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REVIEW ARTICLE

Crucial role of protein oligomerization in the pathogenesis of Alzheimer's and Parkinson's diseases

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Misfolding and aggregation of the proteins amyloid-β, tau and alpha-synuclein is the predominant pathology underlying the neurodegenerative disorders, Alzheimer's and Parkinson's disease. While end stage insoluble products of aggregation have been well characterised in human and animal models of disease, accumulating evidence from biophysical, cellular and *in vivo* studies has shown that soluble intermediates of aggregation, or oligomers, may be the key species that mediate toxicity and underlie seeding and spreading in disease. Here, we review the process of protein misfolding, and the intrinsic and extrinsic processes that cause the native states of the key aggregating proteins to undergo conformational change to form oligomers and ultimately fibrils. We discuss the structural features of the key toxic intermediate, and describe the putative mechanisms by which oligomers may cause cell toxicity. Finally, we explore the potential therapeutic approaches raised by the oligomer hypothesis in neurodegenerative disease.

Introduction

Protein misfolding and aggregation are molecular processes known to occur in all neurodegenerative disorders, including the commonest diseases, Alzheimer's disease (AD) and Parkinson's disease (PD). During misfolding, intrinsically disordered monomeric proteins (such as Aβ and tau in AD, and α-syn in PD), undergo conformational changes from their native states into highly ordered β-sheet rich fibrillar amyloid structures [1]. The detection of these amyloid structures in post-mortem brain tissue has allowed accurate post-mortem diagnosis of subtypes of neurodegenerative diseases. As the presence of intracellular and extracellular fibril deposition is seen predominantly in disease, it is this insoluble end-stage species of protein aggregation that has traditionally defined disease. However, soluble transient intermediate species called oligomers are generated during this process of aggregation. There is accumulating evidence that the soluble oligomeric forms of such proteins may be the key toxic species involved in disease, whilst the role of the end-stage fibrillar aggregate, or inclusion body, is less clear. Post-mortem sporadic PD brain exhibits an increase in oligomeric α-syn [2]. Direct application of *In vitro* formed α-syn oligomers, or overexpression of α-syn mutants induces a range of

Abbreviations
α-syn, alpha-synuclein; AD, Alzheimer's disease; ApoE, apolipoprotein E; ApoJ, apolipoprotein J; APP, amyloid precursor protein; Aβ, amyloid-β peptide; CSF, cerebrospinal fluid; FRET, foster resonance energy transfer; GBA, glucocerebrosidase; hFPD, human prefoldin; IDP, intrinsically disordered protein; IL-1β, interleukin 1β; MAPK, mitogen-activated protein kinase; mPTP, mitochondrial permeability transition pore; NAC, non-amyloid-component; NFT, neurofibrillary tangle; NFTs, neurofibrillary tangle; NMDA-R, *N*-methyl-D-aspartate receptor; NSAIDS, non-steroidal anti-inflammatory drugs; PD, Parkinson's disease; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD2, manganese superoxide dismutase; TFEB, transcription factor EB; Tg, transgenic; TIRF, total internal reflection fluorescence; TLR, toll-like receptor.

cellular damage affecting membrane integrity, mitochondria, ER, autophagy, and synaptic transmission, and ultimately leading to cell toxicity [3–5]. *In vivo* studies, expression of α-syn mutants that promote oligomer formation (but inhibit fibril formation) results in dopaminergic neuronal death, [3,6] while conversely α-syn mutants that form fibrils did not lead to dopaminergic neuronal loss. Comparable with the literature on PD, an increase in soluble oligomeric forms of both Aβ and tau, key aggregating proteins, has also been reported in the brain of AD patients, and there is a correlation between the soluble Aβ and tau concentrations and cognitive decline in both AD patients and animal models [7]. In addition, functional studies have shown that toxicity can be induced by accumulation of soluble oligomers of either Aβ or tau rather than the monomers or fibril [8–11].

Taken together, multiple lines of evidence now suggest that oligomeric species of aggregating proteins are key pathological modulators involved in disease initiation and progression. In this review, we discuss major questions to understand the role of the oligomer in neurodegenerative diseases;

1 What are the native states and conformational evolution as proteins misfold from monomers into variable aggregating species (e.g. soluble β-rich oligomers and amyloid fibrils)?
2 What are the environmental triggers (intrinsic or extrinsic) of conformational changes from monomeric to oligomeric species?
3 What are the possible mechanisms underlying the toxicity by oligomers of aggregating proteins in neurodegenerative diseases?
4 What are the potential therapeutic strategies to target oligomeric species?

Box 1
Native structure of key protein responsible for aggregation in PD and AD

α-syn: A small acidic protein of 14.5 kDa and 140 amino acids. There are three distinct domains consisting of N-terminal lipid-binding α-helix, amyloid-binding central domain (non-amyloid-component, NAC) and C-terminal acidic tail. (a) The amino-terminal sequence (residues 1–60) that contains highly conserved hexamer motif is the site of mutations A53T, A30P and E46K found in familial PD cases. (b) The central region (residues 61–95) is termed the NAC and this region can undergo conformational changes from a random coil to β -sheet structure to form amyloid β-like fibrils. (c) The carboxy-terminal of α-syn (residue 96–120), acidic rich residues, is responsible for regulating aggregation and residue serine 129 is one of the well-known phosphorylation sites. Dopamine also interacts with those residues for α-syn [119,120].

Aβ: The peptides are produced by a series of enzymatic cleavages of the amyloid precursor protein. Most Aβ peptides in human brain tissue are either 40 or 42 residues in length [121].

Tau: The longest tau isoform is 441 amino acid residues. N1 and N2 are the polypeptide sequences, P1 and P2 are proline-rich regions and R1-R4 are the microtubule-binding domains. In monomeric tau, β structure sequences are present in R2 and R3 regions allowing self-assembly into filaments [122].

Native states and the role of oligomeric species in neurodegenerative diseases

Natively, the proteins of the major components in neurodegenerative diseases such as α-syn, Aβ and tau are classified as intrinsically disordered proteins (IDP) that lack a fixed or stable three-dimensional structure. Instead, they retain conformational freedom in association with other molecules, and can adopt a range of different structures, a property that is key to their wide biological functions (Box 1). Consistent with the structure of IDPs, α-syn consists of a positively charged N-terminal repeat region that allows an interaction between α-syn and lipids (in which α-syn adopts an α-helical structure); a hydrophobic central region that allows aggregation into β-sheet structure (the NAC, or 'non-Aβ-component'), and a negatively charged acidic C-terminal region that does not associate with membranes. In cells, α-syn exists as primarily monomeric with high conformational flexibility, and adopts either an unfolded state in the cytosol, or an α-helical structure when membrane bound [12]. Some studies have suggested that α-syn may exist as a folded tetramer, but the true native conformer state(s) remains unresolved [13,14]. While the exact physiological function of the native conformations of α-syn are not known, α-syn has been reported to play a major role in SNARE complex assembly in neurotransmitter release, synaptic vesicle recycling, dopamine metabolism, and regulation of mitochondrial ATP synthase efficiency [15].

