, personal communication), and this binding may be responsible for exerting toxic effects, perhaps functioning in a similar manner to the active site of an enzyme (J. Kelly, personal communication). On the basis of this hypothesis, there might not be one single toxic species *per se*, but instead, this toxic surface might be most prominent on one or more species.

There are many intriguing observations and hypotheses, but little definitive data regarding the different molecular species that are involved in protein aggregation and their relationships to cellular toxicity. Also, much of the information that is currently available is from *in vitro* studies. Furthermore, the order of events in the protein aggregation process is unknown. Protein aggregation could take the form of a linear pathway — from monomers to oligomers to protofibrils to fibrils — or a series of parallel processes in which monomers are added directly to growing fibres, with alternative species originating from side pathways (FIG. 3). Perhaps abnormally folded monomers can induce toxicity and, if so, it is possible that only those monomers that can form aggregates are toxic to cells. There may be multiple abnormal conformations of the monomer. For example, in prion disease, different conformations of the abnormally folded monomeric prion protein[49,50] are believed to underlie the differential prion infectivity across the species barrier.

Alternative models for fibril formation are crisply articulated in two studies of the *in vitro* assembly of the yeast prion Sup35 NM aggregation domain, which

Box 1 | Neurodegenerative diseases

Huntington's disease (HD) and other polyglutamine disorders
Eight disorders are currently known. HD is a progressive and fatal disorder caused by degeneration of the neurons of the striatum with moderate degeneration in other brain regions. It is caused by an expanded CAG repeat in the huntingtin gene, and the disease manifests when 38 or more consecutive glutamines are present in the huntingtin protein. The CAG length threshold for disease is 36–40 (REFS 2,83,84). Intranuclear, cytoplasmic and neuritic inclusions containing aggregated huntingtin protein are a pathological hallmark[1,2].

Alzheimer's disease (AD)
A progressive, fatal dementia that usually strikes in late life. Genetic forms can have early disease onset[85]. The characteristic lesions of AD are the extracellular amyloid plaques, composed of aggregates of the amyloid-β (Aβ) peptide (a fragment of the amyloid precursor protein (APP), and intracellular neurofibrillary tangles, which contain accumulations of hyperphosphorylated forms of the neurofilament-associated protein tau.

Parkinson's disease (PD)
A progressive movement disorder characterized by resting tremor, bradykinesia and rigidity with cognitive and emotional symptoms[9]. Sporadic PD begins in late life, whereas genetic forms can result in early disease onset. The characteristic lesion of PD, the Lewy body, is an eosinophilic cytoplasmic inclusion containing fibrillar deposits of the α-synuclein protein and can also include neurofilaments, the α-synuclein associated protein synphilin-1, and parkin. Familial PD can be caused by genetic mutations in α-synuclein, parkin, DJ-1, PTEN induced putative kinase-1 (PINK1) and leucine-rich repeat kinase-2 (LRRK2).

Amyotrophic lateral sclerosis (ALS)
A progressive, debilitating neuromuscular disorder that generally strikes in mid to late life and that can rapidly progress to death. Most cases are sporadic, though autosomal dominant familial forms can arise owing to mutation in superoxide dismutase (SOD1). Protein aggregation is increasingly recognized as a feature of ALS[86].

Other neurodegenerative diseases with protein aggregation include prion diseases, familial British dementia, BRI2 gene-related dementia, familial Danish dementia, frontotemporal dementia, meningo-vascular amyloidoses, multisystem atrophy, progressive supranuclear palsy, familial and sporadic cerebral amyloid angiopathies, congophilic angiopathy and others (reviewed in REFS 87–89).

