Trends in Neurosciences

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Review

How do protein aggregates escape quality control in neurodegeneration?

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Protein aggregates are hallmarks of neurodegenerative diseases. The protein quality control (PQC) system normally prevents proteins from misfolding and accumulation; however, proteins somehow escape this control on disease. Here we review advances in the role of PQC in protein aggregation and neurodegeneration. We focus primarily on the protein Tau, which aggregates in Alzheimer's disease (AD) and other tauopathies. We also examine recent advances in amyloid fibril structures and the process of fibril formation via phase separation, which are shedding new light on the role of PQC in protein aggregation diseases. While specific components of the quality control system appear to be altered in disease, most chaperones and degradation factors are unchanged at the cellular end stage. Advancing the understanding of quality control factors in neurodegeneration, particularly in the early stages of disease, is among the key challenges for neurodegeneration research.

Highlights

Amyloid fibrils in neurodegeneration often escape the protein quality control (PQC) system.

The PQC system maintains protein homeostasis and can decide between the refolding and degradation of misfolded substrates.

Cryo-EM analyses of recombinant and ex-patient amyloid fibrils show dramatic diversity in architecture, which may require a revision of paradigms on the quality control of protein fibrils.

Phase-separation interactions are gaining growing interest as a potentially important step in fibril formation. These interactions propose a mechanism for cellular derailment via π–π interactions and the rewiring of protein interactomes.

Progress in understanding similarities and heterogeneity across neurodegenerative diseases, and how this affects the role of PQC in neurodegeneration, may open new possibilities for therapeutic interventions.

Protein aggregation as a key player in neurodegeneration

One of the hallmarks of neurodegenerative diseases is the accumulation of aberrantly folded proteins, which leads to toxicity and neuronal death [1–4]. **PQC** (see Glossary) is, therefore, crucial to maintain neuronal health. Most proteins rely on their native state for their function and the structure of this native state is determined by the amino acid sequence (Figure 1, arrow 1) [5,6]. Hydrophobic amino acids will be buried inside the hydrophobic core of the protein, with the polar – hydrophilic – residues facing the aqueous cellular environment. Besides the pathway leading to the native state, proteins can funnel into off-pathway intermediates with low free energy, which may result in the formation of protein aggregates (Figure 1, arrow 5).

Intriguingly, one specific subset of proteins – intrinsically disordered proteins (IDPs) – does not have a defined 3D structure (Figure 1, arrow 2). These proteins are active while being unfolded and fluctuate over a wide range of conformations, which enables their involvement in a multitude of regulatory and signalling processes (Figure 1, arrow 3) [7]. These fluctuations, however, make them also susceptible to environmental changes, and as their **hydrophobic stretches** are not buried inside a protein core, IDPs are more prone to adopt aberrant conformations and aggregate in **amyloid fibrils** (Figure 1, arrow 4). IDPs have a reduced content of hydrophobic residues, which makes it impossible for them to adopt a globular, folded structure [8]. A fibril structure allows the bringing together of the few hydrophobic side chains in a 2D layer. Stacking these layers on top of each other allows interaction of the hydrophobic side chains with identical layers below or above in the fibril structure. This explains the apparent paradox that otherwise soluble IDPs are frequently found in protein fibrils.

Amyloid fibrils are characteristic of several neurodegenerative diseases, including AD, Parkinson's disease (PD), and Huntington's disease (HD). Interestingly, the proteins aggregating in these diseases are often IDPs, like Tau, α-synuclein, and Huntingtin (Htt) [9]. The PQC system

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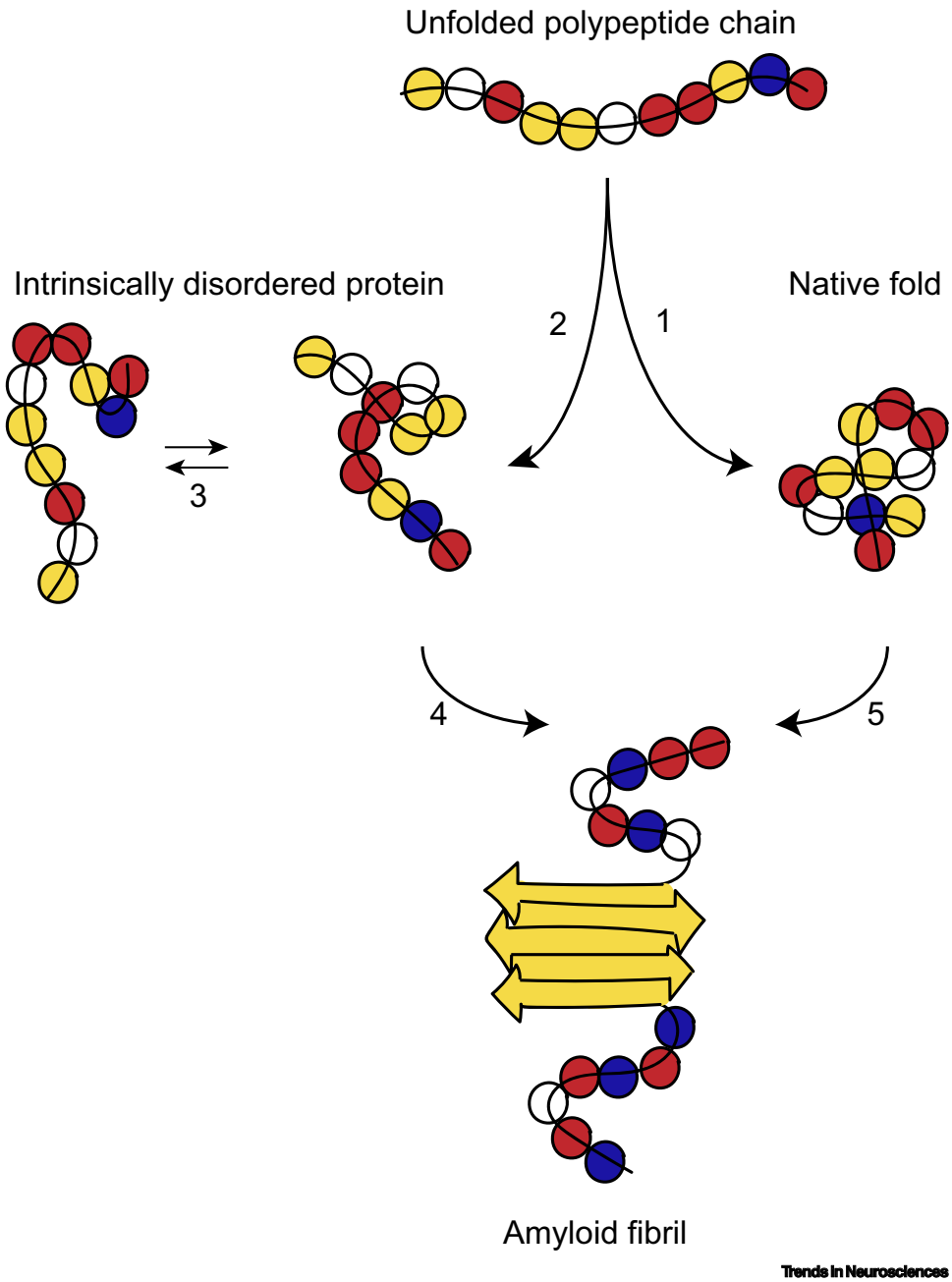