During pathology, protein misfolding and self-assembly leads to the native monomeric state

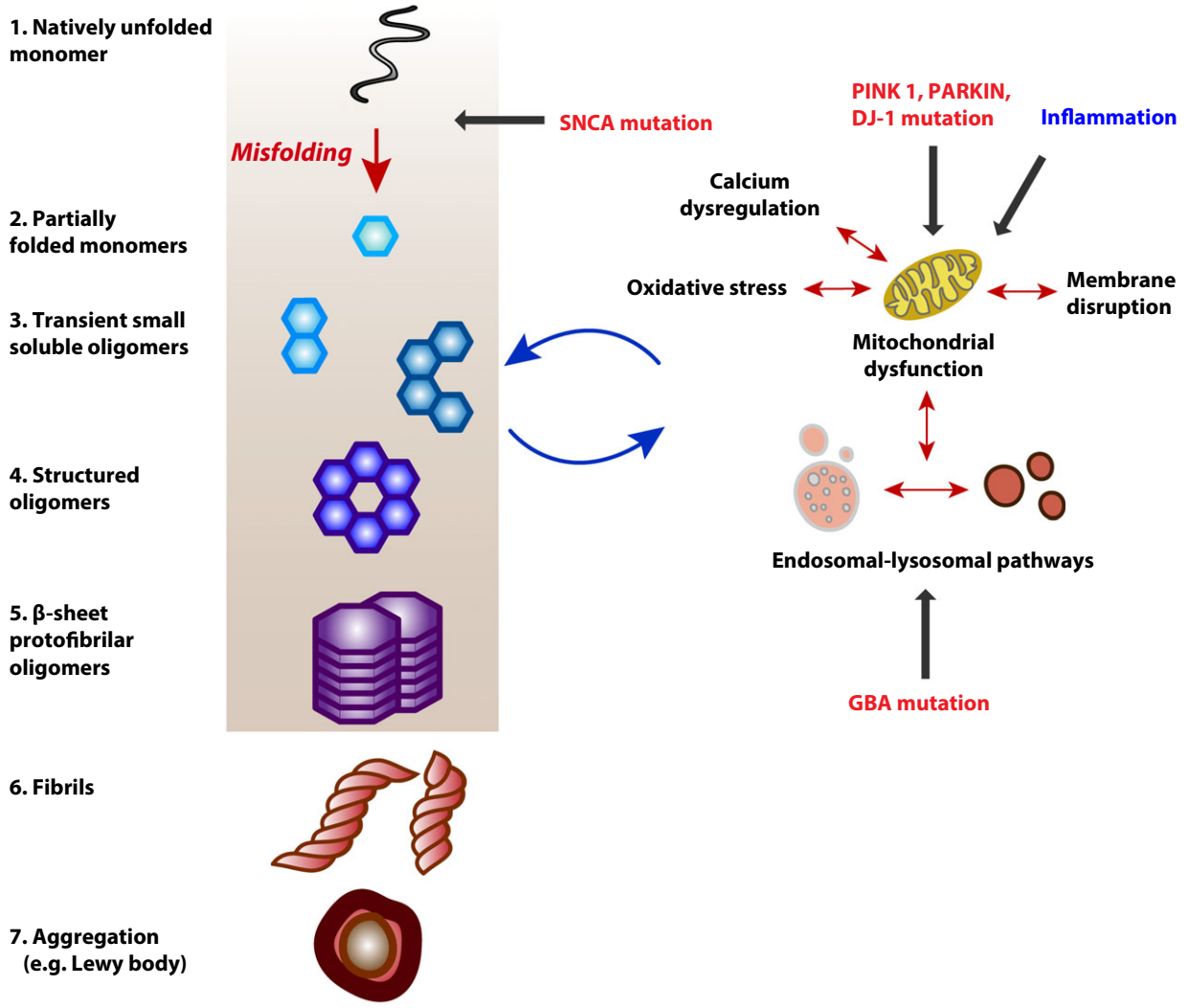

Fig. 1. Structural evolution during aggregation. Intrinsically disordered proteins such as α-syn, Aβ, phosphorylated tau that mostly exist as (1) unfolded monomers can be transformed into (2) partially folded conformation by pathological stimuli. Internal stimuli such as genetic mutations in SNCA, or environmental stress such as inflammation can trigger misfolding. The partially folded monomers can initiate aggregation and transform into (3) transient small soluble oligomers in the earlier stages and (4) later β -sheet structures. In turn, accumulation of oligomeric species facilitates the pathological cascade. The β -sheet structure protofibrilar oligomers (5) can transform intracellularly into (6) fibrils and finally accumulate as (7) fibrillary aggregates.

undergoing a structural change to first form small soluble amorphous oligomers, then later oligomers with high β-sheet content, protofibrils, and finally insoluble amyloid fibrils (Fig. 1). Transformation from an unfolded protein to a partially folded protein, that can undergo self assembly, may occur in conditions such as lowered pH, or increased temperature that alter the hydrophobicity and net charge resulting in the acquisition of ordered structure [16]. Oligomerisation is simply promoted by agitation but high initial concentrations, temperature, lipids, the presence of fibrillar seeds or iron accelerate the oligomerisation process [17–19]. While 'oligomer' is a widely used term, it is defined as an assembly of misfolded proteins that maintain their solubility in a range of sizes from dimers to protofibrils. For example, it is reported that the dimeric form of α-syn is unstable and transient with discrete structural conformations [20], but there is little study of the trimeric form of α-syn. A metastable a-helical tetramer is generally believed to be a non-toxic species [21]. Although a heterogeneous range of size and structures are generated during the oligomerisation process, the pathological properties of the 'toxic oligomer' are proposed to be β-sheet rich, and have

high numbers of exposed hydrophobic residues, which allows the species to integrate into membranes and induce calcium fluxes through channel or pore formation [22].

Once misfolding occurs, aggregates may be generated in cells by the initial formation of small soluble oligomers from monomers, followed by sequential monomeric addition eventually forming mature fibrils, a process termed primary nucleation. Alternatively, disassembly or fragmentation of fibrils can generate aggregate intermediates. Finally, seeding or templating in which monomers are added onto a seed, or template, of cross β-sheet structure can also lead to the generation of oligomeric intermediates [23]. Importantly, α-syn fibrils propagate to neighbouring neurons, where the seeding/templating property of the cross β-sheet structure of fibrils, may underlie disease spread and progression [24]. Perhaps both the complexity of the different aggregation processes (primary nucleation, secondary nucleation or fragmentation), coupled with the diversity of experimental conditions used to aggregate proteins, has led to a large range of aggregation intermediates reported in the literature, and used in different experimental paradigms. It is increasingly clear that these different structures, or strains, of proteins may possess different properties in relation to inducing toxicity, or seeding and spread [25,26].

Intrinsic and extrinsic triggers for oligomerisation

Key aggregating proteins are known to misfold and oligomerise following exposure to various extrinsic (e.g. oxidative stress, dopamine) or intrinsic challenges (e.g. high concentration, overexpression, presence of mutants) [27]. As transition from monomers to oligomers is a crucial step to generating the neurotoxic intermediate species that then drives pathogenesis, an understanding of why oligomerisation occurs in pathological states is important. Although it remains unclear exactly how the transition is triggered, it has been suggested that a critical concentration of the monomers of the aggregating proteins is required. Genetic evidence demonstrates that patients with duplication and triplication of the SNCA gene exhibit elevated levels of α-syn protein, associated with widespread early onset aggregation and neuronal death [28,29]. Furthermore, variants in SNCA are a common genetic risk factor for developing sporadic PD, and risk variants in the SNCA genomic region and intergenic sequences are reported to act by regulating expression of α-syn, which may drive aggregation [30]. Interestingly normal ageing is also associated with increased levels of monomers [31].

Extrinsic factors, or biological processes, that accelerate or trigger oligomerisation interestingly overlap with the mechanisms underlying the oligomer-induced toxicity, highlighting the bi-directional effects of protein aggregation and oxidative stress, protein aggregation and inflammation, and protein aggregation and impaired clearance. Therefore, once both processes have been triggered, they act synergistically such that the cellular stress increases the accumulation of protein aggregates, which in turn exacerbates the cellular stress. Here, we address two potential triggers for intracellular oligomerisation, the redox balance of the cell, and post-translational modifications of the aggregating proteins themselves.

Under healthy conditions, the generation of reactive oxygen species (ROS) is balanced by the antioxidant system while in neurodegenerative states, an imbalance allows excess accumulation of ROS that can accelerate oligomerisation [32]. Interestingly, it is reported that ROS induced by hydrogen peroxide accelerates the formation of dimers and soluble β-sheet oligomers, and thus promotes α-syn aggregation under oxidative stress [33–35]. Oxidised unfolded monomers of α-syn accelerate fibril formation and oxidised α-syn is also toxic to dopaminergic neurons [36,37]. In addition, treatment of α-syn with oxidising agents is reported to accelerate crosslinking of tyrosines, which results in triggering oligomerisation of the protein [20,38]. Oxidation of Met35 promotes the formation of Aβ protofibrils from monomers and a study has reported that the Met35-oxidised Aβ interacts with lipid membranes which can disrupt ion-channel functions [39–41]. With consistency, oxidative stress has also been reported to lead to an increase in tau phosphorylation which facilitates aggregation [42].