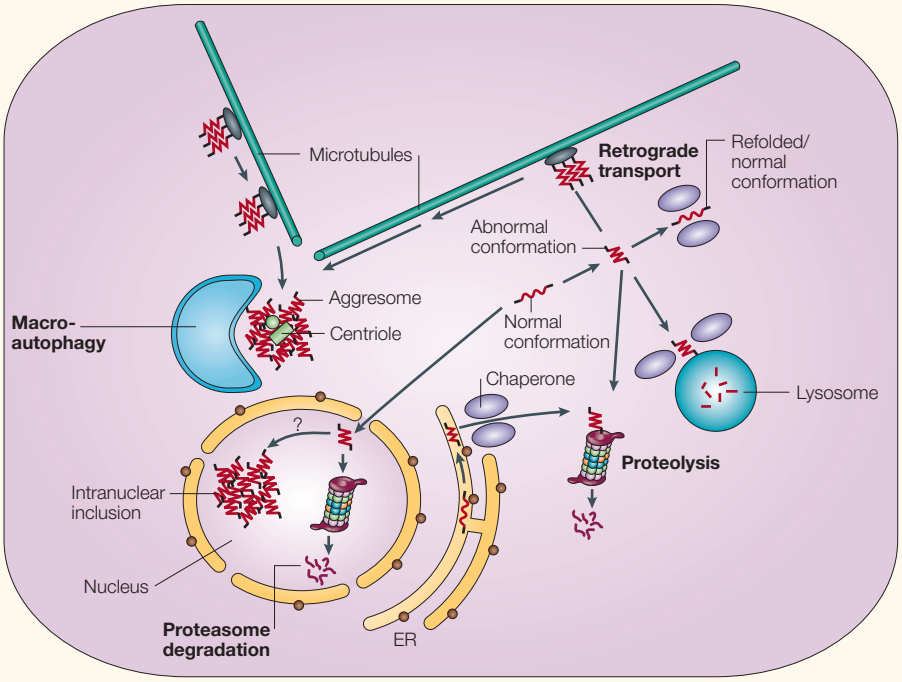

Figure 2 | **Cellular defence mechanisms against aggregated abnormal proteins.** A protein can undergo a change to an abnormal conformation. Abnormally folded proteins can be refolded by molecular chaperones. If the protein cannot be refolded, it can be targeted to the endosome/lysosome system or to the proteasome for degradation. Alternatively, protein aggregates can be transported by dynein-mediated retrograde transport on microtubules toward the centriole, where they can form an aggresome, which can ultimately be degraded by macroautophagy. If the abnormal protein is present in the endoplasmic reticulum (ER), it can be processed and delivered to the cytoplasmic proteasomal machinery for degradation. There are also proteasomes within the nucleus that can degrade abnormal proteins. However, the mechanisms underlying intranuclear inclusion formation and removal are poorly understood. Cellular components are not drawn to scale.

conclude that fibril growth is mediated by the addition of either oligomers[51] or monomers[52] to the growing fibril. The limited structural information available on metastable oligomeric states, which reveals surprising structural similarities between protofibrils and fibrils[53], is consistent with either mechanism. The situation may be different for human disease proteins *in vivo*, where protein concentrations are considerably lower, the medium is far more complex, and heterogeneous nucleation by other cellular components might contribute to fibril formation.

The events in the fibrillization pathway have been studied in most detail for Aβ. Soluble Aβ can be extracted from post-mortem AD brain and the level of this material seems to correlate with cognitive deficits. Such soluble material could represent monomers or soluble oligomers[54,55]. Several groups have identified soluble oligomeric Aβ aggregates and have called them Aβ diffusible ligands (ADDL), paranuclei, protofibrils or other terms[56–59] Aβ42 can form pentameric or hexameric units, which further assemble to form beaded superstructures similar to early protofibrils[60]. Such oligomers may be precursors to protofibril or filament formation. One important difference between these oligomers and those observed in other diseases is that Aβ oligomers are probably extracellular. One might then ask how these structures elicit a toxic effect from outside the cell. There is some speculation that ADDLs bind to the tyrosine kinase receptor Fyn, although the significance of this is still not clear.

Elegant studies of the role of oligomers in toxicity were carried out by Selkoe, Walsh, Teplow and colleagues. Aβ was injected directly into the brain of anaesthetized rats, and yielded toxic effects on the synaptic plasticity index called long-term potentiation[61]. The investigators then pretreated the injected Aβ with insulin degrading enzyme, which selectively degrades Aβ monomer. This pretreated material was still toxic — implying that the toxic species is a soluble oligomer. The oligomers can be purified using size-exclusion chromatography, and oligomer-induced toxicity can be blocked with co-injection of an oligomer-specific monoclonal antibody[62].

Several different aggregated forms of α-synuclein have also been described. These include oligomeric globular assemblies, protofibrils, and ring-like structures, which might be circularized protofibrils. The nature of the toxic species is uncertain. Biophysical studies indicate that ring-like intermediates could form pores in membranes[63], but a role for these structures in pore formation in cells or *in vivo* remains speculative.

The study of polyglutamine fibrillization is still in an early stage of research. In an *in vitro* study using bacterially expressed exon-1 of huntingtin with normal or expanded repeats, several different kinds of huntingtin aggregates were identified[64]. A time-course study indicated that oligomeric globular assemblies formed early, followed by protofibrils, later followed by fibril formation, indicating a sequential pathway from monomer to oligomer to protofibril to fibre. However, there is as yet no direct evidence to support this possibility. A recent study has replicated the observation of spherical and protofibrillar forms of huntingtin aggregates[65]. Chaperone proteins could 'partition' monomers and decrease the spherical and annular oligomers. As chaperones have previously been shown to decrease toxicity of mutant huntingtin, these observations would be consistent with the hypothesis that oligomeric assemblies are a toxic species.