Figure 1. Schematic illustration of protein-folding pathways. Top: An unfolded polypeptide chain produced from the ribosomes and awaiting a specific fate. The sequence of amino acids determines the outcome of the folding process. Proteins that rely on their native state for their function are guided onto the pathway leading to their native state (1). Some proteins do not have a defined 3D structure (2) but rather fluctuate between different conformations depending on their function (3). Upon external influence or stresses, proteins can adopt an aberrant conformation, which may lead to the formation of amyloid fibrils (4,5).

appears to fail in these diseases, thereby allowing the proteins to aggregate. Here, we elucidate the role of the PQC system in fibril formation and its possible failure in neurodegenerative diseases. We touch on the different mechanisms of fibril formation and the heterogeneity of amyloid

Glossary

π–π interactions: type of noncovalent interaction involving the unsaturated π-system, such as aromatic rings or amino acids with side chains with double bonds like arginines and glutamines. These interactions play an important role in LLPS.

Amyloid fibrils: type of protein aggregates that are defined by a higher second-order structure and are resistant to degradation. They are characteristic of neurodegenerative diseases and can be toxic for cells.

Chaperone-assisted autophagy (CASA): a specific autophagy pathway loaded by concerted action of the heat shock inducible HSP70 system in cooperation with the small heat shock protein HSPB8 and the NEF BAG3.

Disaggregation: the process of disassembly of protein aggregates, which is facilitated by molecular chaperones. Amyloid fibrils can be disaggregated via the mechanism of entropic pulling, where a local high concentration of the chaperones on the fibrils creates a force that will break the fibrils apart.

Hydrophobic stretches: hydrophobic amino acids or hydrophobic stretches have side chains of amino acids that are repelled by water. For this reason, hydrophobic amino acids or stretches are often buried inside the protein core or shielded from the aqueous cellular environment by chaperones.

Liquid–liquid phase separation (LLPS): the formation of membraneless organelles that reversibly form at high local concentration. Macromolecules, including a subset of proteins, condense together in a droplet-like way driven by a specific grammar of a large number of low-affinity interactions. IDPs are particularly prone to LLPS.

Molecular chaperones: proteins that can assist other proteins in folding, refolding, and degradation. Molecular chaperones are crucial for cellular health and are involved in a plethora of cellular processes.

Post-translational modifications (PTMs): once a protein is produced (translated), modifications can be added to the protein that can influence its function, called PTMs. Common PTMs are, for instance, phosphorylation, ubiquitylation, and methylation.

Protein quality control (PQC) system: network in the cell to maintain protein homeostasis. It describes the

fibrils, stressing the importance of a versatile and adequately responding quality control system to maintain cellular health. We use the Tau protein as the main paradigm, due to its heterogeneity in fibril formation and involvement in several neurodegenerative diseases, collectively referred to as tauopathies.

system that ensures proper protein folding, refolding, and degradation to maintain the functioning of the proteins and the degradation of aberrant substrates.

Amyloid fibrils characterize neurodegenerative diseases

As a first example of the role of PQC in neurodegenerative diseases, we focus on AD, starting with an overview of protein aggregation phenomena in this disorder. Among the hallmarks of AD is the intracellular aggregation of the microtubule-associated protein Tau (MAPT). Under normal conditions, Tau has a cellular function in stabilizing microtubules. In disease, hyperphosphorylation of Tau releases the protein from the microtubules, which makes the Tau protein susceptible to aggregation into so-called neurofibrillary tangles (NFTs) [10]. In AD, intracellular NFT formation is typically preceded by extracellular plaques containing aggregated Amyloid-β [10,11]. Spreading of Amyloid-β starts in the posterior cingulate, the precuneus, the middle temporal lobe, and the lateral orbitofrontal cortex [11]. In intermediate stages, Amyloid-β spreads to isocortical areas except the primary motor and primary sensory areas. In the last stage of Amyloid-β spreading, the protein can also accumulate in the primary motor and primary sensory areas [12]. The process of Tau spreading starts in brain regions affecting memory, like the entorhinal cortex and the hippocampus, but it gradually spreads through the brain to also affect other regions, like the primary motor and sensory cortex [12–15].

For all neurodegenerative diseases, aggregating proteins are linked to toxicity and eventually neuronal degeneration. A shared trait of neurodegenerative disorders is the age-related onset [16]. In some cases, the age-related onset can be linked to genetic causes. In HD, for instance, a disease that has a defined genetic cause, the age of onset is strongly influenced by the length of the CAG repeats in the Htt-gene [17,18]. In AD and PD, a fraction of disease cases are familiar forms of the disease, which can be linked to specific genetic risk factors, but most patients suffer sporadic forms of the disease and have no defined genetic cause [19,20]. The age-related onset even in the absence of apparent genetic causes suggests that alterations in cellular processes that arise with age, including possibly a decrease in the efficiency of the PQC system, may be key players in the etiology of neurodegenerative diseases (Box 1).

Among the components of the PQC system that could be susceptible to age-related decline are **molecular chaperones**. We next ask, is there a decline in the activity or levels of molecular chaperones in neurodegeneration?

Molecular chaperone control protein fidelity

Molecular chaperones are proteins involved in many cellular processes and essential for cellular health. Chaperones assist in protein folding and are key players in deciding between folding, refolding, or degradation. A fraction of proteins folds spontaneously and independent of chaperones, reaching the native state autonomously [5,21]. More complex proteins, however, typically rely on molecular chaperones, which guide them ‘on pathway’ to reach their native state. Hsp70 and Hsp90 are the two major chaperone systems in the protein-folding pathway [22]. Both chaperones are ATP dependent and regulated by co-chaperones to cater for a great diversity of substrate proteins [23]. Co-chaperones such as ATPase-stimulating J-domain proteins (JDPs) and nucleotide exchange factors (NEFs) allow Hsp70 to undergo the full cycle of binding, hydrolysis, and release and are indispensable for proper Hsp70 functioning.

Hsp70 binds to early folding intermediates by scavenging short hydrophobic stretches. Of note, Hsp70 does not act as an active folding accelerator, but rather blocks further folding of the

Box 1. The PQC network

The cellular PQC network comprises numerous components to maintain protein homeostasis and ensure the integrity of the proteome and cellular health. The tasks of the PQC network can be roughly divided into three major categories: protein folding, refolding, and degradation.