Phosphorylation is the most widely studied post-translational modification that is known to promote the propensity of aggregating proteins to form oligomers. In PD, Lewy bodies found in post-mortem brain with PD contain phosphorylated α-syn at residue S129 [43], and the soluble non-fibrillar fractions from the brain with PD were also found to be phosphorylated at S129 [44]. Tau has been long reported to be a phosphoprotein and is known to be mostly phosphorylated at the site of Ser-Pro and Thr-Pro motifs. Phosphorylation negatively regulates microtubule assembly [45]. Tau oligomers are known to exist in a variety of states which are either hyper-phosphorylated or unphosphorylated forms. Under physiological conditions, phosphorylation is crucially required for tau to regulate its binding to microtubules which determines their

stability in neurons, while hyper-phosphorylated tau results in abnormal protein aggregates in brain [46]. For Aβ, serine residues 8, 26 and tyrosine residue 10 have been proposed for potential phosphorylation sites but the most studied is the serine 8 site, and the phospho-
serine-8-specific antibodies have shown the presence of phosphorylated Aβ in both patients with AD and animal models [47]. Aβ phosphorylated at serine 8 has shown to be localised to amyloid plaques under pathological conditions and *in vitro* studies have shown that phosphorylation enhances the formation of oligomeric species [48].

Mechanisms of toxicity induced by oligomers

Since the earliest reports of a potential role of oligomers in disease, there has been an increase in studies that compare the different species of aggregating proteins *in vitro* and *in vivo*, in an attempt to elucidate the specific 'toxic' species and its potential mechanism of action. It is worth highlighting that different studies employ different methods to generate oligomeric intermediates, and different methods to characterise them, and it remains a challenge to compare and validate findings across studies. Broadly however, soluble oligomeric species show high surface to volume ratio and hydrophobic like properties [49] and are prone to bind to membranes which leads to pore formation and membrane permeability [3]. As well as exhibiting structural differences to the monomeric state, oligomers may initiate aberrant cellular processes through specific and non-specific interactions with receptors, mitochondria, synaptic vesicles, and membranes leading to aberrant signalling or cellular dysfunction. In the next section, we will discuss major mechanisms underlying the oligomer-induced toxicity reported to date.

Mitochondrial dysfunction

Mitochondrial dysfunction is a key feature in the pathogenesis of Parkinson's disease, and has been described in other neurodegenerative diseases. Mitochondria play a major role in neurons in the generation of ROS and redox signalling, cellular respiration and ATP production, calcium buffering, and cell death [50]. We, and others, have shown that α-syn oligomers induce mitochondrial depolarisation and impair respiration through an interaction with complex I [51,52]. Oligomeric α-syn also promotes calcium-induced mitochondrial depolarisation and swelling, and cytochrome c release, demonstrating α-syn-induced mitochondrial dysfunction is dependent upon both complex I as well as mitochondrial uptake of exogenous calcium [53]. α-syn oligomers have been demonstrated to interact with the outer mitochondrial membrane Tom20 and impair mitochondrial protein import. Early opening of mitochondrial permeability transition pore (mPTP) is considered to be one of the key modulators involved in programmed cell death which occurs during neuronal cell loss through neurodegeneration [54,55]. We recently demonstrated that α-syn oligomers localise to the inner mitochondrial membrane and come into close proximity to the ATP synthase, and are able to directly induce classical PTP opening in isolated brain mitochondria, and in intact mitochondria in human neurons [56].

Similarly, application of oligomeric species of tau is also reported to cause mitochondrial dysfunction, such as a decrease in complex I activity [57,58]. Lasagna *et al*. [58], showed decreased complex I level in tau oligomer injected hemisphere compared to the side with either with injected fibrils or monomers. In line with the study, it has been reported that there is a reduction in levels of the 24- and 75-kDa subunits of complex I and modifications of mitochondrial encoded complex I subunit mRNA in response to tau accumulation but not formation of neurofibrillary tangles (NFT) [57,59]. Tau oligomers co-localise with porin, a mitochondrial protein, and suggest that tau oligomers could disrupt microtubule stability and trafficking [58].

Membrane disruption and calcium dysregulation

The protein α-syn binds to synthetic and biological lipids, and its interaction with membrane surfaces can initiate and accelerate its aggregation. This process may be dependent on the high local concentration of α-syn at the membrane, exposure of hydrophobic residues leading to self-assembly, or the physiochemical properties of the surrounding lipids [60,61]. Furthermore, once oligomers are formed, they are able to disrupt membranes and this is due to their structural composition, with a high lipophilic component promoting membrane interaction, and the cross β-sheet structure leading to integration into the lipid bilayer [62]. The consequence of such membrane disruption is the ability to induce ion fluxes across the membrane, although whether this mechanism is through pore formation [26] or membrane destabilising is not clear. Calcium is the most pleiotropic ion that is capable of triggering intracellular pathways in response to external stimuli [5,63,64]. In the brain, calcium dysregulation due to aberrant calcium signalling or disrupted calcium homeostasis has been reported in

neurodegenerative diseases [64]. We have reported that α-syn oligomers induce cytosolic calcium influx in neurons, and this results in an increase in cytosolic calcium before inducing cell death; cell toxicity is rescued by exclusion of extracellular calcium [5], confirming the importance of oligomer-induced membrane disruption in inducing neuronal toxicity. In other *in vitro* studies, oligomeric α-syn was able to induce calcium flux across both artificial membrane and neuronal membrane, using a pore-forming mechanism [26]. The significance of oligomers generated during the disease course (rather than synthetic oligomers) generating calcium influx has been investigated by Drews *et al*. [65], showing that Aβ oligomers from human cerebrospinal fluid (CSF) can permeabilise membranes and induce calcium influx in both control and patients with AD. However, Aβ oligomer is also reported to induce calcium influx into neuronal cells and interestingly even low picomolar concentration of the oligomers comparable to the concentration of species detected in CSF, induces calcium influx [65]. The same group has investigated how Aβ oligomers initiate the pathway of damage, using picomolar concentrations of both Aβ (1–40) and Aβ (1–42) [66]. Notably, α-syn oligomers and Aβ oligomers have also been reported to trigger calcium deregulation through receptor -mediated mechanisms, involving an interaction between the oligomer complex and PrP^c [67,68]. Therefore, oligomer-induced calcium signalling may be a common phenomenon in neurodegeneration, and calcium-mediated toxicity a unifying event by oligomers in all neurodegenerative diseases.

Calcium dysregulation is not only a primary mechanism mediating the oligomeric toxicity, but also a key stimulator to initiate or accelerate oligomerisation. For instance, high levels of intracellular calcium induced by thapsigargin depletes endoplasmic reticulum calcium stores, promotes oligomerisation of α-syn, which in turn exacerbates calcium dysregulation [69].

Endosomal–lysosomal pathways

Failure of the endosomal–lysosomal pathways that remove aggregated protein is another mechanism that underlies oligomer-mediated toxicity. The presence of oligomeric species of amyloidogenic proteins leads to incomplete clearance, which promotes further aggregation. It was reported by Lee and colleagues that α-syn oligomers are cleared through the lysosome and that inhibition of lysosomal activity accelerates its aggregation and toxicity [70]. Elevated activity of autophagy conversely reduces the accumulation of oligomeric α-syn in neuronal cell lines [71]. An *In vivo* study has shown that the overexpression of α-syn induces the accumulation of aggregates, and overexpression of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, was able to reverse the lysosomal defects and accumulation of aggregates [72]. Interestingly, mutations in the GBA gene are an important genetic risk factor for PD. The glucocerebrosidase (GBA) gene encodes a lysosomal enzyme that has shown to be linked to α-syn oligomerisation [73]. Among patients carrying GBA mutation, brain samples of insoluble fractions from patients with parkinsonism contained oligomeric α-syn, but only monomeric forms were detected in the non-parkinsonism group [74]. A decrease in lysosomal glucocerebrosidase activity has also been suggested to promote the production of stable α-syn oligomers [75].

