Targeting protein aggregation for the treatment of degenerative diseases

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Abstract | The aggregation of specific proteins is hypothesized to underlie several degenerative diseases, which are collectively known as amyloid disorders. However, the mechanistic connection between the process of protein aggregation and tissue degeneration is not yet fully understood. Here, we review current and emerging strategies to ameliorate aggregation-associated degenerative disorders, with a focus on disease-modifying strategies that prevent the formation of and/or eliminate protein aggregates. Persuasive pharmacological and genetic evidence now supports protein aggregation as the cause of postmitotic tissue dysfunction or loss. However, a more detailed understanding of the factors that trigger and sustain aggregate formation and of the structure–activity relationships underlying proteotoxicity is needed to develop future disease-modifying therapies.

Amyloid fibrils
Lateral assemblies of protein aggregates adopting a cross-β-sheet structure. These aggregates bind to Congo red, thioflavin T and analogous aromatics.

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Transthyretin (TTR)[1], immunoglobulin light chain (LC)[2], serum amyloid A (SAA)[3] and amyloid-β (Aβ)[4] are examples of more than 30 human proteins that seem to cause a range of degenerative disorders owing to their misfolding and/or misassembly into various aggregate structures[5]. These amyloid diseases are named after their pathological hallmarks: cross-β-sheet aggregates, or amyloid fibrils[6,7]. Amyloid fibrils in a specific disease are generally composed predominantly of one protein[5]. Amyloid fibrils from different diseases and composed of different proteins exhibit similar structural features[6].

In affected patient tissues, protein aggregation and deposition mainly occur at the normal extracellular or intracellular location of the aggregation-prone protein. However, there is increasing evidence for the presence of both intracellular and extracellular aggregates in nearly all of the aggregation-associated degenerative diseases[8–10]. Moreover, evidence indicates that aggregates can travel between intracellular and extracellular locations. This suggests that intracellular toxicity might also contribute to proteinopathy that was once thought to result predominantly from extracellular aggregation, for example, of Aβ aggregates contributing to Alzheimer disease (AD)[11–16]. Furthermore, the cellular uptake and release of protein aggregates seem to contribute to the spreading of these aggregates within a multicellular organism and to the associated pathology and tissue damage[17–20]. However, the mechanism (or mechanisms) by which the processes of intracellular and/or extracellular aggregation cause pathology remains unclear.

Strong genetic, pharmacological, biochemical and pathological evidence supports the hypothesis that human amyloid diseases result from the process of protein aggregation, or amyloidogenesis[21–28] (FIG. 1). It is important to recognize that there is an incomplete understanding of aggregation, both *in vitro* and in multicellular organisms, because probes to monitor the different types of aggregates formed or structures yielded during this dynamic process are not available. In the absence of more detailed information about the ensemble of aggregate structures present in a patient, it is probably useful to think about aggregates as a range of structures, from small, relatively unstructured oligomers to structurally well-defined cross-β-sheet amyloid fibrils, recognizing that some structures may only be significantly populated in particular tissues or in certain cellular compartments. Which of the aggregate types are toxic and their mechanism of cytotoxicity is unclear. A current hypothesis suggests that it is the smaller diffusible oligomers, which exhibit a range of structures, rather than the insoluble cross-β-sheet amyloid fibrils that drive the degenerative pathology[29]. However, these diffusible oligomers could result either from fragmentation of fibrils into small pieces that are no longer capable of supporting a cross-β-sheet amyloid structure or from unsuccessful degradation of fibrils by the lysosome or proteasome, or they could be formed independently. Thus, we hypothesize that it is important to consider protein aggregation *in vivo* as a dynamic process with many components. Even with this incomplete

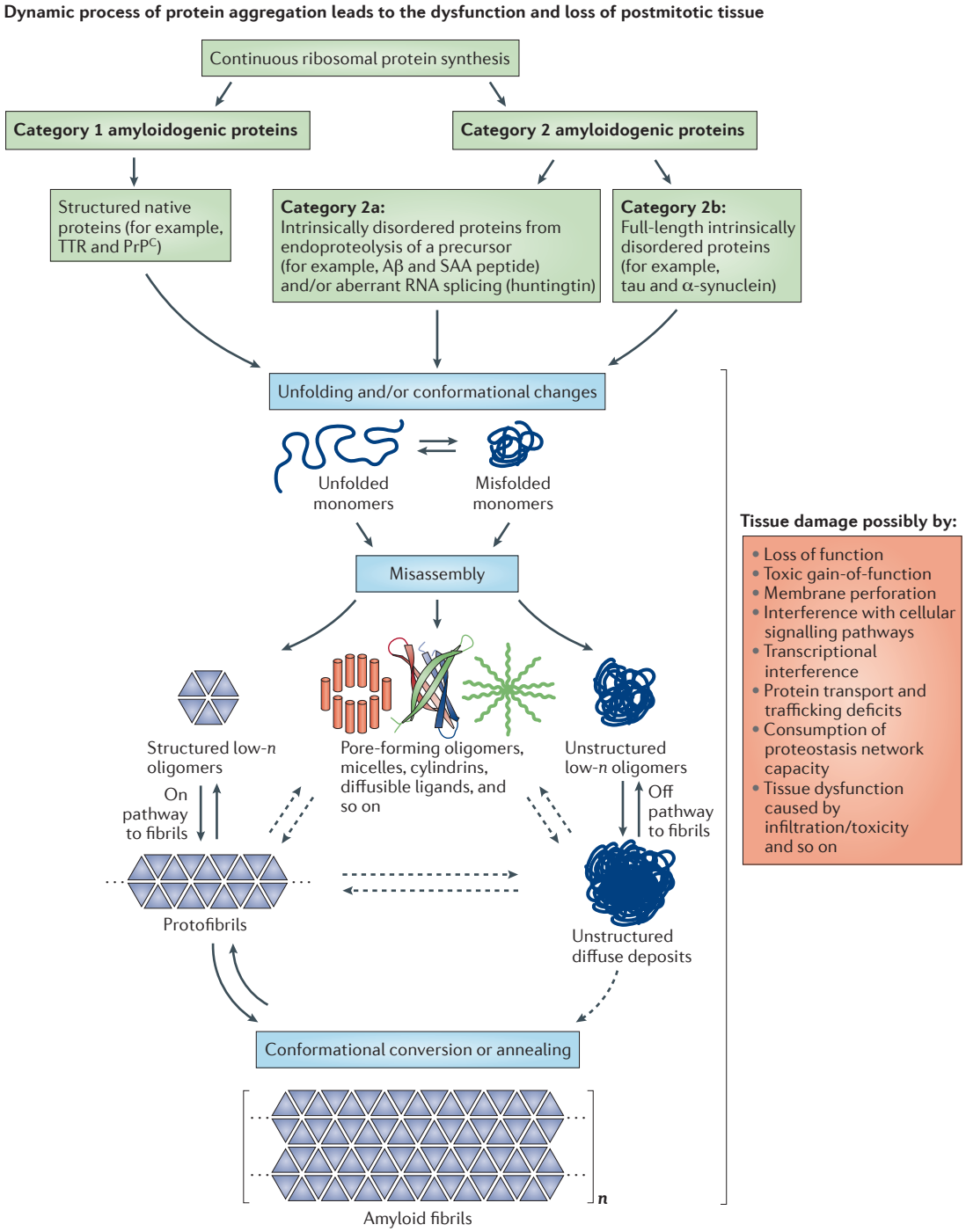


Figure 1 | **Amyloidogenesis: a process of aggregation influenced by the physical chemistry of the protein as well as by cellular and extracellular components.** Amyloidogenic proteins associated with degenerative disorders can be divided into two categories based on their native structure. Category 1 proteins, such as transthyretin (TTR) and cellular prion protein (PrP^C), exhibit a well-defined native three-dimensional structure, whereas category 2 proteins are intrinsically disordered. Both intrinsically disordered polypeptides produced by endoproteolytic processing of a precursor protein (category 2a), such as amyloid-β (Aβ) generated by cleavage of the β-amyloid precursor protein, and full-length intrinsically disordered proteins (category 2b), such as tau and α-synuclein, can be amyloidogenic. The critical step in amyloidogenesis is the misfolding and aggregation of category 1 proteins or the misassembly of category 2 proteins into a range of aggregate structures, including β-sheet-rich structures and amyloid fibrils. The structures associated with the amyloid cascade are depicted along with their hypothesized mechanisms of proteotoxicity (shown on the far right). The ensemble of structures is probably influenced by, and partly generated by, the biology of the organism: for example, incomplete degradation of amyloid could give rise to novel structures, or aggregation on cell membranes could yield aggregate structures that can only form in the presence of certain lipids and/or carbohydrates. SAA, serum amyloid A.