To ensure proper protein folding, the PQC machinery relies on the action of molecular chaperones. Hsp70 and Hsp90 are two major chaperone families important in guiding proteins on their folding pathways (Figure I) (extensively reviewed in [22]). Besides guiding proteins towards the folding pathway, molecular chaperones are also involved in the handling of misfolded proteins. Misfolded proteins can either be refolded or degraded, and molecular chaperones play an important role in this. The cellular fate of misfolded proteins depends on the specific combination of chaperones, co-chaperones, and other factors.

On proteotoxic stress (e.g., temperature increase, the presence of reactive oxygen species) PQC reacts mainly at the level of transcription, by activating so-called HSFs like HSF-1. These transcription factors can activate downstream pathways leading to upregulation of molecular chaperones or activation of degradation pathways, to buffer the proteotoxic stress.

Neurodegenerative diseases are characterized by an imbalance of protein homeostasis, possibly by a compromised PQC machinery. Recent analysis of the protein levels of Alzheimer's patients revealed a decrease of the Hsp90 paralogs and upregulation of the specific cellular pathway leading to autophagy (Hsp70–BAG3–sHSPs), which are all under HSF-1 regulation [77], stressing the important role for the PQC network in health and disease.

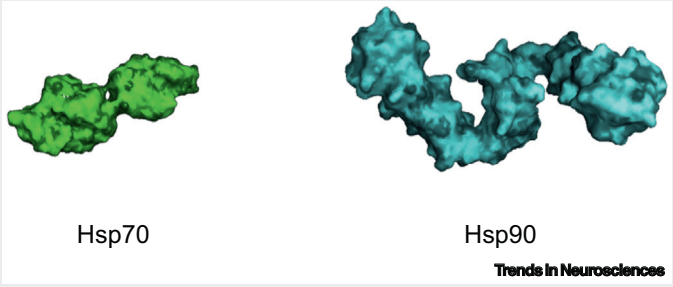

Figure I Schematic illustration of amyloid-fibril-formation pathways. External factors can trigger the formation of aggregation nuclei from protein monomers (arrow 1). Once the aggregation nuclei are formed, these can convert into amyloid fibrils (arrow 2). On the addition of monomers to the end of the existing fibrils, the fibrils are elongated (arrow 4). The surface of the existing amyloid fibril can also seed the formation of new aggregating nuclei from protein monomers, which is the process of secondary nucleation (arrow 3).

substrate when at physiological levels [24]. Release of the substrate from Hsp70 allows the substrate to fold on its own into the native state or rebind to another Hsp70 molecule. Hsp90 shortcuts this repetitive cycling by breaking the Hsp70 block, by binding to protein stretches characteristic of late folding stages [25]. This allows the protein to enter a productive folding route. Both Hsp70 and Hsp90 do not actively fold the protein, nor do they change the folding kinetics; they assist the substrate to fold on its own [24].

Both Hsp70 and Hsp90 chaperones interact with proteins that form amyloid fibrils in disease, including Tau, α-synuclein, and Htt, and they are key to the cellular response that prevents fibril formation in healthy neurons [22,26–29]. To discuss chaperone interaction with fibrils at the molecular level, we need to zoom in onto fibril structure. We do that using Tau as a paradigm.

The challenging versatility of amyloid fibrils

Tau is a remarkable protein in amyloid fibril formation, as different tauopathies are characterized by distinct Tau conformations (Figure 2) [30–34]. Fibril formation is a kinetic process that can be influenced by a variety of factors resulting in different amyloid structures (Box 2). Monomeric Tau undergoes post-translational alternative splicing resulting in six different isoforms. In its sequence Tau has four pseudo-repeats responsible for binding to microtubules. Alternative splicing results in isoforms with either three (3R) or four (4R) repeats of the microtubule-binding domain (MTBD).

The different tauopathies can be divided into 3R (Pick's disease), 4R [e.g., corticobasal degeneration (CBD), progressive supranuclear palsy] or 3R/4R (e.g., AD) tauopathies,