Structurally oligomers are naturally resistant to cytosolic proteases but interestingly there is a report that granulovacuolar degeneration found in early tau aggregation is derived from the endosomal–lysosomal system, and these deposits are the most likely substrate for initial seeding or nucleation of tau aggregation [76]. Another hypothesis is that nucleation of tau generates oligomeric tau aggregates by capturing normal tau (or mutant tau) in the process. Tau oligomers can only be cleared via the endosomal–lysosomal processing pathway and these oligomers contribute to further congestion and dysfunction in lysosomal processing. In addition, tau aggregation propagates itself by autocatalytic binding of tau and ultimate formation of tau fibrils. In the case of amyloid precursor protein and presenilin mutations, it has been reported that a congested endosomal–lysosomal pathway delays the critical time point to remove proteins, in particular membrane bound proteins from mitochondria, and this can accelerate aggregation and neuronal dysfunction in AD [77].

Oxidative stress

Oxidative stress is caused by the imbalance between ROS such as O_2^-, H_2O_2 and O, and antioxidants such as glutathione or enzymes such as manganese superoxide dismutase (SOD2) [78,79]. Overproduction of ROS has been well established in neurodegenerative diseases [80]. In terms of oligomeric-mediated toxicity, there has been accumulating data showing oligomeric species of aggregating proteins produce high levels of ROS [65,81]. Acute application of recombinant β-sheet-rich oligomers dramatically induced higher production of ROS in neuron and astrocytes co-cultures, while monomers, unstructured oligomers, and insoluble fibrils did not [4,23,82,83]. We have demonstrated that

the oligomer-induced ROS is dependent on metal ions, and that deferoxamine (an iron chelator), penicillamine (a copper chelator), and clioquinol (a highly lipophilic copper and zinc chelator) applied directly to the oligomers before application to cells, reduced the ability of the α-syn oligomers to induce ROS both rat primary and iPSC-derived cortical neurons [83] The same 'toxic' α-syn oligomer is also able to induce an increase in lipid peroxidation, and both oligomer-induced lipid peroxidation and oligomer-induced cell death, could be prevented by pre-incubation of cells with isotope-reinforced polyunsaturated fatty acids (D-PUFAs) that prevent oxidation of lipids [84].

It seems also evident that ROS-mediated injury is a key pathological mechanism in AD. For instance, AD brains display an increase in levels of malondialdehyde and 4-hydroxynonenal, and lipid peroxidation markers. De Felice *et al*. [85], has demonstrated the mechanism underlying oligomer-induced ROS. According to this study, Aβ oligomer stimulates excessive formation of ROS through a mechanism dependent on *N*-methyl-D-aspartate receptor (NMDA-R) activation. Interestingly, in the AD brain Aβ-mediated mitochondrial oxidative stress causes hyperphosphorylation of tau, which may trigger the cascade pathways leading to neuronal dysfunction and eventually cell death [86].

Inflammation

Inflammation is considered a critical component in neurodegenerative disease pathogenesis, since microglia can be activated in response to misfolded proteins. Therefore, one hypothesis is that release of protein aggregates from neurons activates microglia which consequently initiates an inflammation response [87]. Activation of microglia results in elevation of the level of cytokines, chemokines, ROS and interlukine that are crucially involved in neuronal death [82]. It has been demonstrated that α-syn aggregates released from neurons are taken up by astroglial cells where they trigger an inflammatory response [70]. Furthermore, specific structures of α-syn oligomers released by neurons can activate inflammatory responses in microglia and astrocytes through the Toll-like receptor (TLR) pathways [88–90]. Similar to α-syn pathology, in the case of tau, there are reports showing that formation of neurofibrillary tangle (NFTs) is caused by local microglial cell-driven neuroinflammation even without activation of peripheral immune activation. Recent studies have shown that bacterial LPS-induced systemic inflammation increased tau pathology through cyclin-dependent kinase 5 activation [91]. Microglia-induced tau phosphorylation may be initiated by IL-1β receptor activation and p38 mitogen-activated protein kinase (MAPK)-mediated signal transduction, as it has been shown that aged mice acutely expressing IL-1β display increased tau pathology [92]. In addition, a study with tau transgenic (Tg) mouse model showed that early immunosuppression helped delay the progression of tau pathology [93].

Therapeutic approaches

Based on the evidence reviewed here, it is clear that intracellular oligomerisation generates aggregation intermediates that are responsible for driving toxicity, and that furthermore spread to the extracellular space and seed pathology in neighbouring cells in neurodegenerative diseases [94,95]. Therefore, the main strategies of the therapeutic approaches have focused on either targeting or inhibiting the aggregation process *per se*, or modulating the environmental stresses that accelerate/exacerbate aggregation.

Antibody therapeutics; extracellular clearance by immunisation

There has been a number of trials based on antibodies targeting the aggregating protein. It should be recognised that targeting antibodies to the CNS need to address the challenge of crossing the blood–brain barrier which results in limited accessibility of most macromolecules to the brain. Antibodies engineered to incorporate sequences to allow permeability of blood–brain barrier naturally, or through using active transport can be employed. Recently, developments in single chain antibodies, such as camelid raised antibodies called nanobodies, allow increased stability and solubility as well as easy production, and may cross the blood–brain barrier [96]. In fact, immunotherapy has been tried in AD and PD, however, there is no data to show a significant improvement in clinical symptoms or mortality. For example, clinical trials such as Bapineuzumab and solanezumab did not show any beneficial effect in patients with both moderate and mild forms of AD. There is a number of possible explanations for these failures, including robust antibody fragment binding and the selection of the protein species to target. Bapineuzumab is a murine monoclonal antibody targeting the N-terminal region of Aβ and has been shown to bind to amyloid plaques. Solanequmab binds to the mid-domain of the Aβ peptide recognising soluble monomers but not oligomers, and failure in these trials may indicate the importance of careful targeting of the soluble oligomeric species. Nonetheless, it will be technically challenging to develop an antibody that can bind

selectively and stabilise and remove the oligomeric species. Additionally, immunisation approaches need to target early disease, at the point at which monomers are forming the toxic oligomeric species, and not the later fibrils. Therefore, another explanation given for the failure of the clinical trials has been the time point selected to treat is too late, while recognising that it is difficult to accurately diagnose patients in the prodromal or preclinical phases of disease. In order to capture early disease time points, such trials of antibodies need to be combined with more advanced biomarker or diagnostic tools to detect early, or prodromal, disease. Finally, a direct measurement of oligomer levels would be important to demonstrate the change in oligomer load by the intervention used.

Chaperone therapy (increasing degradation through chaperones, lysosomal up-regulators)

A molecular chaperone is a protein that selectively recognises and binds to exposed hydrophobic surfaces of non-native proteins to mediate the folding of protein [97]. It is also defined as a class of proteins that interact with proteins to stabilize their native structures and subsequently involve them in the pathways of protein degradation, which are capable of removing misfolding proteins such as the ubiquitin–proteasome system for degradation of α-syn misfolding [98,99]. Based on the initial studies proving that chaperones protect neurons against protein aggregation-induced toxicity in AD and PD, there is accumulating evidence of their specific interaction with oligomeric species to interfere with the process of aggregation. For instance, α-syn oligomer formation is significantly reduced in Heat shock protein 70 (Hsp70) chaperone-stimulated conditions. Complexes of α-syn are reported to be neutralised by Hsp90 chaperone in an ATP-dependent manner [100,101]. In addition to their role in counterbalancing aggregation, binding of the molecular chaperone to oligomer can directly inhibit the oligomer-induced cell toxicity. More specifically molecular chaperones can convert the small size oligomers into larger nontoxic aggregates with a decrease in the surface-to-volume ratio which can then enable autophagy to perform its clearance [102]. For example, the Hsp27 chaperone has been shown to increase the size of preformed Aβ43 oligomers so that they are unable to exert their toxicity in mouse neuroblastoma cell culture [103]. Biochemical studies have also shown stabilisation of oligomeric Aβ 42 in the presence of the human prefoldin (hFPD) chaperone [104].