Amyloidogenesis
The process of protein aggregation in an organism whereby physical chemical forces and biological modifiers together influence the aggregate structural ensembles afforded.

knowledge of the aggregated structures present in patients, the therapeutic strategies that are currently being developed are focused on preventing active protein aggregation and/or removing diffusible proteotoxic aggregates, as well as on ameliorating the toxic effects of aggregates, while maximizing the physiological function of these proteins[22–27,30–32].

Amyloidogenesis

There are two categories of amyloidogenic proteins (FIG. 1). In the case of proteins that initially adopt a well-defined, folded and three-dimensional structure (category 1 proteins), substantial evidence supports the idea that a partial loss of this well-defined structure is required for their aggregation[33–36]. Early studies on TTR and LC demonstrated that conformational changes alone are sufficient to enable these proteins to misassemble into a range of aggregate structures, including amyloid fibrils[33,34]. These observations provide substantial evidence that supports the conformation-change hypothesis as the basis for their aggregation. The same forces that drive intramolecular protein folding, including hydrogen bond formation and the hydrophobic effect, also mediate intermolecular aggregation and amyloidogenesis when the misfolded protein is present at sufficient concentrations[33,37].

Amyloidogenic proteins that are intrinsically disordered are classified as category 2 proteins[38,39]. In general, category 2 polypeptides undergo thermodynamically linked aggregation and conformational changes, thus giving rise to more β-sheet-rich structures. These linked changes often require a nucleation step (FIG. 2). Category 2 polypeptides can be further divided into two subcategories. The first of these, category 2a, comprises intrinsically disordered polypeptides that result from the endoproteolysis of a precursor protein. The best-studied examples are the Aβ peptides, which arise from β-secretase and γ-secretase endoproteolysis of β-amyloid precursor protein (APP)[40,41]. Under pathological conditions, Aβ peptides form extracellular aggregates in the brain parenchyma and/or the walls of blood vessels in the brain and, to a lesser extent, inside brain neurons; these processes have been linked to AD and cerebral Aβ angiopathy. The SAA peptide, the aggregation of which causes AA amyloidosis[3], is another well-studied intrinsically disordered category 2a protein. The SAA peptide is derived from the endoproteolysis of the SAA protein, which is produced in high concentrations as a consequence of chronic inflammation. The aggregation propensities of intrinsically disordered proteins, such as SAA and Aβ, are strongly influenced by their concentration, their post-translational modifications and the exact amino acid sequence afforded by the endoproteolytic processing event (or events).

The huntingtin protein (HTT)[42] — the aggregation of which underlies Huntington disease — is also thought to be a category 2a protein. The majority of publications suggest that an amino-terminal fragment of HTT, which includes the polyglutamine (polyQ) stretch, is released following proteolysis and/or aberrant RNA splicing, triggering the formation of aggregates, and is more toxic than full-length HTT[43,44]. Other mechanisms underlying HTT cytotoxicity have been suggested, including altered protein structure of the expanded polyQ stretch within full-length HTT and altered protein–protein interactions of full-length polyQ-expanded HTT, including disruptions in microtubule- and actin-based transport pathways inside neurons. Other polyQ disorders may result from the misfolding and/or misassembly of category 1 proteins: for example, spinal and bulbar muscular atrophy, which is a neuromuscular disorder apparently caused by the aggregation of an expanded polyQ tract in the androgen receptor[45]. It is clear that more thorough observations are needed with regard to whether full-length and/or fragment polyQ-expanded proteins are driving pathology. Nonetheless, the number of CAG repeats in several polyQ-expanded proteins, including HTT, correlates with the age of onset and disease severity, suggesting that the probability of misassembly correlates with proteotoxicity[46].

Category 2b polypeptides are full-length proteins that are intrinsically disordered and that aggregate without the requirement for endoproteolysis. This classification includes proteins such as tau[47], the aggregation of which is associated with forms of frontotemporal dementia and AD, and α-synuclein[48], the amyloidogenesis of which is thought to cause Parkinson disease. These category 2b proteins aggregate intracellularly. The propensity of tau and α-synuclein to aggregate is influenced principally by their concentration but also by post-translational modifications, such as phosphorylation and nitrosylation at certain residues in sporadic forms, or by point mutations in familial forms of these maladies[49,50]. It should be noted that recent findings suggest that the α-synuclein protein may also adopt a quaternary structure within neurons under specific conditions, thus challenging the previous notion that α-synuclein is solely an intrinsically disordered protein[51–53].

Typically, the misfolding and/or aggregation of category 1 or 2 proteins are thought to cause a gain-of-proteotoxicity phenotype, which disturbs cellular function (or functions) and initiates toxicity cascades (FIG. 1). However, it is worth noting that loss of function may also contribute to the pathogenesis of aggregation-associated diseases. This seems especially relevant for nuclear proteins such as TDP43 (TAR DNA-binding protein 43) and FUS (fused in sarcoma; an RNA-binding protein) that are involved in amyotrophic lateral sclerosis and frontotemporal lobar degeneration-type dementia. Both TDP43 and FUS relocate to and aggregate in the cytoplasm, where they can also be recruited to stress granules and cause transcriptional and translational alterations[54,55]. In the case of the hexanucleotide repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene that is associated with amyotrophic lateral sclerosis and frontotemporal dementia, it seems that sense and antisense RNA aggregates and protein aggregates that result from unconventional translation into polydipeptides are jointly responsible for the neurodegenerative phenotypes[56]. Importantly, protein aggregates can sequester folded proteins and RNA, altering additional cellular functions[57]. In addition, the build-up of protein aggregates can impair proteostasis network capacity and thereby compromise the quality of other cellular protein components.

Proteostasis network
The macromolecular machinery that generates, folds, moves and degrades the proteome. Proteostasis network components include chaperones, the proteasome, trafficking machinery and various enzymes — such as disulfide isomerases — that act on the proteome.