One difficulty of *in vitro* studies of huntingtin polyglutamine is generating sufficient material. The study of synthetic peptides might provide an alternative. Kinetic studies of synthetic peptides indicate that nucleated growth takes place by monomer addition rather than oligomerization, with nucleation corresponding to a folding event within the monomeric polyglutamine peptide[66].

Unfortunately, it is difficult to study the biochemical and biophysical properties of the putative aggregation intermediates. An interesting approach to determine the requisite structure for aggregation was taken by Wetzel's group. They designed polyglutamine-containing peptides with interspersed proline–glycine interruptions every nine glutamines — which would induce periodic β-turns. These peptides formed aggregates as efficiently as pure expanded polyglutamine[67]. These data indicate that polyglutamine aggregation begins by the formation of a compact β-structure with alternating β-strands

Box 2 | **Terminology of aggregation**

Part of the difficulty in the field has been the use of vague or conflicting terminology. We propose the following definitions for terms commonly used in the field.

- **Aggregation: Any abnormal association of misfolded proteins (or parts of proteins). Aggregation is a process that begins with the abnormal association of as few as two molecules and that has the potential to form larger structures that are visible by microscopy. This definition is therefore a morphological one. Small aggregates can be soluble under many conditions, but large aggregates are insoluble under physiological conditions. At the biochemical level, aggregation can be any abnormal association of misfolded proteins (or parts of proteins). This definition is important, as biochemical aggregates might not be visible using conventional microscopic techniques.**
- **Aggregation intermediate: A putative metastable molecular species that may be 'on' or 'off' the pathway to fibril formation and that forms large aggregates and inclusions.**
- **Aggresome: A cytoplasmic inclusion body located in the perinuclear region near the centriole that results from the collection of small aggregates from other parts of the cell through active, microtubule-based transport.**
- **Amyloid: Insoluble fibrillar aggregates composed of amyloid fibrils, which can be seen using electron microscopy (EM) or highlighted by birefringent staining using Congo Red.**
- **Amyloid fibril: A thermodynamically stable, structurally organized, highly insoluble, filamentous protein aggregate. The amyloid fibril is composed of repeating units of β-sheets aligned perpendicular to the fibre axis, with a distinctive X-ray fibre diffraction pattern ('cross β') that is similar to crystalline silk and consistent with high β-sheet content.**
- **Amorphous aggregate: protein aggregates without amyloid fibrils, often with a granular appearance when viewed by EM.**
- **Annular protofibril (or annular oligomeric aggregate). An annular species that has been suggested to form a pathogenic pore in biological membranes.**
- **Inclusion body: A large accumulation of aggregated material in a cell, visible by light microscopy, often well demarcated from other cellular constituents. It can contain amyloid fibrils and amorphous aggregates, as well as other material. Classic examples are Lewy bodies of Parkinson's disease and intranuclear inclusions of Huntington's disease.**
- **Microaggregate: A small accumulation of aggregated protein, detected by EM or other specialized microscopic technique. It can consist of any of the species of aggregates defined here.**
- **Oligomeric aggregate: Small (approximately 5–10 nm) assembly with a globular appearance, probably comprised of about 3–50 monomers, which might represent an aggregation intermediate.**
- **Protofibril: Soluble, short fibril-shaped aggregated structure, usually thinner or shorter than a mature fibril, which might represent an aggregation intermediate.**
- **Protofilament: A proposed single strand of a mature, mutistranded amyloid fibril.**

and β-turns, with an optimal length of seven glutamines per β-strand. Recent cell transfection studies with exon-1 huntingtin have confirmed the propensity of the β-turn-inducing sequences to form aggregates in cultured mammalian cells and to induce toxicity as effectively as pure polyglutamine[68].

Mechanisms of toxicity

Regardless of the nature of the toxic species, it will be crucial to determine the molecular mechanisms of cellular toxicity. Many proposals have been made (reviewed in REFS 4–6,9,30,31,69). A detailed review is beyond the scope of this article, but perhaps most relevant to the current discussion are suggestions involving interference with the cellular defence mechanisms described above. One interesting hypothesis is that abnormal proteins might inhibit the proteasome[70]. Long polyglutamine stretches, even in the usual physiological range, are difficult for proteasomes to digest[71]. Expanded polyglutamine might therefore directly inhibit the proteasome[72,73]; although expression of mutant protein in the nucleus can inhibit cytoplasmic proteasomes and vice versa, indicating that the mechanism is probably indirect[74]. However, it is not clear if this inhibition occurs *in vivo*[75].