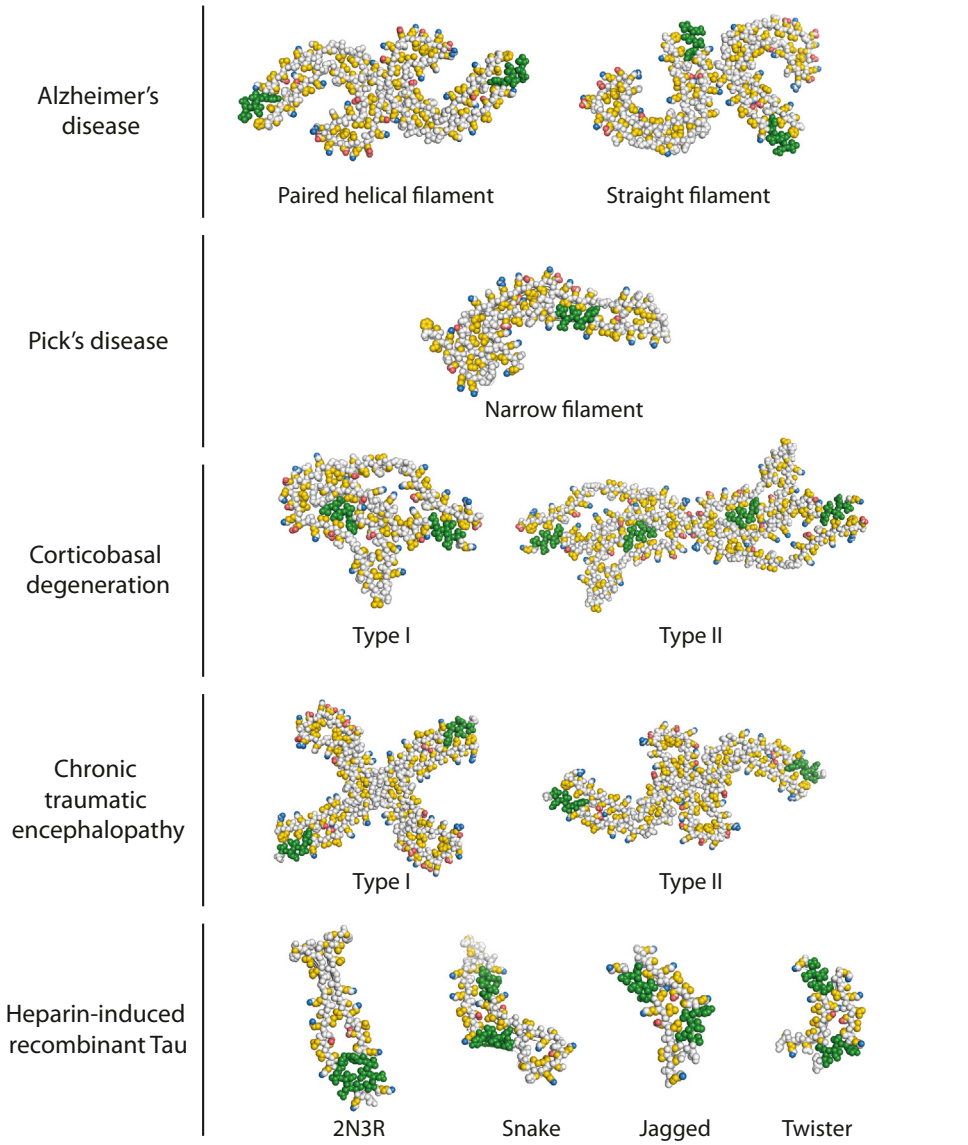

Figure 2. Structural diversity among *in vivo* and recombinant Tau fibrils. Structural representation of the different Tau amyloid fibrils in tauopathies, based on fibrils purified from primary pathological material, and, for comparison, heparin-induced fibrils from recombinant Tau protein. Functional groups are colored according to the YRB script: hydrocarbon groups without substitutions in yellow, negatively charged oxygen of glutamate and aspartate in red, and nitrogen of positively charged functional groups of lysine and arginine in blue, with all remaining atoms in gray [104]. Segments indicating Hsp70-binding sites are colored green. The images were generated using the software PyMOL based on the coordinates of cryoelectron microscope (cryo-EM) structures [104], PBD accession codes: Alzheimer's disease (AD) paired helical filaments, 5O3L; AD straight filaments, 5O3T; Pick's disease (PiD) narrow filament, 6GX5; corticobasal degeneration (CBD) type I, 6TJO; CBD type II, 6VH7; chronic traumatic encephalopathy (CTE) type I, 6NWP; CTE type II, 6NWQ; 3R heparin Tau, 6QJQ; Jagged, 6QJP; Snake, 6QJH; Twister, 6QJM.

Box 2. Molecular mechanism of amyloid fibril formation

Whereas the structure of folded proteins is determined by their amino acid composition, the structure of amyloid fibrils is not determined solely by their amino acids but rather is a kinetic event that can be influenced by environmental factors. In general, the pathway of amyloid fibril formation can be divided into primary and secondary events, of which the latter either refers to the elongation or secondary nucleation pathway (Figure I) [105]. The primary event in fibril formation – or primary nucleation – describes the process of the formation of a fibril nucleus from monomers and is independent of the presence of already-existing fibrils (Figure I, arrow 1).

Primary nucleation can be a homogeneous or heterogeneous event and in either case is the rate-limiting step in the fibril formation process. In case of homogeneous primary nucleation, the kinetics of the event are determined solely by the concentration of monomers. In heterogeneous primary nucleation, the presence of external factors – like surface-binding or crowding agents – can influence the kinetics of the reaction. Once the aggregation nuclei are formed, the nuclei are converted into amyloid fibrils (Figure I, arrow 2).

Elongation and secondary nucleation are steps in the fibril formation process that are determined by the concentration of already-existing fibrils. In the case of elongation, this is exclusively determined by the fibril concentration, where monomeric protein is added to the ends of the pre-existing fibrils; the fibrils are elongated by new monomers (Figure I, arrow 4). Secondary nucleation depends on the concentration of both the pre-existing fibrils and the monomer. The surface of the pre-existing aggregates accelerates the formation of new aggregate nuclei from monomers (Figure I, arrow 3) [106].

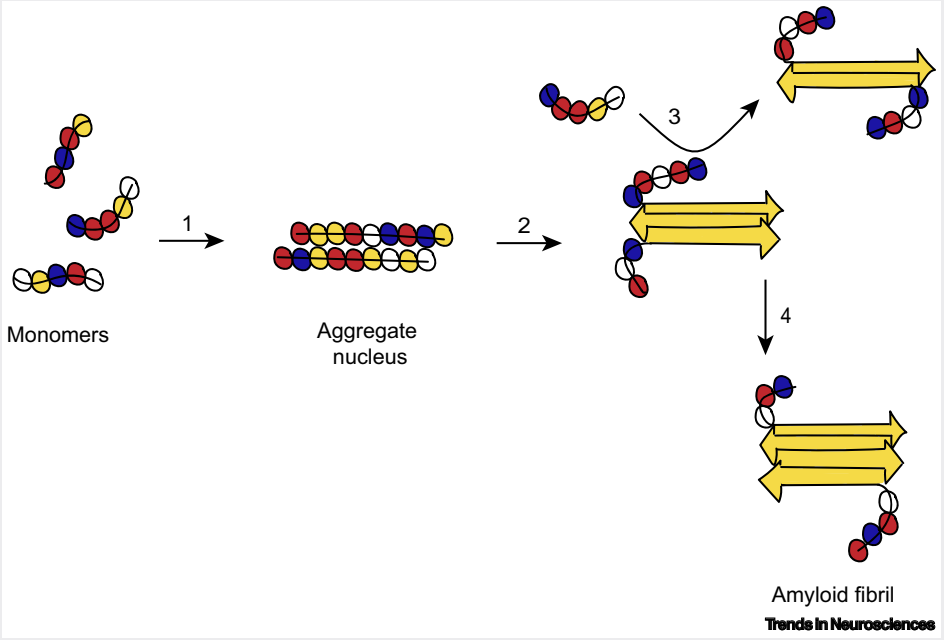

Figure I. Structure of the Hsp70 and Hsp90 chaperones. 3D structure of Hsp70 (green) and Hsp90 (blue) [22,25]. The structural representations were generated using the software PyMOL.

depending on the Tau isoforms incorporated into the fibril. Despite differences in isoform composition, the fibrils of each tauopathy do share some similarities. The fibril cores comprise β-sheets and loops and turns between the sheets. The exact residues incorporated in the fibril core differ per tauopathy, but the core almost solely comprises residues from the MTBD [33]. One specific motif incorporated in the core of all Tau fibrils is the ^{305}KVQIVYK^{311} motif. This site is implied to be important for the fibrillization of Tau *in vitro* [35], but is also a binding site for Hsp70 [36]. This raises the question of to what extent this motif is indispensable for Tau fibril formation, as it may be occupied by Hsp70 chaperones *in vivo* [35].