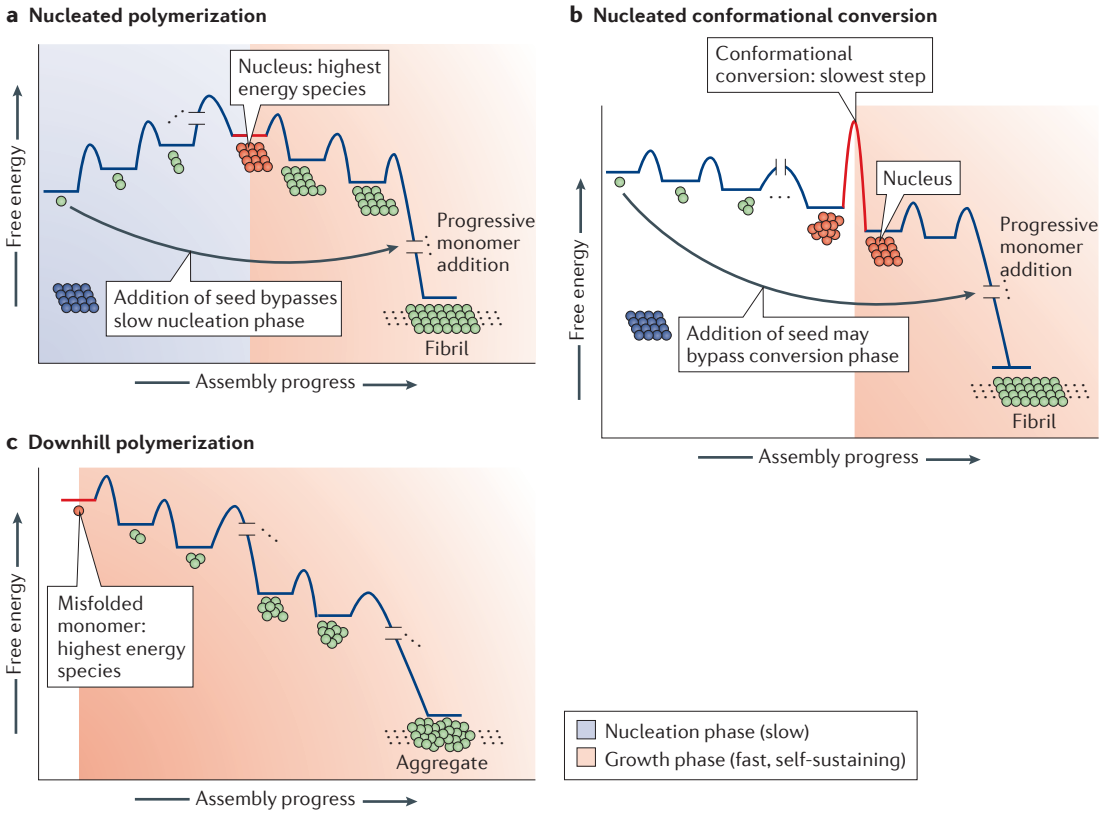


Figure 2 | **Mechanisms of protein aggregation.** **a** | In nucleation-dependent polymerization, the initiating step of aggregation involves the formation of a nucleus, which is a sparsely populated high-energy species that has a different conformation from that of the soluble protein. The nucleus is typically rich in β-sheet structure and presumably oligomeric, although monomeric species have been implicated. Once the nucleus is formed, monomers are rapidly added to the growing non-covalent polymer to generate aggregates that are more thermodynamically stable and that can also act as seeds. Aggregation can proceed rapidly by the addition of preformed aggregates, or seeds (indicated by the blue structures), because nucleation is no longer required in a seeded aggregation reaction. **b** | In a nucleated conformational conversion, an equilibrium exists between monomers and structurally heterogeneous oligomers, which are typically (but not always) more stable than monomers. Over time, the oligomers are converted into a nucleus and then into amyloid fibrils, which can conformationally convert neighbouring proximal monomers into amyloid fibres. Seeding can bypass the requirement for a slow nucleated conformational conversion step. **c** | In downhill polymerization, formation of the aggregation-prone misfolded monomer from a natively folded protein is the rate-limiting step (not shown). Subsequent addition of misfolded monomers to the growing polymer is energetically favourable and leads to amorphous aggregate formation as well as the generation of cross-β-sheet amyloid fibrils (not shown). Seeding does not accelerate the rate of aggregation in a downhill polymerization, at least in transthyretin amyloidogenesis, which has been studied largely *in vitro*.

Nucleus
An energetically unfavourable, sparsely populated, typically oligomeric species that is thought to be rich in β-sheet structure. Nucleus formation is the rate-limiting step for efficient aggregation in a nucleation-dependent polymerization; it is followed by rapid monomer addition, which produces a seed.

Seeds
Stable aggregates that result from the addition of monomers to a nucleus or that arise from the fragmentation of fibrils. Seeds enable homotypic protein aggregation without a requirement for nucleus formation, as seeded aggregation bypasses the requirement for a nucleation step.

We define the process of amyloidogenesis as encompassing all misassembled structures formed, irrespective of conformation. Aggregates include low-*n* oligomers of heterogeneous structure, rather disordered micelle-type structures, amorphous aggregates of various sizes, β-sheet-rich structures and cross-β-sheet amyloid structures[29,58,59] (FIG. 1). Misfolding of the monomer could also contribute to proteotoxicity and should not be overlooked. At least three distinct mechanisms of protein aggregation are supported by the literature (FIG. 2). These mechanisms are largely based on *in vitro* data and are consistent with mathematical models of protein aggregation.

The first mechanism posits that protein aggregation occurs by nucleated polymerization, wherein a high-energy, sparsely populated species (typically oligomeric, but monomers have also been implicated) is formed; this nucleus can subsequently efficiently add monomers to form an aggregate structure that is more thermodynamically stable[60–62] (FIG. 2a). A nucleus can have a cross-β-sheet structure, or it can have an alternative structure that may or may not later conformationally convert to a cross-β-sheet structure[63]. As mentioned above, nucleation is often required for the aggregation of both category 1 and category 2 proteins[64,65]. The requirement for nucleus formation can be bypassed by adding seeds (often cross-β-sheet aggregates) of a particular protein, such as Aβ, to a solution of monomers of the same protein (FIG. 2a). This enables efficient aggregation by exogenous seeding, and therefore accelerates aggregation[62]. Indeed, there is now ample evidence that this type of seeding mechanism might be an important step for the progression and spreading of aggregation in

neurodegenerative disorders (discussed in more detail below). Thus, aggregation could begin in a single rogue cell or tissue and spread throughout the brain by cellular uptake of seeds, followed by cellular secretion of the amplified aggregates by either canonical or alternative pathways[8,17,66,67]. On the basis of *in vitro* and *ex vivo* data, it is commonly assumed that seeded polymerizations yield only structures identical to the seeds and not other structures that currently cannot easily be monitored. However, this may not be true in a multicellular organism, in which biological modifiers influence the process. In fact, it is hard to explain why oligomers can be isolated from tissue containing amyloid fibrils if seeded polymerizations only give rise to cross-β-sheet amyloid fibrils.

In the second accepted mechanism of aggregation — a nucleated conformational conversion — an equilibrium exists between monomers and structurally heterogeneous oligomers that are generally, but not always, more stable than the monomers[63] (FIG. 2b). Over time, the oligomers are converted into a nucleus and then into amyloid fibrils. The fibrils could conformationally convert the proximal monomers into amyloid fibres, but this does not seem to occur with all proteins.

A third mechanism of protein aggregation posits that proteins sometimes aggregate by a downhill mechanism (FIG. 2c); this process is not seedable[68]. In the downhill polymerization scenario, formation of the aggregation-prone misfolded monomer from a natively folded protein is the rate-limiting step (not shown in FIG. 2c). Partially folded monomeric proteins give rise to higher-order aggregates that are more thermodynamically stable, including amyloid fibrils; thus, high-energy nucleus formation is not required for efficient aggregation[68]. Subsequent addition of misfolded monomers to the growing polymer is always energetically favourable in this mechanism of aggregation. TTR seems to form amyloid fibrils and other aggregate structures in parallel by a pathway analogous to that shown in FIG. 2c, wherein tetramer dissociation is rate limiting.

Although these models of protein aggregation provide a framework for our understanding, it seems likely that conditions in living organisms are more complex, and it is possible that several different aggregate structures may coexist. Different cellular environments (for instance, lysosomes, the endoplasmic reticulum (ER), the cytoplasm and extracellular space) provide distinct conditions for the formation and maintenance of different aggregate structures, which probably influence the ensemble of aggregate structures present. Efforts are under way to improve detection and quantification of aggregate structures in addition to cross-β-sheet amyloid. The availability of these probes is expected to greatly advance our understanding of the relative abundance of different aggregate structures and their structure–proteotoxicity relationships.