Extracellular chaperones support the specific internalisation of aggregating proteins, particularly in AD. Apolipoprotein J (ApoJ, commonly known as clusterin) and E (ApoE) are two major apolipoproteins in the brain that facilitate the *in vivo* clearance of misfolded proteins [105]. Clusterin, in complex with Aβ, interacts with the cell surface receptor megalin on mouse teratocarcinoma F9 cells and promotes internalisation and the subsequent degradation of Aβ [106]. Similarly, ApoE and Aβ complexes are internalised in smooth muscle primary cultures by endocytosis-mediated by the lipoprotein receptor [107]. However, Aβ also complexes with α2M, another extracellular chaperone also known to be internalised by lipoprotein receptor-related protein (LRP)-mediated endocytosis to degrade Aβ aggregation [108]. *In vivo* studies further support the clearance of Aβ by these extracellular chaperones [109–111]. Taken together, it is suggested that extracellular chaperones and endocytosis mechanisms are important for the control of Aβ turnover, which may be a key therapeutic target.

Antioxidant therapy

Although it still remains unclear whether ROS is a primary driver of pathogenesis or whether it is a secondary consequence of disease, it appears evident that overproduction of ROS is a key pathological mechanisms contributing to neuronal cell death in both AD and PD. Antioxidants may be exogenous or endogenous compounds, and both of them can work by neutralising free radicals through a number of different mechanisms, including scavenging free radicals, reducing metal ions, and preventing lipids from being oxidised, and increasing electron transfer in the respiratory chain of mitochondria [112]. However, despite significant benefits reported in animal studies, there has been little clinical success using antioxidants in both AD and PD. Several reasons may underlie the poor translation of such therapies to clinical benefit. For example, as with the immunotherapy studies, it may be that oxidative stress is an early phenomenon intricately linked to protein aggregation and therefore the trials in late stage disease may be ineffective. The compounds themselves may have limited bioavailability at the correct intracellular target. Finally, scavenging ROS may not be as effective as targeted inhibition of the production of ROS from its source.

Concluding remarks

We provide a range of evidence to support the hypothesis that oligomeric species are crucial in mediating cell toxicity in neurodegenerative diseases. The discovery

of the role of the oligomer has raised with it a number of challenges: the 'toxic' species is likely to be a rare event, among a pool of monomeric protein and aggregation intermediates of differing structures and sizes. Furthermore, it is heterogeneous and transient, and these properties make it difficult to understand the key disease-causing species either in nature or human diseases. Such challenges have complicated the oligomer field, and led to many different biochemical and optical methods used to generate intermediates, and to characterise them. Therefore, it is difficult to standardise the 'oligomer' used in experimental studies, and even more challenging relating the synthetic oligomers to the endogenous intermediates generated during disease. However, the recognition of the importance of the oligomer has spurred progress in methods to enable the detection of rare protein species to address this challenge. Single molecule-based fluorescent approaches are now able to visualise and quantify individual aggregates using single molecule confocal microscopy for labelled aggregates (which relies on the Foster resonance energy transfer (FRET) between two different colour labelled monomers in close proximity during the formation of oligomers [4,19,113], or sm Thioflavin T-based total internal reflection fluorescence (TIRF) microscopy for unlabelled aggregates [114,115]. Proximity ligation assay (PLA) is another method to detect oligomers both *in vitro* and *in vivo* based on closely located monomers during the formation of oligomers. Roberts *et al*. has provided the first visual evidence of elevated α-syn oligomers in PD by generating PLA probes from antibodies raised against α-syn. Commercially available oligomer detection ELISA kits for α-syn, tau and Aβ which utilise conformational or antibodies have been used particularly for biomarker studies in biological fluids such as CSF and blood [116,117]. Lastly, immunohistochemistry with antibodies specifically raised against oligomeric forms has been reported, although such antibodies may also detect other aggregating proteins rather than one specific oligomer [7,118]. It is hoped that such advances, once applied to relevant disease cell models and tissue, will be able to address the outstanding questions in the field.

These include:

1 What is the critical monomeric concentration and required to form intracellular oligomers?
2 Where is the exact location that monomers form oligomers within cells, and when and why does this occur in disease?
3 Which of the oligomeric states are causally involved in neurodegeneration?
4 How do we apply technical advances and detection tools to visualise the oligomeric process in cells and tissue?

Ultimately, we hope to further our understanding of how, where, when and why the oligomerisation process takes place in cells to generate toxic intermediate species. We hope to discover structure–function relationship and structure-toxicity mechanisms within human neurons. Understanding these relationships will allow the field to fully exploit the oligomer hypothesis for improved diagnostics, as a biomarker for disease, and as potential disease targets for therapy.

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Author contributions

M-LC and SG conceived of the ideas and wrote the manuscript.

References

1 Soto C (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat Rev Neurosci* **4**, 49–60.
2 Roberts RF, Wade-Martins R & Alegre-Abarrategui J (2015) Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. *Brain* **138** (Pt 6), 1642–1657.
3 Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S *et al*. (2011) In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci USA* **108**, 4194–4199.
4 Cremades N, Cohen SI, Deas E, Abramov AY, Chen AY, Orte A, Sandal M, Clarke RW, Dunne P, Aprile FA *et al*. (2012) Direct observation of the interconversion of normal and toxic forms of alpha-synuclein. *Cell* **149**, 1048–1059.
5 Angelova PR, Ludtmann MH, Horrocks MH, Negoda A, Cremades N, Klenerman D, Dobson CM, Wood NW, Pavlov EV, Gandhi S *et al*. (2016) Ca2+ is a key factor in alpha-synuclein-induced neurotoxicity. *J Cell Sci* **129**, 1792–1801.
6 Chung CY, Koprich JB, Siddiqi H & Isacson O (2009) Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. *J Neurosci* **29**, 3365–3373.
7 Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW & Glabe CG (2003) Common

structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486–489.
8 Gómez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE & Hyman BT (1997) Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol* **41**, 17–24.
9 Hardy J & Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356.
10 Sakono M, Zako T, Ueda H, Yohda M & Maeda M (2008) Formation of highly toxic soluble amyloid beta oligomers by the molecular chaperone prefoldin. *FEBS J* **275**, 5982–5993.
11 Barry AE, Klyubin I, Mc Donald JM, Mably AJ, Farrell MA, Scott M, Walsh DM & Rowan MJ (2011) Alzheimer's disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. *J Neurosci* **31**, 7259–7263.
12 Burre J, Sharma M & Sudhof TC (2014) Alpha-synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. *Proc Natl Acad Sci USA* **111**, E4274–E4283.
13 Bartels T, Choi JG & Selkoe DJ (2011) Alpha-synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature* **477**, 107–110.
14 Wang W, Perovic I, Chittuluru J, Kaganovich A, Nguyen LT, Liao J, Auclair JR, Johnson D, Landeru A, Simorellis AK *et al.* (2011) A soluble alpha-synuclein construct forms a dynamic tetramer. *Proc Natl Acad Sci USA* **108**, 17797–17802.
15 Ludtmann MH, Angelova PR, Ninkina NN, Gandhi S, Buchman VL & Abramov AY (2016) Monomeric alpha-synuclein exerts a physiological role on brain ATP synthase. *J Neurosci* **36**, 10510–10521.
16 Uversky VN, Li J & Fink AL (2001) Evidence for a partially folded intermediate in alpha-synuclein fibril formation. *J Biol Chem* **276**, 10737–10744.
17 Kostka M, Högen T, Danzer KM, Levin J, Habeck M, Wirth A, Wagner R, Glabe CG, Finger S, Heinzelmann U *et al.* (2008) Single particle characterization of iron-induced pore-forming alpha-synuclein oligomers. *J Biol Chem* **283**, 10992–11003.
18 Iljina M, Garcia GA, Horrocks MH, Tosatto L, Choi ML, Ganzinger KA, Abramov AY, Gandhi S, Wood NW, Cremades N *et al.* (2016) Kinetic model of the aggregation of alpha-synuclein provides insights into prion-like spreading. *Proc Natl Acad Sci USA* **113**, E1206–E1215.
19 Horrocks MH, Lee SF, Gandhi S, Magdalinou NK, Chen SW, Devine MJ, Tosatto L, Kjaergaard M, Beckwith JS, Zetterberg H *et al.* (2016) Single-molecule imaging of individual amyloid protein aggregates in human biofluids. *ACS Chem Neurosci* **7**, 399–406.
20 Souza JM, Giasson BI, Chen Q, Lee VM & Ischiropoulos H (2000) Dityrosine cross-linking promotes formation of stable alpha -synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J Biol Chem* **275**, 18344–18349.
21 Kara E, Lewis PA, Ling H, Proukakis C, Houlden H & Hardy J (2013) Alpha-synuclein mutations cluster around a putative protein loop. *Neurosci Lett* **546**, 67–70.
22 Campioni S, Mannini B, Zampagni M, Pensalfini A, Parrini C, Evangelisti E, Relini A, Stefani M, Dobson CM, Cecchi C *et al.* (2010) A causative link between the structure of aberrant protein oligomers and their toxicity. *Nat Chem Biol* **6**, 140–147.
23 Cremades N & Dobson CM (2018) The contribution of biophysical and structural studies of protein self-assembly to the design of therapeutic strategies for amyloid diseases. *Neurobiol Dis* **109**(Pt B), 178–190.
24 Volpicelli-Daley LA, Luk KC, Patel TP, Tanik SA, Riddle DM, Stieber A, Meaney DF, Trojanowski JQ & Lee VM (2011) Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron* **72**, 57–71.
25 Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, Van den Haute C, Melki R & Baekelandt V (2015) Alpha-synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* **522**, 340–344.
26 Danzer KM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, Kretzschmar H, Hengerer B & Kostka M (2007) Different species of alpha-synuclein oligomers induce calcium influx and seeding. *J Neurosci* **27**, 9220–9232.
27 Feng LR, Federoff HJ, Vicini S & Maguire-Zeiss KA (2010) Alpha-synuclein mediates alterations in membrane conductance: a potential role for alpha-synuclein oligomers in cell vulnerability. *Eur J Neurosci* **32**, 10–17.
28 Farrer M, Kachergus J, Forno L, Lincoln S, Wang DS, Hulihan M, Maraganore D, Gwinn-Hardy K, Wszolek Z, Dickson D *et al.* (2004) Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. *Ann Neurol* **55**, 174–179.
29 Byers B, Cord B, Nguyen HN, Schüle B, Fenno L, Lee PC, Deisseroth K, Langston JW, Pera RR & Palmer TD (2011) SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate alpha-synuclein and are susceptible to oxidative stress. *PLoS One* **6**, e26159.
30 Tagliafierro L & Chiba-Falek O (2016) Up-regulation of SNCA gene expression: implications to synucleinopathies. *Neurogenetics* **17**, 145–157.
31 Petersen K, Olesen OF & Mikkelsen JD (1999) Developmental expression of alpha-synuclein in rat

hippocampus and cerebral cortex. *Neuroscience* **91**, 651–659.
32 Garcia-Garcia A, Zavala-Flores L, Rodriguez-Rocha H & Franco R (2012) Thiol-redox signaling, dopaminergic cell death, and Parkinson's disease. *Antioxid Redox Signal* **17**, 1764–1784.
33 Hashimoto M, Hsu LJ, Xia Y, Takeda A, Sisk A, Sundsmo M & Masliah E (1999) Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein in vitro. *NeuroReport* **10**, 717–721.
34 Batelli S, Albani D, Rametta R, Polito L, Prato F, Pesaresi M, Negro A & Forloni G (2008) DJ-1 modulates alpha-synuclein aggregation state in a cellular model of oxidative stress: relevance for Parkinson's disease and involvement of HSP70. *PLoS One* **3**, e1884.
35 Deb I, Poddar R & Paul S (2011) Oxidative stress-induced oligomerization inhibits the activity of the non-receptor tyrosine phosphatase STEP61. *J Neurochem* **116**, 1097–1111.
36 Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM & Ischiropoulos H (2004) Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. *J Biol Chem* **279**, 47746–47753.
37 Yu Z, Xu X, Xiang Z, Zhou J, Zhang Z, Hu C & He C (2010) Nitrated alpha-synuclein induces the loss of dopaminergic neurons in the substantia nigra of rats. *PLoS One* **5**, e9956.
38 Norris EH, Giasson BI, Ischiropoulos H & Lee VM (2003) Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involve distinct mechanisms of protein modifications. *J Biol Chem* **278**, 27230–27240.
39 Palmblad M, Westlind-Danielsson A & Bergquist J (2002) Oxidation of methionine 35 attenuates formation of amyloid beta -peptide 1-40 oligomers. *J Biol Chem* **277**, 19506–19510.
40 Hou L, Kang I, Marchant RE & Zagorski MG (2002) Methionine 35 oxidation reduces fibril assembly of the amyloid abeta-(1-42) peptide of Alzheimer's disease. *J Biol Chem* **277**, 40173–40176.
41 Barnham KJ, Ciccotosto GD, Tickler AK, Ali FE, Smith DG, Williamson NA, Lam YH, Carrington D, Tew D, Kocak G *et al*. (2003) Neurotoxic, redox-competent Alzheimer's beta-amyloid is released from lipid membrane by methionine oxidation. *J Biol Chem* **278**, 42959–42965.
42 Pérez M, Cuadros R, Smith MA, Perry G & Avila J (2000) Phosphorylated, but not native, tau protein assembles following reaction with the lipid peroxidation product, 4-hydroxy-2-nonenal. *FEBS Lett* **486**, 270–274.
43 Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K & Iwatsubo T (2002) Alpha-synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* **4**, 160–164.
44 Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, Barbour R, Huang J, Kling K, Lee M *et al*. (2006) Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J Biol Chem* **281**, 29739–29752.
45 Jameson L, Frey T, Zeeberg B, Dalldorf F & Caplow M (1980) Inhibition of microtubule assembly by phosphorylation of microtubule-associated proteins. *Biochemistry* **19**, 2472–2479.
46 Šimić G, Babić Leko M, Wray S, Harrington C, Delalle I, Jovanov-Milošević N, Bažadona D, Buée L, de Silva R, Di Giovanni G *et al*. (2016) Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. *Biomolecules* **6**, 6.
47 Kumar S, Wirths O, Theil S, Gerth J, Bayer TA & Walter J (2013) Early intraneuronal accumulation and increased aggregation of phosphorylated Abeta in a mouse model of Alzheimer's disease. *Acta Neuropathol* **125**, 699–709.
48 Kumar S, Rezaei-Ghaleh N, Terwel D, Thal DR, Richard M, Hoch M, Mc Donald JM, Wüllner U, Glebov K, Heneka MT *et al*. (2011) Extracellular phosphorylation of the amyloid beta-peptide promotes formation of toxic aggregates during the pathogenesis of Alzheimer's disease. *EMBO J* **30**, 2255–2265.
49 Bucciantini M, Calloni G, Chiti F, Formigli L, Nosi D, Dobson CM & Stefani M (2004) Prefibrillar amyloid protein aggregates share common features of cytotoxicity. *J Biol Chem* **279**, 31374–31382.
50 Abramov AY, Berezhnov AV, Fedotova EI, Zinchenko VP & Dolgacheva LP (2017) Interaction of misfolded proteins and mitochondria in neurodegenerative disorders. *Biochem Soc Trans* **45**, 1025–1033.
51 Reeve AK, Ludtmann MH, Angelova PR, Simcox EM, Horrocks MH, Klenerman D, Gandhi S, Turnbull DM & Abramov AY (2015) Aggregated alpha-synuclein and complex I deficiency: exploration of their relationship in differentiated neurons. *Cell Death Dis* **6**, e1820.
52 Devi L, Raghavendran V, Prabhu BM, Avadhani NG & Anandatheerthavarada HK (2008) Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J Biol Chem* **283**, 9089–9100.
53 Luth ES, Stavrovskaya IG, Bartels T, Kristal BS & Selkoe DJ (2014) Soluble, prefibrillar alpha-synuclein oligomers promote complex I-dependent, Ca2+-induced mitochondrial dysfunction. *J Biol Chem* **289**, 21490–21507.