Recombinant heparin-induced Tau fibrils are shorter and more dynamic than the patient-derived fibrils [33]. The 4R isoform can form four structurally different fibrils, whereas the 3R isoform can only form one. The 3R-recombinant fibril appears to be a co-assembly of two molecules stacked against each other. The *in vitro* heparin-induced fibril core comprises only a few residues of the MTBD [33]. For *in vivo* fibrils, the ordered core comprises the majority of the MTBD residues, while the remainder of the protein forms the fuzzy coat (reviewed in [37]). Differences in the flanking regions of the filament core may also affect the fibril morphology and thus contribute to the great variety of Tau fibrils [38,39]. The variety of fibril structures comprising the same monomeric protein can possibly also be explained by the 'proteoform concept'. This concept describes the phenomenon of distinct protein molecules that arise from the same gene [40]. Modifications such as alternative splicing and various pretranslational modifications and **post-translational modifications (PTMs)** give rise to chemically different forms of proteins, although they originate from the same gene. These alternative forms of proteins may assemble into different conformations, as has been shown for α-synuclein [41].

The structural heterogeneity of Tau fibrils underscores the importance of a versatile and adequately responding quality control. Different fibrillar structures have different build-ups of the fibril core, but also have different flanking regions. Besides differences in fibril morphology, differences in flanking regions may affect the recruitment of aberrant binding partners, which trigger a variety of pathways to toxicity, potentially leading to neuronal death. The difference between different *in vivo* Tau fibrils may result in different cellular responses by the PQC system. However, while there are many different fibril structures, even of the same protein, it is always the same chaperone machinery that needs to take care of them.

Driving forces of protein aggregation

The fact that a single protein can adopt entirely different fibril structures strengthens the thought that fibril formation is susceptible to environmental influences and is not solely determined by the sequence of the protein. Understanding what determines the formation of different fibrillar structures and which cellular – quality control – processes may be involved in this is of great importance for the field.

Liquid–liquid phase separation (LLPS) is gaining growing interest in the context amyloid fibril formation. LLPS is a proteome-wide phenomenon, as approximately 80% of the proteome is a candidate for LLPS and approximately 40% of the proteome can undergo spontaneous phase separation [42]. Driving forces for the process of LLPS are proteins with low-complexity domains (LCDs), which tend to have a high frequency of aromatic residues, arginines, and glutamines. These residues can engage in **π–π interactions**, which contribute to the formation of membrane-less organelles [43].

LLPS serves several biological functions in the organization and regulation of proteins; for instance, in the formation of ribonucleoproteins (RNPs) and stress granules (SGs) [44–48]. RNPs are a complex between RNA and RNA-binding proteins (RBPs) and are important for RNA processing and modification. On cellular stress, stress-inducible RNPs – or SGs – are formed intracellularly. and their formation leads to a local enhancement of the protein concentrations. Under healthy conditions, the SG droplets form a steady state, which is a reversible process as SGs can dissolve on stress removal. As the contents of the SGs are often proteins with LCDs, they form a source for aggregation in case of a failing control mechanism [49]. Mutations in multiple RBPs, like fused in sarcoma (FUS) and heterogeneous nuclear RNP A1 (hnRNPA1) impair the re-solubilization of SGs [50–52]. Mutations in FUS and hnRNPA1 are among the genetic risk factors for the progressive neurodegenerative disease amyotrophic lateral sclerosis (ALS). This raises the question of whether fibril formation via LLPS plays a role in other neurodegenerative diseases as well.

Droplet formation in tauopathies

Tau can undergo phase separation *in vitro* [53]. As an IDP, Tau has an uneven distribution of charge, which facilitates intermolecular interactions and can trigger droplet formation. Hyperphosphorylation or oligomerization of Tau – which is observed in disease – strengthens the interaction of Tau with one of the RNPs, TIA1 [54]. This interaction facilitates the formation of SGs [54] in animal models of AD and FTDP-17 [54,55]. These findings raise the question of whether the association of TIA1 in Tau tangles may be a molecular scar on fibrils that may have grown out of TIA1-containing droplets (Figure 3) [47,48].

PTMs as a source of fibril diversity

In line with the 'proteoform concept', the aggregation state of Tau can be influenced by PTMs. As an IDP, Tau lacks a defined secondary and tertiary structure and is therefore more susceptible to PTMs [56]. Can all of these different modifications be of importance in fibril formation and toxicity? It appears that PTM patterns differ between AD patients. A subset of PTMs, however, is abundant in specific disease stages and appears to be indicative of disease