Amyloid versus the aggregation process

The extracellular and/or intracellular cross-β-sheet amyloid fibril deposits in tissues of patients with amyloid diseases are recognized as the histopathological hallmarks and still serve as the basis for a definitive diagnosis of the systemic amyloidoses and a diagnostic confirmation of brain disorders, such as AD, post-mortem[7]. Thus, it has been proposed that the accumulation of protein deposits is the primary influence driving pathogenesis ("amyloid hypothesis") (BOX 1). Whereas the protein deposits in human amyloid diseases often consist of aggregates of diverse structure, the focus has been on the cross-β-sheet amyloid fibrils because of their well-defined structure and thus ease of detection[6]. Amyloid fibrils bind to thioflavin T, Congo red and other aromatic chromophores that exhibit fluorescence and/or birefringence. These attributes help pathologists to identify amyloid in tissue. Amyloid fibrils are typically covered by or co-deposited with glycosaminoglycans, the amyloid P glycoprotein and other intracellular and extracellular macromolecules in humans.

It remains unclear to what extent amyloid fibrils are by themselves the toxic entities, or if other forms of misassembled proteins — for example, non-uniformly structured oligomers — are contributors to cytotoxicity or the primary drivers of proteotoxicity. Whether this cytotoxicity derives from intracellular and/or extracellular aggregates is unknown. It also remains unclear whether the conversion of the smaller non-cross-β -sheet aggregates into cross-β-sheet amyloid fibrils would offer some protection (BOX 1). Amyloid load often does not correlate with disease status, calling into question whether amyloid fibrils are the main driver of neurodegeneration; this topic has been discussed intensively in the AD field[69]. It has been suggested that amyloid formation may even be protective: according to this hypothesis, the more toxic structurally diverse aggregates are converted into the less toxic cross-β-sheet amyloid fibrils, minimizing exposure of hydrophobic surfaces and open hydrogen bond valencies that mediate aberrant interactions with proteins, nucleic acids and carbohydrates[70]. Experimental evidence suggests that neuronal survival is increased in cultured cells when mutant HTT is sequestered in inclusion bodies, concomitant with a decrease in the more diffuse aggregated forms of cellular mutant HTT[71]. Moreover, in patients with light chain or TTR amyloidosis who show clinical benefit from disease-modifying therapies, amyloid clearance occurs only in some but not the majority of patients. Amyloid clearance needs to be studied more systematically, especially in the amyloidoses for which disease-modifying therapies exist, so that clear conclusions can be drawn[30,72,73]. Numerous laboratories have hypothesized that smaller diffusible aggregates of poorly characterized structure, known as oligomers and protofibrils, that often escape histological detection are the toxic species driving postmitotic tissue degeneration in the amyloidoses[29,58]. Acute cellular toxicity assessments *in vitro* and *in vivo* also indicate that smaller soluble and diffusible aggregates are notably more toxic than amyloid fibrils[74–76] and that conversion of toxic oligomers to amyloid fibrils can reduce toxicity[75,77]. Our understanding of structure–proteotoxicity relationships is currently incomplete, largely owing to technical limitations. Thus, we urgently need better technologies to specifically detect and quantify amyloid and non-amyloid aggregate types, which could also be useful as much-needed biomarkers for diagnosis of and monitoring response to therapy in the amyloidoses, and we need more relevant proteotoxicity assessments.

Box 1 | **The amyloid cascade hypothesis**

The amyloid cascade hypothesis was first postulated in the context of systemic amyloidosis in the 1960s[264] and as a framework for Alzheimer disease (AD) in the early 1990s[265–267]. AD is characterized by the aggregation of two proteins: amyloid-β (Aβ), which is deposited in the form of extracellular amyloid plaques, and tau, which becomes hyperphosphorylated and aggregates intracellularly to form neurofibrillary tangles[268]. The amyloid cascade hypothesis for AD, which incorporates genetic, biochemical and histological evidence, posits that the deposition of Aβ in the brain is due to an imbalance between its production and clearance, which initiates a sequence of events that ultimately lead to AD. It was proposed that the sequence of events in the rare familial forms is similar to that in the common sporadic forms of AD. Although it was originally thought that amyloid plaques containing fibrillar forms of Aβ were the pathogenic entities[267], some findings seem to contradict this: for example, reports that the amount of amyloid does not correlate with disease status[269]. In other words, clinically normal individuals can have a substantial amyloid load that is similar in amount to severely symptomatic patients[270]. More recent evidence points to a stronger neurotoxic effect from smaller, diffusible protein aggregates — often referred to as oligomers — and suggests that they may represent the disease-driving force[271,272]. It could be that oligomers and amyloid fibrils are part of a dynamic structure–proteotoxicity relationship and that numerous structures contribute to AD pathogenesis. The amyloid cascade hypothesis for AD proposes that Aβ aggregation is the primary event and that the aggregation of tau, inflammation and other changes observed in the brains of patients with AD are downstream[271,273]. If this is correct, prohibiting the formation of Aβ aggregates, as well as reducing or removing them, should be therapeutically useful. However, so far, clinical trials targeting Aβ have not exhibited the ability to slow AD progression. This may be due to several reasons, including enrolment in clinical trials of patients in which AD has already progressed to later stages and/or the drugs being pursued are targeting the wrong Aβ species. These results have led investigators to question whether Aβ is the right primary target and have challenged the validity of the amyloid cascade hypothesis for AD. Although the amyloid cascade hypothesis was postulated to explain the temporal relationship between Aβ and tau aggregation in AD, the hypothesis in a broader sense posits that the conversion of native proteins into non-native protein aggregates is the primary event in amyloid pathogenesis. Moreover, this hypothesis states that aggregation results in alterations in the cellular microenvironment that lead to pathological changes and, eventually, cell malfunction and degeneration. In this broader sense, the amyloid hypothesis provides a framework for all amyloid disorders. Recent advances in the treatment of familial amyloid polyneuropathy, a transthyretin amyloid disease (BOX 2), support the notion that protein misfolding and aggregation are indeed the primary drivers and that dramatically slowing the process of active aggregation is disease modifying[22,34].

There is mounting evidence that the process of amyloidogenesis is an important driver of neurodegeneration. Amyloidogenesis is dynamic: that is, there is a constant flux of newly synthesized proteins aggregating into a range of transient structures[78,79]. These fleeting and structurally heterogeneous aggregates evolve into progressively larger aggregates[80] and, if the conditions are right, into amyloid fibrils. Presumably, most of the non-fibrillar aggregate structures (FIG. 1) are intrinsically unstable, owing to exposed hydrophobic surfaces and/or the presence of unsatisfied hydrogen bonds, and can engage in aberrant molecular interactions. Amyloid fibrils are less able to engage in inappropriate molecular interactions because of the more extensive burial of hydrophobic residues and the formation of intermolecular hydrogen bonds[6]. It is now clear that nearly all proteins that are capable of forming amyloid are also capable of forming a range of structurally diverse aggregates that seem to be more toxic than amyloid fibrils to cultured cells, but whether this is the case in more relevant proteotoxicity assays and in humans remains unclear.

Autosomal dominant inherited familial amyloid diseases are caused by mutations that can destabilize the native structure of category 1 proteins and thus enable their partial unfolding and render them aggregation prone[33,34,36]. Similarly, mutations in the genes encoding intrinsically disordered category 2 proteins can facilitate aggregation and cause familial forms of amyloidosis[48,81]. An increased concentration of a category 2 protein — for example, α-synuclein through gene duplication in Parkinson disease or Aβ due to APP triplication in Down syndrome-linked AD, or through variations within promoter or regulatory regions of the genes encoding these proteins — can lead to aggregation-associated degenerative pathologies[82–84].

Strategies that target protein aggregation

Protein reduction strategies. The first successful treatment of a human amyloid disease that was not caused by an underlying pathology (such as cancer or inflammation) occurred in the context of the TTR amyloid disease familial amyloid polyneuropathy (FAP) (BOX 2). This strategy involved surgically mediated gene therapy[85,86] (TABLE 1). Familial TTR amyloid diseases are caused by the aggregation of predominantly mutant TTR and, to a lesser extent, wild-type (WT) TTR[87]. As the liver is the source of 95% of the TTR secreted into the bloodstream, replacing the liver of a patient with heterozygous polyneuropathy with a liver from a normal subject, which secretes only WT TTR, abrogates the secretion of mutant TTR into the blood. This resulted in increased serum concentrations of the more kinetically stable, less aggregation-prone WT TTR tetramers (FIG. 3a), which slowed the progression of peripheral and autonomic neuropathy and significantly extended the lifespan of patients with FAP[88,89]. Amyloid clearance seems to occur slowly in patients who have undergone transplantation. The newly available positron emission tomography (PET) amyloid imaging agents should enable the rate of TTR amyloid clearance to be studied more rigorously[90].