54 Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, Downward J, Latchman DS, Tabrizi SJ, Wood NW *et al.* (2009) PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell* **33**, 627–638.
55 Di Maio R, Barrett PJ, Hoffman EK, Barrett CW, Zharikov A, Borah A, Hu X, McCoy J, Chu CT, Burton EA *et al.* (2016) Alpha-synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. *Sci Transl Med* **8**, 342ra78.
56 Ludtmann MHR, Angelova PR, Horrocks MH, Choi ML, Rodrigues M, Baev AY, Berezhnov AV, Yao Z, Little D, Banushi B *et al.* (2018) α-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. *Nat Commun* **9**, 2293.
57 Kim SH, Vlkolinsky R, Cairns N, Fountoulakis M & Lubec G (2001) The reduction of NADH ubiquinone oxidoreductase 24- and 75-kDa subunits in brains of patients with Down syndrome and Alzheimer's disease. *Life Sci* **68**, 2741–2750.
58 Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR & Kayed R (2011) Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol Neurodegener* **6**, 39.
59 Sims NR, Finegan JM, Blass JP, Bowen DM & Neary D (1987) Mitochondrial function in brain tissue in primary degenerative dementia. *Brain Res* **436**, 30–38.
60 Galvagnion C, Buell AK, Meisl G, Michaels TC, Vendruscolo M, Knowles TP & Dobson CM (2015) Lipid vesicles trigger alpha-synuclein aggregation by stimulating primary nucleation. *Nat Chem Biol* **11**, 229–234.
61 Galvagnion C, Brown JW, Ouberai MM, Flagmeier P, Vendruscolo M, Buell AK, Sparr E & Dobson CM (2016) Chemical properties of lipids strongly affect the kinetics of the membrane-induced aggregation of alpha-synuclein. *Proc Natl Acad Sci USA* **113**, 7065–7070.
62 Fusco G, Chen SW, Williamson PTF, Cascella R, Perni M, Jarvis JA, Cecchi C, Vendruscolo M, Chiti F, Cremades N *et al.* (2017) Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers. *Science* **358**, 1440–1443.
63 Mosharov EV, Larsen KE, Kanter E, Phillips KA, Wilson K, Schmitz Y, Krantz DE, Kobayashi K, Edwards RH & Sulzer D (2009) Interplay between cytosolic dopamine, calcium, and alpha-synuclein causes selective death of substantia nigra neurons. *Neuron* **62**, 218–229.
64 Ludtmann MHR & Abramov AY (2018) Mitochondrial calcium imbalance in Parkinson's disease. *Neurosci Lett* **663**, 86–90.
65 Drews A, Flint J, Shivji N, Jönsson P, Wirthensohn D, De Genst E, Vincke C, Muyldermans S, Dobson C & Klenerman D (2016) Individual aggregates of amyloid beta induce temporary calcium influx through the cell membrane of neuronal cells. *Sci Rep* **6**, 31910.
66 Drews A, De S, Flagmeier P, Wirthensohn DC, Chen WH, Whiten DR, Rodrigues M, Vincke C, Muyldermans S, Paterson RW *et al.* (2017) Inhibiting the Ca(2+) Influx Induced by human CSF. *Cell Rep* **21**, 3310–3316.
67 Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, Kerrisk ME, Vortmeyer A, Wisniewski T, Koleske AJ *et al.* (2013) Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer abeta oligomer bound to cellular prion protein. *Neuron* **79**, 887–902.
68 Ferreira DG, Temido-Ferreira M, Miranda HV, Batalha VL, Coelho JE, Szegö ÉM, Marques-Morgado I, Vaz SH, Rhee JS, Schmitz M *et al.* (2017) alpha-synuclein interacts with PrP(C) to induce cognitive impairment through mGluR5 and NMDAR2B. *Nat Neurosci* **20**, 1569–1579.
69 Nath S, Goodwin J, Engelborghs Y & Pountney DL (2011) Raised calcium promotes alpha-synuclein aggregate formation. *Mol Cell Neurosci* **46**, 516–526.
70 Lee HJ, Khoshaghideh F, Patel S & Lee SJ (2004) Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. *J Neurosci* **24**, 1888–1896.
71 Lu JH, Tan JQ, Durairajan SS, Liu LF, Zhang ZH, Ma L, Shen HM, Chan HY & Li M (2012) Isorhynchophylline, a natural alkaloid, promotes the degradation of alpha-synuclein in neuronal cells via inducing autophagy. *Autophagy* **8**, 98–108.
72 Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J & Björklund A (2013) TFEB-mediated autophagy rescues midbrain dopamine neurons from alpha-synuclein toxicity. *Proc Natl Acad Sci USA* **110**, E1817–E1826.
73 McNeill A, Magalhaes J, Shen C, Chau KY, Hughes D, Mehta A, Foltynie T, Cooper JM, Abramov AY, Gegg M *et al.* (2014) Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain* **137** (Pt 5), 1481–1495.
74 Choi JH, Stubblefield B, Cookson MR, Goldin E, Velayati A, Tayebi N & Sidransky E (2011) Aggregation of alpha-synuclein in brain samples from subjects with glucocerebrosidase mutations. *Mol Genet Metab* **104**, 185–188.
75 Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA & Krainc D (2011) Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* **146**, 37–52.