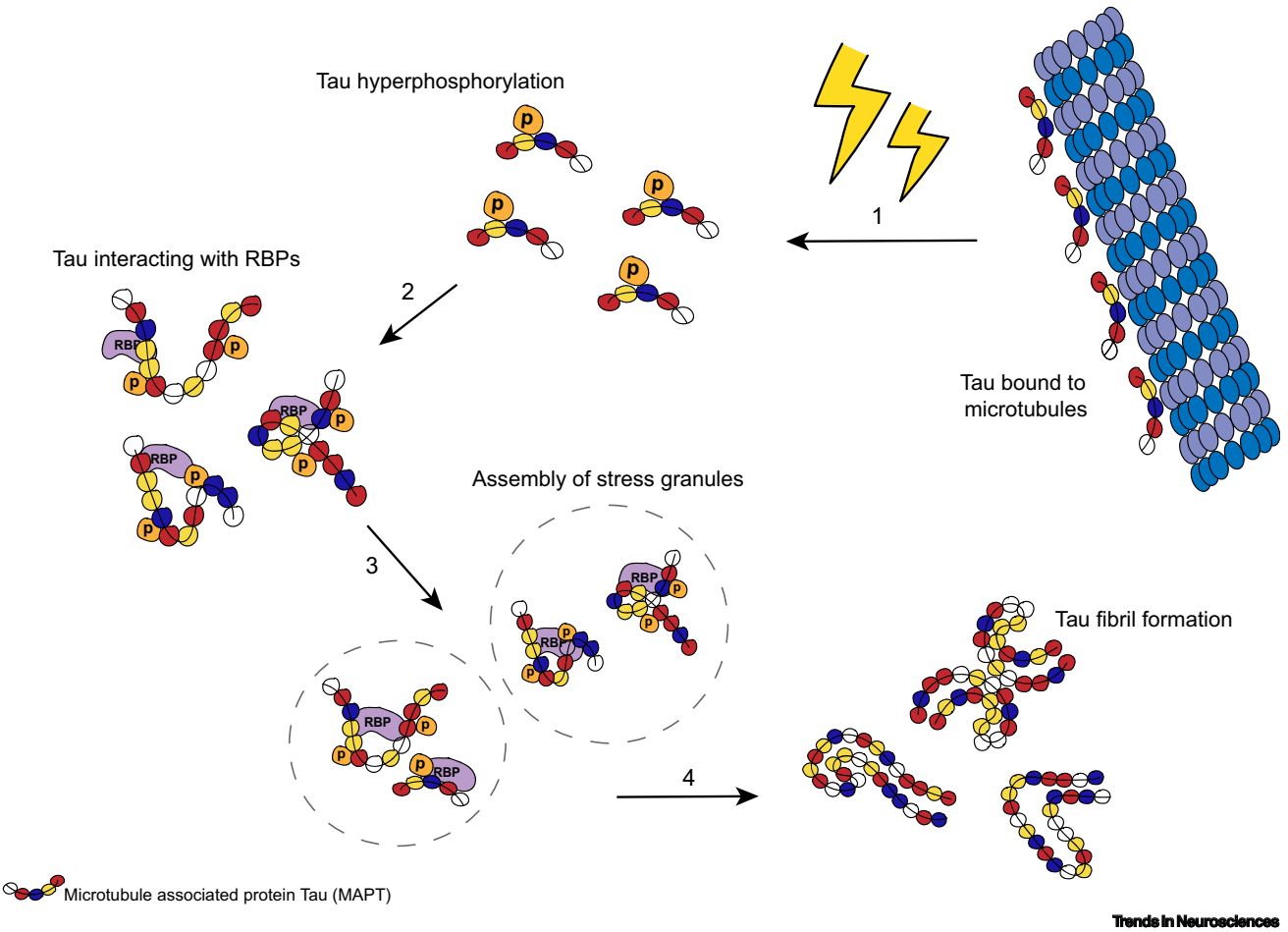

Figure 3. Liquid–liquid phase separation (LLPS) in fibril formation. Schematic representation of a proposed pathway for Tau fibril formation facilitated by LLPS. (1) On cellular stress and hyperphosphorylation, Tau detaches from the microtubules. (2) Hyperphosphorylation and oligomerization trigger the colocalization of phospho-Tau with RNA-binding proteins (RBPs) like TIA1. (3) The interaction between RBPs like TIA1 and Tau triggers the formation of stress granules (SGs). (4) TIA1 in SGs – via an unknown mechanism – facilitates the fibril formation and propagation of Tau, contributing to neurodegeneration.

progression [57]. Also, the ordered cores of CBD fibrils are acetylated and ubiquitylated. Acetylation is known to decrease protein solubility and may enhance Tau aggregation. Ubiquitylation is known for being a trigger for both proteasomal and selective autophagy degradation but is also implied to play a structural role by stabilizing fibril filaments [58,59]. If the fibrils are ubiquitylated and ubiquitylation is a trigger for degradation of the substrate, why are these fibrils not degraded by the cell?

Failing degradation system

The two major degradation pathways for many cellular elements are the ubiquitin-proteasome system (UPS) and autophagy (Figure 4). The UPS recognizes ubiquitin-tagged substrates that need to be degraded. The ubiquitin tag of the substrates is recognized and the substrate is fragmented into smaller peptides, which can be recycled in the cell [60]. Before processing by the proteasome, ubiquitin is removed from the substrate by deubiquitinating enzymes (DUBs) and can be recycled as well. Three of these DUBs are associated with the mammalian proteasome and are thus of importance in protein clearance via this pathway [61].

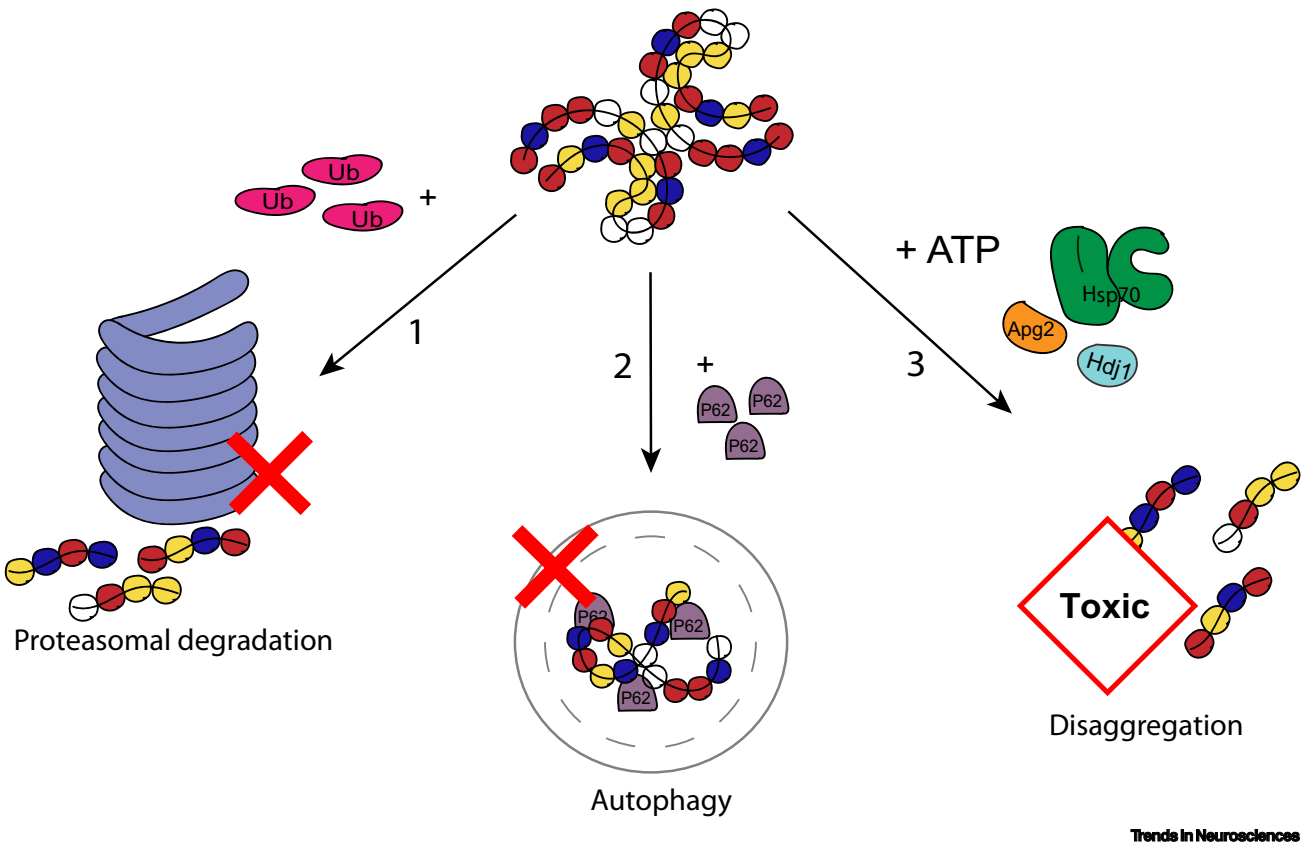

Figure 4. Cellular degradation pathways contributing to neurodegeneration. Schematic illustration of degradation pathways involved in protein processing and fibril disassembly in neurodegenerative diseases. (1) Proteasomal degradation: Ubiquitin-positive substrates can be targeted for degradation by the proteasome. (2) Autophagy: Cargo receptors recognize misfolded or aggregated proteins and transfer them to the autophagosome for degradation. Impairment of the autophagy pathway may contribute to protein accumulation as observed in neurodegeneration (3). Disaggregation: The Hsp70 disaggregation machinery controls protein levels of amyloidogenic proteins and can disaggregate amyloid fibrils. However, the species released from the fibrils may be toxic and seed competent, questioning the desirability of the disaggregation process.