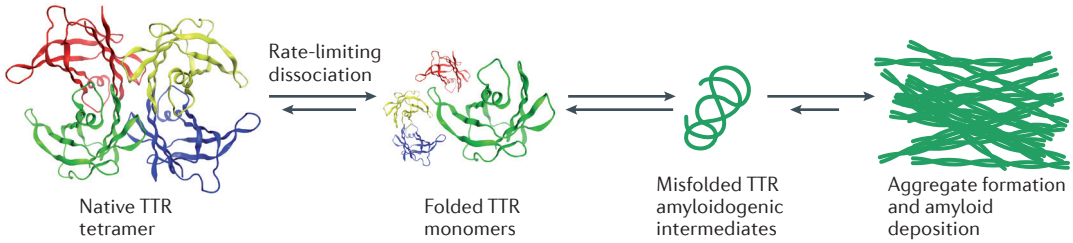
As amyloid clearance does not seem to correlate with clinical benefit, amyloid fibrils per se are unlikely to directly cause the neuronal dysfunction and death associated with FAP[91–93]. However, the fragmentation or incomplete catabolism of amyloid fibrils could contribute to the range of oligomers formed during the process of aggregation (FIG. 1), of which some seem to be more proteotoxic[76]. Liver transplantation might also be useful to treat other familial amyloid diseases wherein the amyloidogenic protein is predominantly secreted by the liver: for example, hereditary fibrinogen amyloidosis[94].

Although the pioneering liver transplant strategy has improved the health and prolonged the lives of a few thousand patients with FAP and demonstrated that intervention is possible after the start of neuropathy, it is not without its challenges. These include the reduced ability to slow disease progression in patients with advanced polyneuropathy, the 10% mortality due to surgical complications, limited organ availability, risk of infection associated with life-long immunosuppression and the emergence of WT TTR aggregation-associated cardiomyopathy upon ageing[95]. In addition, because liver transplant-mediated

Box 2 | **Transthyretin amyloidogenesis**

Transthyretin (TTR) is a natively folded tetrameric category 1 protein rich in β-sheet structures that is secreted into the blood by the liver, into the vitreous by the retinal pigment epithelial cells and into the cerebrospinal fluid (CSF) by the choroid plexus[274–276]. In most people, TTR tetramers are composed of four identical subunits; however, the TTR tetramers in heterozygotic patients with amyloidosis comprise wild-type (WT) and mutant subunits[277]. The established function of TTR in the bloodstream is to transport ~0.5 equivalents of retinol-binding protein bound to vitamin A[278]. TTR carries a substantial quantity of thyroxine (T_4) in the CSF, but in the blood the two T_4-binding sites of TTR are 99% unoccupied owing to the presence of additional T_4 carriers[279]. Substantial biophysical data suggest that in the case of the ≥125 distinct mutations linked to the familial TTR amyloidoses[280] (Mutations in Hereditary Amyloidosis database), incorporation of mutant TTR subunits into a TTR tetramer otherwise composed of WT subunits destabilizes the heterotetramer — leading to faster TTR tetramer dissociation kinetics (kinetic destabilization) and/or an increased population of misfolded aggregation-prone TTR monomers (thermodynamic destabilization)[281,282]. Kinetic destabilization is especially relevant for aggregation and probably also for disease progression, as TTR tetramer dissociation is the rate-limiting step in the amyloidogenesis cascade (see the figure)[25,26]. The subsequent monomer misfolding required for TTR aggregation is much faster than tetramer dissociation[33,68,282–285]. Misfolded TTR monomers efficiently aggregate by a downhill polymerization mechanism (FIG. 2c). Thus, TTR aggregation is not susceptible to seeding, at least as determined by kinetic approaches[68].

Depending on the destabilizing mutation inherited in the familial TTR amyloidoses, TTR aggregation can compromise the function of one or more of the following: the autonomic and/or peripheral nervous systems (familial amyloid polyneuropathy)[1,286], the heart (familial amyloid cardiomyopathy)[287–290], the eyes[96] and the meningocerebrovascular system in the brain[291]. The recently renamed WT TTR amyloidosis — formerly known as senile systemic amyloidosis — results in a type of cardiomyopathy that affects as much as 15% of the population over 80 years of age[287,288,292,293]. It was recently reported that WT TTR aggregation in and around the vasculature may be a more prominent cause of vascular diseases than previously thought[294].



gene therapy does not alter mutant TTR secretion by the choroid plexus into the cerebrospinal fluid or by the retinal pigment epithelial cells into the vitreous of the eye, aggregation of mutant TTR in the eye[96] and in meningeal vessels[97] seems to lead to ophthalmological and neurological symptoms in patients treated by liver transplantation. These challenges warrant the development of the alternative treatments discussed below.

The protein reduction strategy has also been used to successfully treat amyloid LC (AL) amyloidosis[27,98]. AL amyloidosis develops when a proliferating plasma cell (a cancer cell similar to that causing multiple myeloma) secretes a LC protein that is aggregation prone owing to its destabilization and proclivity for misfolding and misassembly[27,34,99]. The misfolding and/or aggregation of LC within the circulatory system lead to dysfunction of multiple organ systems, including the kidney and heart[72,100,101]. The concentration of LC is reduced by eliminating the clonal plasma cells that secrete LC into the blood using various chemotherapy agents, including proteasome inhibitors[27,102] (TABLE 1). This therapeutic strategy (FIG. 3b) leads to a dramatic and rapid improvement in patient health, especially in cardiac function, even though there is overwhelming evidence that the amyloid fibrils are not cleared from the hearts of these patients on this timescale[72]. Although the presence of amyloid in the heart is deleterious, it seems that it is the process of active LC aggregation — including the apparent uptake of proteotoxic misfolded LC monomers or oligomers by cardiomyocytes and other relevant cells — that causes the severe multiple organ system toxicity and pathology[72,94,95]. Unfortunately, ~30% of the patients with AL amyloidosis presenting with severe cardiomyopathy are too sick to tolerate the chemotherapy regimen[98]. Thus, other strategies to ameliorate aggregation-associated LC proteotoxicity are still needed.

An analogous protein reduction strategy has been used to successfully treat systemic AA amyloidosis, which arises in a subset of individuals with chronic inflammatory diseases (for example, rheumatoid arthritis, Crohn disease and recurring infections)[103]. The SAA protein is an acute-phase protein; therefore, sustained high concentrations are produced in response to chronic inflammation. Although high SAA concentrations seem to be a prominent risk factor for the onset and progression of AA amyloidosis[104], they are insufficient to cause it. Apparently, other factors are needed to develop AA amyloidosis, including the endoproteolysis of the SAA protein, which generates an intrinsically disordered amyloidogenic SAA fragment[105]. Kidney damage seems to result from the aggregation of the SAA fragment[3]. Treating the underlying inflammatory disease with anti-inflammatory drugs (TABLE 1) reduces the concentration of SAA and of its amyloidogenic fragments (FIG. 3c), which ameliorates renal dysfunction, providing strong evidence that aggregation of SAA fragments causes AA amyloidosis[106–111].