76 Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM & Mandelkow E (2009) Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. *Hum Mol Genet* **18**, 4153–4170.
77 Wischik CM, Harrington CR & Storey JM (2014) Tau-aggregation inhibitor therapy for Alzheimer's disease. *Biochem Pharmacol* **88**, 529–539.
78 Holley AK, Bakthavatchalu V, Velez-Roman JM & St Clair DK (2011) Manganese superoxide dismutase: guardian of the powerhouse. *Int J Mol Sci* **12**, 7114–7162.
79 Flynn JM & Melov S (2013) SOD2 in mitochondrial dysfunction and neurodegeneration. *Free Radic Biol Med* **62**, 4–12.
80 Gandhi S & Abramov AY (2012) Mechanism of oxidative stress in neurodegeneration. *Oxid Med Cell Longev* **2012**, 428010.
81 Iljina M, Tosatto L, Choi ML, Sang JC, Ye Y, Hughes CD, Bryant CE, Gandhi S & Klenerman D (2016) Arachidonic acid mediates the formation of abundant alpha-helical multimers of alpha-synuclein. *Sci Rep* **6**, 33928.
82 Chen WW, Zhang X & Huang WJ (2016) Role of neuroinflammation in neurodegenerative diseases (Review). *Mol Med Rep* **13**, 3391–3396.
83 Deas E, Cremades N, Angelova PR, Ludtmann MH, Yao Z, Chen S, Horrocks MH, Banushi B, Little D, Devine MJ *et al.* (2016) Alpha-synuclein oligomers interact with metal ions to induce oxidative stress and neuronal death in Parkinson's disease. *Antioxid Redox Signal* **24**, 376–391.
84 Angelova PR, Horrocks MH, Klenerman D, Gandhi S, Abramov AY & Shchepinov MS (2015) Lipid peroxidation is essential for alpha-synuclein-induced cell death. *J Neurochem* **133**, 582–589.
85 De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST & Klein WL (2007) Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem* **282**, 11590–11601.
86 Liu Z, Li T, Li P, Wei N, Zhao Z, Liang H, Ji X, Chen W, Xue M & Wei J (2015) The ambiguous relationship of oxidative stress, tau hyperphosphorylation, and autophagy dysfunction in Alzheimer's disease. *Oxid Med Cell Longev* **2015**, 352723.
87 Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS *et al.* (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J* **19**, 533–542.
88 Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, Joong Lee S, Masliah E, Hwang D, Lee HJ *et al.* (2013) Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. *Nat Commun* **4**, 1562.
89 Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, Wenning GK & Stefanova N (2013) Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. *Glia* **61**, 349–360.
90 Rannikko EH, Weber SS & Kahle PJ (2015) Exogenous alpha-synuclein induces toll-like receptor 4 dependent inflammatory responses in astrocytes. *BMC Neurosci* **16**, 57.
91 Kitazawa M, Oddo S, Yamasaki TR, Green KN & LaFerla FM (2005) Lipopolysaccharide-induced inflammation exacerbates tau pathology by a cyclin-dependent kinase 5-mediated pathway in a transgenic model of Alzheimer's disease. *J Neurosci* **25**, 8843–8853.
92 Bhaskar K, Konerth M, Kokiko-Cochran ON, Cardona A, Ransohoff RM & Lamb BT (2010) Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* **68**, 19–31.
93 Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ & Lee VM (2007) Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* **53**, 337–351.
94 Danzer KM, Kranich LR, Ruf WP, Cagsal-Getkin O, Winslow AR, Zhu L, Vanderburg CR & McLean PJ (2012) Exosomal cell-to-cell transmission of alpha synuclein oligomers. *Mol Neurodegener* **7**, 42.
95 Guo JL & Lee VM (2014) Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat Med* **20**, 130–138.
96 Iljina M, Hong L, Horrocks MH, Ludtmann MH, Choi ML, Hughes CD, Ruggeri FS, Guilliams T, Buell AK, Lee JE *et al.* (2017) Nanobodies raised against monomeric a-synuclein inhibit fibril formation and destabilize toxic oligomeric species. *BMC Biol* **15**, 57.
97 Finka A & Goloubinoff P (2013) Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. *Cell Stress Chaperones* **18**, 591–605.
98 Hartl FU, Bracher A & Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* **475**, 324–332.
99 Ebrahimi-Fakhari D, Wahlster L & McLean PJ (2012) Protein degradation pathways in Parkinson's disease: curse or blessing. *Acta Neuropathol* **124**, 153–172.
100 Danzer KM, Ruf WP, Putcha P, Joyner D, Hashimoto T, Glabe C, Hyman BT & McLean PJ (2011) Heat-shock protein 70 modulates toxic extracellular alpha-synuclein oligomers and rescues trans-synaptic toxicity. *FASEB J* **25**, 326–336.

101 Daturpalli S, Waudby CA, Meehan S & Jackson SE (2013) Hsp90 inhibits alpha-synuclein aggregation by interacting with soluble oligomers. *J Mol Biol* **425**, 4614–4628.
102 Bolognesi B, Kumita JR, Barros TP, Esbjorner EK, Luheshi LM, Crowther DC, Wilson MR, Dobson CM, Favrin G & Yerbury JJ (2010) ANS binding reveals common features of cytotoxic amyloid species. *ACS Chem Biol* **5**, 735–740.
103 Ojha J, Masilamoni G, Dunlap D, Udoff RA & Cashikar AG (2011) Sequestration of toxic oligomers by HspB1 as a cytoprotective mechanism. *Mol Cell Biol* **31**, 3146–3157.
104 Sörgjerd KM, Zako T, Sakono M, Stirling PC, Leroux MR, Saito T, Nilsson P, Sekimoto M, Saido TC & Maeda M (2013) Human prefoldin inhibits amyloid-beta (Abeta) fibrillation and contributes to formation of nontoxic Abeta aggregates. *Biochemistry* **52**, 3532–3542.
105 Wyatt AR, Yerbury JJ, Berghofer P, Greguric I, Katsifis A, Dobson CM & Wilson MR (2011) Clusterin facilitates in vivo clearance of extracellular misfolded proteins. *Cell Mol Life Sci* **68**, 3919–3931.
106 Hammad SM, Ranganathan S, Loukinova E, Twal WO & Argraves WS (1997) Interaction of apolipoprotein J-amyloid beta-peptide complex with low density lipoprotein receptor-related protein-2/ megalin. A mechanism to prevent pathological accumulation of amyloid beta-peptide. *J Biol Chem* **272**, 18644–18649.
107 Urmoneit B, Prikulis I, Wihl G, D'Urso D, Frank R, Heeren J, Beisiegel U & Prior R (1997) Cerebrovascular smooth muscle cells internalize Alzheimer amyloid beta protein via a lipoprotein pathway: implications for cerebral amyloid angiopathy. *Lab Invest* **77**, 157–166.
108 Narita M, Holtzman DM, Schwartz AL & Bu G (1997) Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. *J Neurochem* **69**, 1904–1911.
109 Bell RD, Sagare AP, Friedman AE, Bedi GS, Holtzman DM, Deane R & Zlokovic BV (2007) Transport pathways for clearance of human Alzheimer's amyloid beta-peptide and apolipoproteins E and J in the mouse central nervous system. *J Cereb Blood Flow Metab* **27**, 909–918.
110 Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, Fagan AM, Morris JC, Mawuenyega KG, Cruchaga C *et al.* (2011) Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. *Sci Transl Med* **3**, 89ra57.
111 Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J *et al.* (2000) Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest* **106**, 1489–1499.
112 Richard D, Kefi K, Barbe U, Bausero P & Visioli F (2008) Polyunsaturated fatty acids as antioxidants. *Pharmacol Res* **57**, 451–455.
113 Tosatto L, Horrocks MH, Dear AJ, Knowles TP, Dalla Serra M, Cremades N, Dobson CM & Klenerman D (2015) Single-molecule FRET studies on alpha-synuclein oligomerization of Parkinson's disease genetically related mutants. *Sci Rep* **5**, 16696.
114 Ban T, Hamada D, Hasegawa K, Naiki H & Goto Y (2003) Direct observation of amyloid fibril growth monitored by thioflavin T fluorescence. *J Biol Chem* **278**, 16462–16465.
115 Ban T, Hoshino M, Takahashi S, Hamada D, Hasegawa K, Naiki H & Goto Y (2004) Direct observation of Abeta amyloid fibril growth and inhibition. *J Mol Biol* **344**, 757–767.
116 Majbour NK, Vaikath NN, van Dijk KD, Ardah MT, Varghese S, Vesterager LB, Montezinho LP, Poole S, Safieh-Garabedian B, Tokuda T *et al.* (2016) Oligomeric and phosphorylated alpha-synuclein as potential CSF biomarkers for Parkinson's disease. *Mol Neurodegener* **11**, 7.
117 Sengupta U, Portelius E, Hansson O, Farmer K, Castillo-Carranza D, Woltjer R, Zetterberg H, Galasko D, Blennow K & Kayed R (2017) Tau oligomers in cerebrospinal fluid in Alzheimer's disease. *Ann Clin Transl Neurol* **4**, 226–235.
118 Zhang X, Sun XX, Xue D, Liu DG, Hu XY, Zhao M, Yang SG, Yang Y, Xia YJ, Wang Y *et al.* (2011) Conformation-dependent scFv antibodies specifically recognize the oligomers assembled from various amyloids and show colocalization of amyloid fibrils with oligomers in patients with amyloidoses. *Biochim Biophys Acta* **1814**, 1703–1712.
119 Lashuel HA, Overk CR, Oueslati A & Masliah E (2013) The many faces of alpha-synuclein: from structure and toxicity to therapeutic target. *Nat Rev Neurosci* **14**, 38–48.
120 Emamzadeh FN (2016) Alpha-synuclein structure, functions, and interactions. *J Res Med Sci* **21**, 29.
121 Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K & Xu HE (2017) Amyloid beta: structure, biology and structure-based therapeutic development. *Acta Pharmacol Sin* **38**, 1205–1235.
122 Kolarova M, García-Sierra F, Bartos A, Ricny J & Ripova D (2012) Structure and pathology of tau protein in Alzheimer disease. *Int J Alzheimers Dis* **2012**, 731526.