Targeting of substrates to the proteasome is strongly dependent on chaperones. Both Hsp70 and Hsp90 are important in proteasomal degradation, with the co-chaperone CHIP as their common nominator. In healthy neurons, degradation of tau released from microtubules is constantly mediated by the concerted action of Hsp90 and CHIP [62]. CHIP acts as a delivery factor for the proteasome, by coupling the ubiquitin to the substrate to be degraded [63]. The accumulation of ubiquitin-positive proteins in protein aggregates is a hallmark of several neurodegenerative diseases and may imply an impairment of the proteasome, a phenomenon that is observed in several pathologies [57,64–66]. Whereas ubiquitin-positive substrates normally are linearized before they are degraded, the narrow pore of the proteasome and the rigid composition of the amyloid fibrils may prevent this in neurodegenerative diseases [67]. This may possibly render the UPS unable to degrade amyloid fibrils.

Fibril targeting by autophagy

Besides the UPS, the other major degradation system is autophagy. It is a conserved catabolic process targeting, among others, aggregates linked to neurodegeneration. There are three main types of autophagy, all converging on the lysosome, which is the catabolic unit: (i) (macro) autophagy, which refers to the specific removal of parts of the cytoplasm, either selectively or in bulk [68]; (ii) chaperone-mediated autophagy, relying on a specific subset of chaperones [69]; and (iii) microautophagy, where invaginations in the lysosome directly sequester parts of the cytoplasm [70].

Selective autophagy mainly relies on cargo receptors capable of phase separating a wide array of cargoes, including protein aggregates [71–73]. The cargo receptors p62/SQSTM1, NBR1, and TAX1BP1 selectively recognize ubiquitinated substrates, undergo LLPS and are then targeted to the lysosome in a process termed aggrephagy. Lysozymes are suitable to destroy large species such as protein aggregates and their oligomeric precursors. Efficient autophagy may, therefore, be key to keeping healthy neurons free of fibrils [74]. Interestingly, p62 colocalizes with Tau tangles in AD (reviewed in [75]). The association of this autophagy receptor with fibrils in disease may be a marker of failed destruction of Tau tangles or their precursors.

While canonical chaperone-mediated autophagy relies on HSC70-HSPA8 and LAMP2A, **chaperone-assisted autophagy (CASA)** depends on BAG3 [76]. Interestingly, a recent meta-analysis by our group of proteome changes in AD pointed to a major contribution by this co-chaperone [77]. BAG3 links the Hsp70 system to the small heat shock protein network, initiating the removal of aggregates in an orchestrated way [78]. BAG3 is of particular interest in neurodegeneration, as it switches the catabolic gears of aged neurons, favoring autophagy over the proteasome [79].

Although the two degradation systems should be able to degrade the amyloid fibrils or their precursors, their effectiveness in doing so in neurodegenerative disease seems to be reduced. Why is it that the fibrils are not cleared via one of these two systems? Do fibrils reflect an inert end stage or can **disaggregation** facilitate the disassembly of fibrils?

The (un)desirable disaggregation of fibrils

In vitro-generated amyloid fibrils of α-synuclein, Htt, and Tau are susceptible to degradation by molecular chaperones [80–83]. The mammalian disaggregation machinery – comprising the Hsc70-Hdj1-Apg2 complex – can disaggregate fibrils from various proteins. It is a key question, however, whether this is desirable *in vivo*, as oligomers derived from recombinant Tau and α-synuclein fibrils may be toxic and seed competent (Figure 4) [80,83].

Susceptibility to disaggregation varies across fibrillar structures, which may be explained by differences in fibril architecture. The fibrils from the 3R isoform of Tau are most efficiently disaggregated, but also appear to be the least stable considering their fibril structure [33,83]. The availability of Hsp70-binding sites for disaggregation may also be crucial for the differences in disaggregation susceptibility. The position of the Hsp70-binding sites in fibril structures differ across pathologies. For α-synuclein, it has been shown that Hsp70 binds outside the fibril core. For Tau, the predicted Hsp70-binding sites are either inside or outside the fibril core depending on the pathology. Interestingly, although the Hsp70 sites are inside the core of heparin-induced *in vitro* fibrils, and thus protected from the solvent, they appear to be sufficiently dynamic to be subject to Hsp70-mediated disaggregation [84].

It remains to be elucidated whether all species of Tau fibrils in various tauopathies can be disaggregated. This process may be energetically costly, given the size of the fibrils, and the fate of the species that arise from disaggregation is unclear, so whether the process is overall favorable from a cellular perspective remains to be elucidated. Disassembly of fibrils may have detrimental effects for neurons, as toxic species or seed-competent species could be generated. Considering that oligomeric intermediates may be the most toxic species, a key challenge for PQC is to act in the early phase of fibril formation rather than disassembling the fibrillar structures.

Do aggregating proteins escape from the control?

Neurodegenerative diseases are often age related, implying that age-related changes in PQC may be relevant. With age, stress induction of chaperones is compromised and so are autophagy and proteasomal degradation [85]. Impairment of these degradation systems would disrupt the clearance of misfolded or aggregating proteins, thereby facilitating the formation and accumulation of fibrils.

The concept of 'liquidity' is gaining major momentum in selective autophagy research [86–88]. In this concept, the mobility of cargo within a phase-separating unit destined for the lysosome is a crucial parameter that determines the cargo's degradation. Protein aggregates have incredibly rigid structures and their mechanical strength has been argued to be comparable with that of steel [89]. Such physical properties could impact the formation of optimal phase-separated units, negatively impacting their downstream processing by the lysosome.

Although age-related changes in PQC may be important events in the onset of neurodegeneration, a significant subset of the human population still ages without developing these pathologies. This suggests that, at least in some instances, a failing cellular quality control step is involved in the processes leading to neurodegeneration. A transcriptomic analysis comparing diseased brains and age-matched controls revealed the suppression of many Hsp70 (co-)chaperones [90], while changes on the protein level are targeted to specific components of the PQC [77]. On the protein level, the Hsp70–BAG3–sHSP pathway is strongly upregulated in AD compared with non-AD brain, suggesting activation of CASA [77]. All of these components are under the control of heat shock factor 1 (HSF-1), which is a transcription factor regulating gene expression in response to cellular stress, suggesting that AD may involve ongoing, chronic cellular stress in. Upregulated CASA appears counterintuitive given that AD patients show accumulation of Tau fibrils. The fact that fibrils accumulate in AD despite upregulation of a clearance pathway (CASA) may, therefore, indicate that CASA either is inefficient or became inefficient in disease to counter the accumulation of Tau fibrils.