Table 1 | **Potentially disease-modifying emerging therapeutic strategies**

Therapeutic strategy or agent	Disease	Stage of development	Refs
Protein reduction			
Anti-inflammatory drugs	AA amyloidosis	Regulatory agency approved	106–111,295,296
mRNA reduction	AA amyloidosis	Preclinical	127,128
β-secretase inhibitors and modulators	Alzheimer disease	Clinical trials	117,118
γ-secretase modulators	Alzheimer disease	Clinical trials	122,297
Chemotherapy	AL amyloidosis	Regulatory agency approved	27,102,298,299
Proteasome inhibitors	AL amyloidosis	Regulatory agency approved	27,102,298,299
mRNA reduction	AL amyloidosis	Preclinical	127
Liver transplantation-mediated gene therapy	TTR amyloidoses	Standard of care	85,86,300
	Hereditary fibrinogen amyloidosis		94
RNAi, antisense oligonucleotides	TTR amyloidoses	Clinical trials	125,126,301
Protein stabilization			
Kinetic stabilizers (tafamidis and diflunisal)	TTR amyloidoses	Regulatory agency approved	22–24
Protein quality control			
Small-molecule activators of the heat shock response	Huntington disease and other degenerative disorders	Preclinical	159,160
Small-molecule activators of the unfolded protein response	TTR amyloidoses	Preclinical	146,177
	AL amyloidoses		147
HSP90 inhibition	Alzheimer disease	Preclinical	302
	Huntington disease		162,303
	Parkinson disease		163
Protein reduction and quality control			
Small-molecule HSC70 inhibitors	Alzheimer disease	Preclinical	174–176
Deubiquitylation enzyme inhibitors	Alzheimer disease	Preclinical	178,179
	Parkinson disease		178,179
Amyloid remodelling			
EGCG	Different amyloidogenic proteins	Preclinical	190–196,304
	TTR cardiomyopathy	Clinical report	189
	Parkinson disease	Preclinical	198
Amyloid removal			
Variants of Hsp104 disaggregase	Parkinson disease	Preclinical	217,218
	Amyotrophic lateral sclerosis		217,218
	Frontotemporal dementia		
Catalytic antibodies	TTR amyloidoses	Preclinical	184
	Alzheimer disease		185
Protein reduction and amyloid removal			
Passive immunization, amyloid-β and tau	Alzheimer disease	Clinical trials	204,208, 210–213,305,306
Active immunization	Alzheimer disease	Clinical trials	202–206
Passive immunization	Parkinson disease	Preclinical	210–215

AA, amyloid A; AL amyloidosis, amyloid LC amyloidosis; EGCG, epigallocatechin-3-gallate; HSP, heat shock protein; LC, immunoglobulin light chain; RNAi, RNA-mediated interference; TTR, transthyretin.

Seeding of SAA fragment aggregation by injecting SAA-fragment amyloid fibrils hastened the onset of AA amyloidosis in a mouse model, providing further compelling evidence that the process of aggregation causes the characteristic renal dysfunction[66]. Seeded protein aggregation by a prion-like mechanism is now considered to be a major driver for propagation and intra-organismal spreading of protein aggregation for many amyloid diseases (discussed in more detail below)[8,17]. Not surprisingly, disease severity is also influenced by the exact sequence of the SAA protein that is transcriptionally upregulated by inflammation and generated through aberrant endoproteolytic processing[112].

The protein reduction strategy is also being pursued aggressively in AD research. One approach is to inhibit or modulate the activity of the β-secretases and/or γ-secretase in an effort to reduce Aβ concentration[113–116] (FIG. 3d). After prolonged development phases, due to several challenges including a very limited number of hits in screens, a poor balance of pharmacokinetics, potency and safety, and the lack of effective blood–brain barrier penetration, a new generation of small-molecule β-secretase inhibitors are being evaluated in clinical trials[116–118] (TABLE 1). BACE1 (β-site APP-cleaving enzyme 1) is the main β-secretase enzyme in the brain that initiates Aβ generation (FIG. 3d). Promising BACE1 inhibitors are emerging in Phase I clinical trials, raising hope that a good safety profile, good pharmacokinetics and significant Aβ-load reduction by BACE1 targeting are achievable, which will hopefully translate into a disease-modifying AD treatment. Other compounds exhibiting BACE1 selectivity over BACE2, which is a homologue with low neuronal expression, are being developed by several companies and are beginning to emerge in the patent literature. Complete inhibition of BACE1 will probably not be necessary to ameliorate AD pathogenesis, owing to the striking concentration dependence of Aβ aggregation, although *Bace1*-knockout mice exhibit mild phenotypes[119].

A multi-protein complex with four essential subunits, γ-secretase conducts intramembrane cleavage (FIG. 3d) of numerous substrates[120]. The nicastrin and presenilin enhancer 2 (PEN2) subunits are identical in every γ-secretase complex, whereas the anterior pharynx defective 1 (APH1) subunit has two subtypes — APH1A and APH1B — as does the catalytic subunit, which is either presenilin 1 (PSEN1) or PSEN2. Challenges arose when it was discovered that γ-secretase cleaves not only APP to produce the Aβ peptides but also more than 100 protein substrates, one of which is Notch, an important cellular signalling protein[121]. Studies and clinical trials undertaken so far suggest that direct γ-secretase inhibition may be problematic as a long-term pharmacological strategy for AD owing to the strong mechanism-based toxicity observed (TABLE 1). Current efforts are focused on modulating γ-secretase substrate specificity using small molecules that function by an allosteric mechanism (TABLE 1), through which shorter, non-amyloidogenic Aβ fragments are generated without compromising the cleavage of other important γ-secretase substrates[121,122]. Emerging knowledge about the assembly, regulation and specificity of the γ-secretase complexes is expected to guide these endeavours and has been reviewed elsewhere[120,121].

Another protein reduction approach is to inject antibodies (passive immunization) into patients with AD. These antibodies bind to Aβ peptides to promote their clearance and thus reduce Aβ aggregation[123] (TABLE 1). Based on clinical trial data so far, this seems to be a promising approach for reducing amyloid load and continues to be pursued intensively. An active immunization strategy (vaccination) using the appropriate antigen could ultimately produce these protein reduction antibodies[124]. Immunization strategies for amyloid disorders are discussed in more detail below.

Gene silencing by RNA-mediated interference (RNAi) or antisense oligonucleotide technology (FIG. 3e) is another protein reduction strategy that is being actively pursued. The reduction of both WT and mutant TTR protein levels in the bloodstream has been successfully achieved by applying either of the gene silencing technologies in the liver[125,126] (TABLE 1). TTR FAP clinical trials to test TTR RNAi (Alnylam Pharmaceuticals) and to evaluate the efficacy of an antisense TTR oligonucleotide approach (Isis Pharmaceuticals, Inc.) (FIG. 3e) are currently enrolling patients. Analogous mRNA reduction approaches are also being explored in tissue culture and animal models for other amyloidoses, including AL amyloidosis[127,128].

Protein stabilization strategy. A different approach for preventing the detrimental effects of misfolding and aggregation of category 1 proteins is the 'protein stabilization' or 'kinetic stabilization' strategy. Portuguese family members carrying the FAP V30M *TTR* mutation, but who exhibited a very mild polyneuropathy phenotype or no obvious pathology[129], were found to be compound heterozygotes, expressing V30M *TTR* from one allele and T119M *TTR* from the other allele. This resulted in the secretion of TTR heterotetramers comprising both V30M and T119M subunits in the expected stoichiometries[25]. Biophysical studies showed that T119M TTR subunit incorporation into tetramers otherwise composed of the disease-associated V30M TTR subunits, proportionately reduces the rate of tetramer dissociation and thus the rate of aggregation[25,26], by destabilizing the dissociative TTR transition state and thereby increasing the activation energy for tetramer dissociation[130,131]. This kinetic stabilization mechanism provides strong evidence that the process of aggregation or amyloidogenesis is the cause of these maladies: that is, the V30M subunits within the context of natively folded tetramers are not in themselves proteotoxic[33,131]. We hypothesize that V30M TTR aggregates expose hydrophobic surface area and unsatisfied hydrogen bonds that enable aberrant interactions with proteins, lipids, nucleic acids and carbohydrates, rationalizing how the process of TTR aggregation becomes proteotoxic. Although TTR concentration is not reduced in this strategy, the proportion of TTR that can dissociate, misfold and aggregate is dramatically reduced by this interallelic *trans*-suppression kinetic stabilization mechanism[132].