Mutant α-synuclein can also lead to proteasomal inhibition[21,22]. This is especially interesting as systemic exposure to proteasome inhibitors can cause a Parkinson's-like syndrome in rats, accompanied by loss of dopaminergic neurons in the substantia nigra, loss of dopamine terminals in the striatum and a progressive movement disorder responsive to dopamine agonist treatment[76].

Another possibility is that abnormal proteins could alter autophagy. Mutant α-synuclein can impair chaperone-mediated autophagy[35]. In some models, mutant polyglutamine induces an autophagic response, although apparently an incomplete one, as further stimulation of autophagy can protect against huntingtin toxicity in cells and in mice.

A more general proposal for the relationship between aggregation and toxicity is that toxicity depends on interactions with, and possibly recruitment of, other cellular constituents during the aggregation process. Evidence supporting this possibility has come mostly from studies of polyglutamine[77–81]. Exposure of hydrogens of main-chain carbons and nitrogens might facilitate abnormal hydrogen bond interactions with other proteins in the cell, perhaps in abnormal β-conformations[7]. Another possibility is that exposure of hydrophobic side chains could lead to abnormal interactions with other cellular components — perhaps with cellular membranes through the formation of membrane pores[63,82]. Therefore, perhaps no single species is toxic *per se*, but instead the active process of aggregation, and interactions with other proteins in the cell, causes toxicity.

Conclusions

Converging evidence indicates that inclusion body formation might be a protective response of the cell against misfolded proteins. However, we suggest that the aggregation process itself is likely to be related to toxicity, and that there may be common mechanisms of toxicity among the aggregation diseases. An important question is whether the toxic species is the misfolded monomer or a form of soluble aggregate such as oligomer or protofibril. Another related question is whether soluble oligomeric species are on the pathway to fibre formation or are a side reaction. It will be crucial to gain a better understanding of the exact molecular composition, reactivity and molecular structure of these oligomers, as well as the abnormally folded monomers. Because the hypothetical pathways for fibril formation are based on data obtained *in vitro*, a challenging but important task for future studies will be to

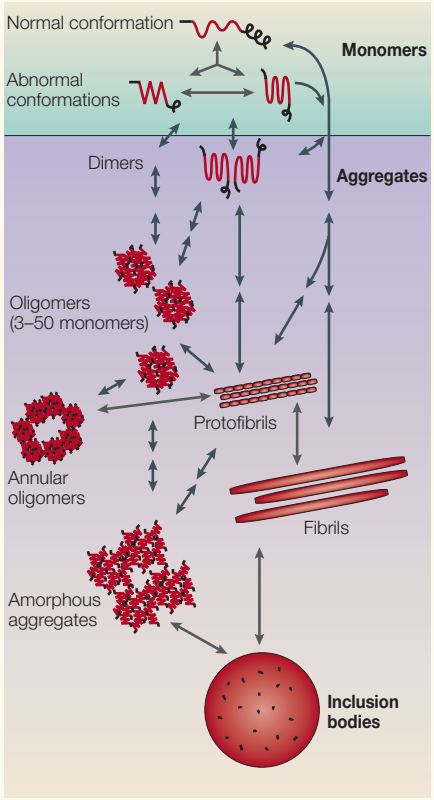

Figure 3 | **Potential pathways for formation of inclusion bodies and other protein aggregates.** Conformational change of the monomer, perhaps with several possible abnormal conformations, initiates the aggregation process. Aggregation (see BOX 2 for definitions) begins as soon as there is an association of two or more abnormal proteins or parts of proteins. Amyloid fibrils might be formed by the linear addition of monomers to the growing fibre (arrows on right), or through intermediate oligomeric assemblies, or species called protofibrils, either of which might be 'off' the pathway to fibril formation. Oligomers or protofibrils might be capable of forming annular rings. Furthermore, amorphous aggregates, which do not contain fibrils, can also be formed, possibly through the oligomeric or protofibrillar intermediates as shown, or through precipitation of monomers (not shown). If an aggregate becomes large enough, it can be visualized by light microscopy, and large well-demarcated aggregates in cells are often termed inclusion bodies. It is currently proposed that the early species in the aggregation process are more toxic than inclusion bodies or large aggregates. Further research is needed to clarify these pathways, determine similarities and differences between the pathways for different diseases and determine the points where inhibition might be beneficial or detrimental for the disease process. Molecular components are not drawn to scale.

dissect these pathways *in vivo*. Ordering the pathway will help to elucidate pathogenesis and to guide therapeutics, as it is possible that inhibiting the process at the wrong step could increase the amount of a toxic intermediate.

Finally, it will be crucial to increase our understanding of the initiating events[4] that lead to formation of the abnormal protein conformation. Some of these initiating events, including phosphorylation or oxidative modification, proteolytic cleavage or other events, could be favourable therapeutic targets.

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Competing interests statement

The authors declare no competing financial interests.

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