In the context of CASA, the BAG3 imbalance in AD could reflect a boost of autophagy as a last attempt to remove the increasing aggregate burden [91]. An alternative, and not mutually

exclusive, explanation is that the BAG3 imbalance signifies that the system is overloaded and becomes part of the aggregated proteome. Given the major role of BAG3 in tuning the UPS/autophagy lysosomal system balance, its upregulation could further disrupt neuronal homeostasis, in turn exacerbating neurodegeneration.

Besides a role for the autophagy pathway, the HSP70-BAG3-HSPB8 complex is also required to dissolve aberrant SGs that contain misfolded proteins via a process called granulostasis [92,93]. This chaperone-mediated pathway of RNP granules is strongly upregulated in AD [77], which possibly indicates the presence of SGs. A failure in the quality control of SGs can lead to the switch between a gel-like state and solid-state droplets. Resolving SGs can be facilitated by ubiquilin-2 (UBQLN2) interacting with Hsp70. UBQLN2 is a ubiquitin adaptor protein involved in resolving SGs but also playing a role in protein degradation. It facilitates proteasomal degradation by recognizing the Hsp70-bound substrates and targets them to the proteasome. Mutations in UBQLN2 have been associated with ALS [94] revealing an interesting potential pathway for fibril formation: defective UBQLN2 prevents binding of the misfolded protein to Hsp70, resulting in impaired shuttling to the proteasome, thereby preventing misfolded proteins from being degraded via this pathway [95]. UBQLN1 knock-in mouse models with this mutation develop inclusion bodies and cognitive deficits with age. Other neurodegenerative diseases besides ALS are also linked to the dysfunction of ubiquitin adaptor proteins. Mutations in the ubiquilin-1 gene are a risk factor for AD and overexpression of this protein alleviates cognitive and motor deficits in an AD mouse model [96]. Deficits in these pathways imply that the misfolded proteins are not invisible to the PQC system. They are recognized by it, but cannot be appropriately directed to the degradation pathways.

The inability of the neuron to clear or prevent protein aggregates in disease reflects dysfunctional quality control. The action of PQC factors, however, may also have negative side effects. Aberrant generation of smaller, soluble fibril fragments by the disaggregation machinery may contribute to the toxicity of aggregates for neurons. On fibril formation, the interactome of the aggregating protein rewires, thereby changing the binding partners. The generation of soluble fibril fragments may stimulate this process. Fibrils can hijack cellular components connected to various pathways, like RNA-related processes [97]. Tau fibrils are known to impair RNA translation, which decreases protein translation and may contribute to cellular changes leading to memory loss in AD [98]. Other hallmarks of neurodegeneration, like an imbalance in axonal transport, can be explained by the altered interactome of Tau fibrils and may facilitate cellular derailment [97,99], underscoring the importance of a well-controlled PQC system.

Future directions for therapeutic interventions

As failures in the PQC machinery can contribute to the development of protein aggregation diseases, boosting the PQC machinery may offer a strategy for suppressing protein aggregation. Molecular chaperones can disassemble amyloid fibrils from various disease-related proteins *in vitro* [80–83]. The jury is still out on disaggregation as therapeutic strategy, as the resulting smaller species appear to be seed competent and toxic for the cell [83]. Potentially more suitable targets for intervention lie earlier in the fibril formation process; for instance, by upregulating the degradation systems.

Boosting the degradation systems using co-chaperones in the cell may contribute to the clearance of oligomeric intermediates, as several neurodegenerative disorders are associated with impaired proteasomal activity [64–66]. CHIP – which is an E3 ubiquitin ligase and important for targeting species towards the degradation pathway – can recognize and mediate the degradation of toxic oligomers of α-synuclein [100]. Interestingly, overexpression of CHIP

reduces hyperphosphorylation of Tau in an AD mouse model [101]. As the levels of CHIP in AD brains negatively correlate with aggregated Tau levels, high levels of CHIP could contribute to lower aggregated Tau levels, thereby playing a protective role [102]. Interventions that lead to the removal of oligomeric intermediates could provide a route to halt neurodegeneration at the earliest stage, but it remains to be examined whether such interventions could lead to new therapeutic strategies.

Concluding remarks

In this review, we addressed recent advances in understanding the role of the PQC system in the molecular mechanisms of protein aggregation. The appearance of amyloid species that are toxic for cells reflects a failure of PQC to sufficiently handle misfolded proteins. The great diversity of amyloid fibrils, from either the same or different proteins, may contribute to the challenge for the PQC system: there is only one PQC system, but there are many types of problematic fibrils. AD is consistently characterized by the accumulation of paired helical filaments and straight filaments [22], like Pick's disease is consistently characterized by the wide paired filaments and narrow paired filaments (Figure 2) [30,31]. However, there appear to be also differences when it comes to Tau deposition. Recently, differences were discovered between AD patients in the trajectories of Tau deposition spreading through the brain [103]. The effectiveness of the PQC response may vary between individuals and between brain regions. This raises the question of whether one cause of PQC failure is triggered differently in different brain regions at the organismal level or whether different routes at the cellular level are dominant in different tissues. In other words, is there one cellular cause for AD or are there multiple pathways that can be derailed but lead to the same pathology (see Outstanding questions)?

The specific upregulation of chaperone pathways in AD patients may reflect a cellular attempt to tackle amyloid fibrils, but for as-yet-unknown reasons this effort fails. An effective PQC is essential for life, but this effectiveness may differ between individuals and decline with age. The appearance of amyloid fibrils represents *per se* a PQC malfunction. This failure may be caused either by a general inability of the PQC system to remove a toxic species or by the derailment or deregulation of an otherwise effective machinery. The roots of losses of PQC may be diverse and they could be triggered, for instance, by extracellular events, cell stress, metabolic failure, neuroinflammatory factors, or genetic disposition. Thus, the origin of PQC decline leading to protein aggregation may differ between patients. The PQC system itself, however, is shared by all cells of our bodies. Future work to address the roles of PQC in neurodegeneration would help us gain better understanding of the pathology on a molecular level and hopefully bring us a step closer to therapeutic interventions for neurodegenerative diseases.

Outstanding questions

What triggers the imbalance in protein homeostasis leading to protein aggregation?

Does protein aggregation result, at least in part, from an accumulation of individual derailments of the PQC system?

Are there specific stages in the process of fibril formation that are particularly amenable to PQC-based interventions to prevent fibril formation and possibly attenuate the degeneration process?

One of the potential reasons for aggregates' escape from degradation pathways is failure of these pathways to effectively recognize aggregates. What are the reasons that protein aggregates miss being recognized by degradation pathways?

Does the protein quality control pathway act differently on amyloid fibrils having different structures?

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Declaration of interests

The authors declare no interests in relation to this work.

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