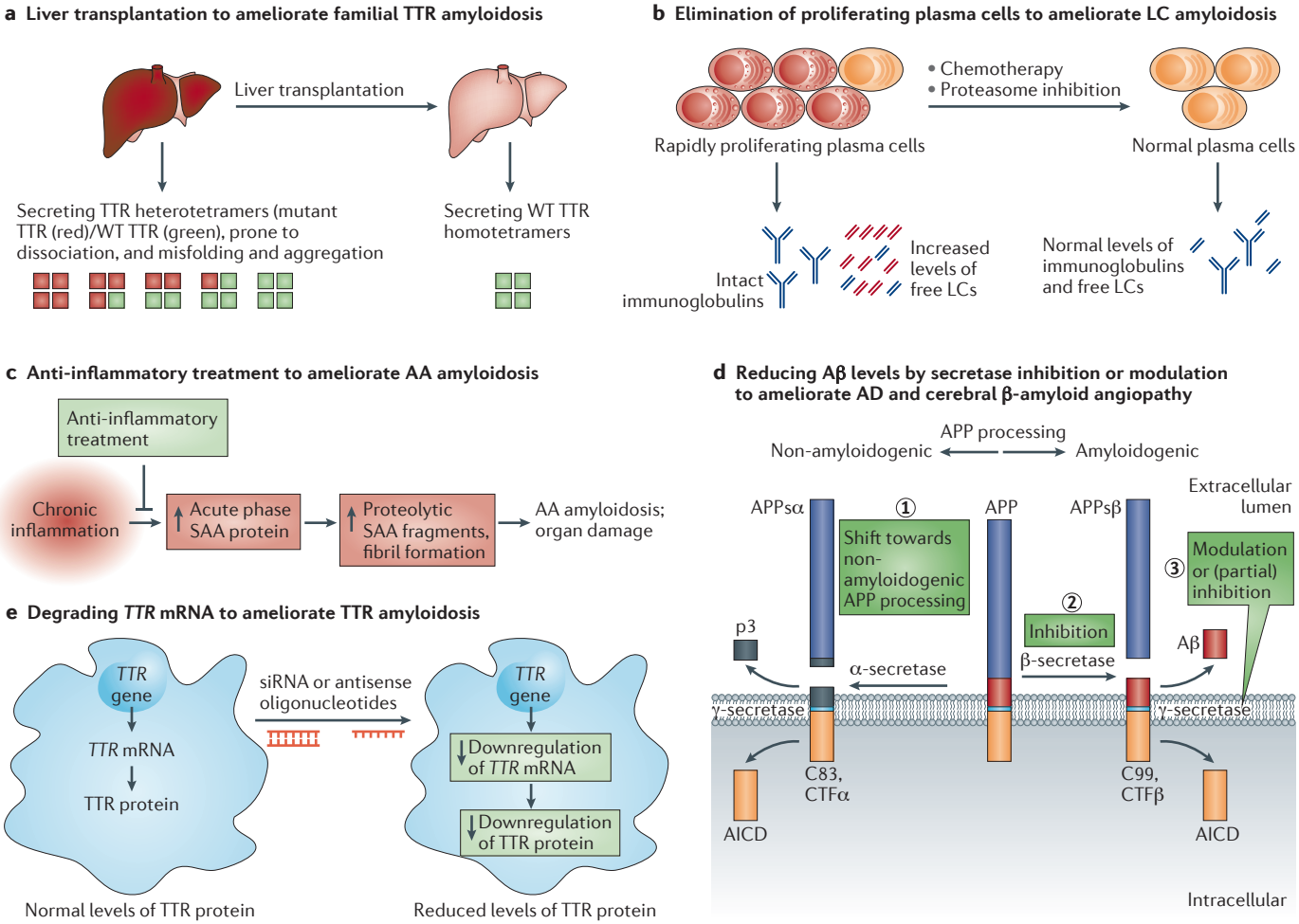


Figure 3 | **Therapeutic strategies to ameliorate amyloidoses.** **a** | Gene therapy by liver transplantation has been used to treat familial amyloid polyneuropathy (FAP). As transthyretin (TTR) is largely produced by the liver, liver transplantation was introduced in the 1990s as a therapy for patients with early-stage FAP. Tetramers composed of wild-type (WT) TTR monomers (depicted by green squares) produced by the donor liver are much less amyloidogenic than the heterotetramers, which include mutant TTR monomers (depicted by red squares), produced by the recipient's liver before transplant. This approach successfully slows the progression of FAP and extends lifespan, but requires organ availability and life-long immunosuppression and is associated with 10% mortality owing to the transplant procedure. **b** | Protein reduction can be mediated by chemotherapy to treat amyloid immunoglobulin light chain (LC) amyloidosis (AL amyloidosis). The amyloidogenic protein in AL amyloidosis is a LC, or a fragment thereof, overproduced owing to a plasma cell dyscrasia. Chemotherapy regimens, with or without autologous stem cell transplantation, can be used to remove the proliferating cancerous plasma cells that produce the amyloidogenic LCs in patients without major cardiac involvement who are well enough to tolerate these aggressive regimens, which leads to apparently durable disease remission. **c** | Anti-inflammatory treatment dramatically reduces acute-phase serum amyloid A (SAA) protein production for the treatment of AA amyloidosis. Systemic AA amyloidosis is a long-term complication in some patients suffering from chronic infection or chronic inflammation (for example, chronic inflammatory arthritis). The amyloidogenic protein is derived by proteolysis of SAA, an acute-phase protein that is transcriptionally upregulated during inflammation. Persistent high concentrations of SAA fragments in the plasma above a critical threshold for aggregation can trigger AA deposition. Treatment of the underlying infectious or inflammatory trigger can reverse this type of amyloidosis, if it is diagnosed early. **d** | Reduction of pathogenic amyloid-β (Aβ) levels by secretase inhibition or modulation has therapeutic potential for Alzheimer disease (AD) and cerebral Aβ angiopathy. The β-amyloid precursor protein (APP) is a transmembrane protein that is constitutively cleaved by secretases. Endoproteolytic processing via the non-amyloidogenic pathway (that is, α-secretase cleavage followed by γ-secretase cleavage (shown on the left)) generates non-amyloidogenic APP fragments. By contrast, processing by β-secretase followed by γ-secretase cleavage produces amyloidogenic Aβ peptides. Aggregation of Aβ peptides into extracellular amyloid fibrils or plaques is a histopathological hallmark and a diagnostic criterion for AD. Reduction of Aβ peptide concentration can be achieved by one or more of three mechanisms: shifting APP processing towards non-amyloidogenic endoproteolysis (mechanism 1); inhibition of β-secretase (mechanism 2); and inhibition or modulation of γ-secretase (mechanism 3). Inhibition of γ-secretase was shown to be feasible experimentally; however, side effects are a concern owing to its many substrates. Interestingly, γ-secretase cleavage generates Aβ peptides of various lengths: as a rule of thumb, the longer the peptide, the more amyloidogenic it is. Modulation of γ-secretase processing of APP to generate shorter, less amyloidogenic Aβ peptides is currently being investigated[122]. **e** | Gene silencing by short interfering RNA (siRNA) or by antisense oligonucleotides has potential for the treatment of FAP. Recently published data from a small placebo-controlled clinical trial have identified siRNA sequences that reduce mutant and WT TTR protein production in patients with FAP[125]. Analogous clinical data on antisense oligonucleotides that degrade *TTR* mRNA and thus reduce TTR production and secretion into the blood from the liver are promising[126]. AICD, APP intracellular domain; APPsα, extracellular domain of APP released on cleavage by α-secretase; APPsβ, extracellular domain of APP released on cleavage by β-secretase; CTF, carboxy-terminal fragment.

The data outlined directly above demonstrate that TTR aggregation is suppressed and that various pathologies are ameliorated via the kinetic stabilization approach. This genetic evidence from humans motivated efforts to discover small molecules that could prevent TTR aggregation through an analogous kinetic stabilization of the TTR tetramer[26,130,131,133,134]. Initial *in vitro* studies showed that when thyroxine (T_4) bound to the T_4-binding sites within TTR, the tetramer was stabilized and TTR amyloidogenesis was inhibited[133]. This finding led to a screening-based[135] and structure-based drug design effort[136,137] to discover small-molecule ligands that selectively and avidly bind to the TTR tetramer over the dissociative transition state, kinetically stabilizing the native non-amyloidogenic tetramer and thereby slowing the rate-limiting step of amyloidogenesis[26,130,131].

More than 1,000 small molecules exhibiting structural complementarity to the T_4-binding sites within TTR have been synthesized during the past 20 years and more than 100 $(ligand)_2$•TTR crystal structures have been solved, enabling the synthesis of very potent kinetic stabilizers that are not thyroid agonists or antagonists[130,134,136,137]. One of these, tafamidis (Vyndaqel; Pfizer)[31,132], was evaluated in a placebo-controlled clinical trial and in a follow-on open-label study[22,23]. Both primary end points were met in the 'efficacy-evaluable' population ($n = 87$) but were just missed in the 'intent-to-treat' population ($n = 125$), which was apparently due to more patients than expected in the intent-to-treat population being selected for liver transplantation during the course of the trial, not as a consequence of treatment failure. However, transplanted patients were classified as treatment failures in the conservative analysis used[22,23]. Tafamidis (Vyndaqel 20 mg once daily) is now approved for the treatment of early-stage TTR-related hereditary amyloidosis (with polyneuropathy, all mutations) by the regulatory agencies of Europe, Japan, Mexico and Argentina. Tafamidis is currently being considered for approval by the neurological division of the US Food and Drug Administration (TABLE 1).

In the process of searching for TTR kinetic stabilizers, we demonstrated that the non-steroidal anti-inflammatory drug (NSAID) diflunisal (Merck) binds to and kinetically stabilizes the TTR tetramer[138,139]. An international randomized double-blind placebo-controlled clinical trial showed the ability of diflunisal to reduce the rate of progressive neurological impairment and to preserve quality of life in patients with FAP, thus enabling diflunisal to be repurposed as a TTR kinetic stabilizer[24] (TABLE 1). Even though diflunisal is not very potent as a TTR kinetic stabilizer, it efficiently kinetically stabilizes TTR at a dose of 250 mg twice daily because of its very high oral bioavailability and its correspondingly very high plasma concentration (~300 μM–1 mM). As diflunisal is a NSAID, it slows renal blood flow, which is a contraindication for those patients exhibiting cardiomyopathy or renal compromise.

Collectively, the tafamidis and diflunisal clinical trial results provide compelling pharmacological evidence that the process of TTR amyloidogenesis causes neurodegeneration and that slowing tetramer dissociation and amyloidogenesis after symptom presentation by small-molecule kinetic stabilizers dramatically slows and, in up to 60% of the patients, stops the progression of neurological impairment. These data strongly support the validity of the amyloid hypothesis: that is, the notion that the process of aggregation causes postmitotic tissue loss[131]. These data motivated the tafamidis cardiomyopathy clinical trial that is currently underway.

Protein quality-control strategy. Ageing is the most significant risk factor for the development of either sporadic or inherited amyloidoses, for reasons that are still not fully understood[140]. One hypothesis, which is gaining experimental support[141], is that the capacity of the cellular and/or extracellular protein homeostasis networks declines with age. Another possibility, which is not mutually exclusive, is that the ability to activate the stress-response signalling pathways that regulate proteostasis network functions declines with age[141–144]. The classic view of amyloid disease aetiology is that the aggregation of a specific protein causes the aggregation-associated degenerative phenotype through a toxic gain-of-function type of mechanism (FIG. 1). However, there is mounting evidence that the intracytosolic aggregation of proteins linked to specific amyloid diseases compromises the folding of the endogenous proteome by sequestering chaperones and chaperonins, resulting in the aggregation of proteins required for critical cellular functions such as controlling transcription[141,145]. Although there is no doubt that the conformational integrity of one protein is compromised in specific amyloid diseases, it now seems that the native conformations, and thus function, of many additional proteins could be compromised owing to consumption of proteostasis network capacity. This suggests that a breakdown in the ability to maintain the proteome is a part of degenerative disease aetiology, if not the primary cause[57,141,145].

Thus, a 'protein quality-control strategy' could be beneficial for ameliorating the amyloidoses. This approach focuses on adapting the intracellular and/or extracellular proteostasis pathways involved in protein folding, trafficking and/or degradation to reduce the aggregation of disease-associated proteins and possibly additional cellular proteins[146–152]. Adaptation of these cellular proteostasis pathways alters the intracellular interactions between destabilized aggregation-prone proteins and the proteostasis network components that compose these pathways, providing the opportunity to attenuate proteotoxic aggregation directly or indirectly. This protein quality-control strategy has been primarily pursued through the activation of organelle-selective stress-response signalling pathways such as the heat shock response (HSR) or the unfolded protein response (UPR) that transcriptionally regulate proteostasis capacity in specific intracellular environments, such as the cytosol or the secretory pathway, respectively[153–157]. Thus, genetic and small-molecule stress-independent activators of individual stress-response signalling pathways are now being developed[146,158,159] (TABLE 1).

Activation of the HSR with celastrol, which increases the cytosolic proteostasis network capacity, has been used in the context of preclinical Huntington disease models to validate the protein quality-control therapeutic

strategy[160]. This molecule primarily functions through the activation of heat shock factor 1 (HSF1; the predominant transcription factor responsible for stress-response upregulation of cytosolic proteostasis network components via the HSR)[154,155]. These small molecules (TABLE 1) exhibit considerable promise for ameliorating pathological intracellular protein aggregation, although not all reports agree on whether HSR activation is beneficial[161].

Another strategy to induce the HSR is to inhibit the cytosolic ATP-dependent chaperone heat shock protein 90 (HSP90). HSP90 binds to and negatively regulates HSF1. Inhibition of HSP90 may prevent its binding to HSF1 and also lead to the misfolding and/or misassembly of many cytosolic proteins that in turn activate HSF1, resulting in transcriptional reprogramming of the cytosolic proteostasis network via the HSR. HSP90 inhibition ameliorates aggregation and proteotoxicity in several experimental models of aggregation-associated degenerative diseases, including Huntington disease, Parkinson disease and AD[162,163]. HSF1 can also be activated indirectly by reducing insulin and insulin growth factor 1-like signalling (IIS), which provides considerable benefits in AD and Huntington disease models, and the FOXO (forkhead box O) transcriptional programme (or programmes) is also likely to contribute[75,164]. Chemical, genetic and pharmacological approaches (TABLE 1) have recently become available to activate HSF1 in the absence of stress or IIS inhibition[158,159].

Increasing cytosolic proteostasis network capacity through the overexpression of the molecular chaperone Hsp70 was also found to significantly reduce proteotoxicity and neurodegeneration in models of Huntington disease[165] and Parkinson disease[166]. However, other studies did not observe the same effect[167], possibly owing to different relative stoichiometry of the proteostasis network components resulting from differences in Hsp70 overexpression. The binding of Hsp70 or the constitutive chaperone Hsc70 to α-synuclein aggregates was shown to reduce toxicity[168] and may even act extracellularly on oligomer formation and protect against trans-synaptic spreading of aggregates[169].

Although the Hsp70–Hsp40–nucleotide exchange factor pathway is often considered to be a pro-folding pathway, there is abundant evidence that this pathway makes critical folding-versus-degradation, or quality-control, decisions[152,170–173]. Thus, small-molecule modulators of this pathway could be used to enhance the degradation of certain aggregation-prone client proteins[174–176]. This approach also falls into the protein reduction therapeutic strategy category. There is evidence that Hsc70 stabilizes tau against degradation in the brain[151], whereas Hsc72 recruits the co-chaperone E3 ubiquitin ligase CHIP, which is known to ubiquitylate tau and facilitate its degradation[176]. Thus, inhibitors of Hsc70 should partition tau in the brain into the Hsc72–CHIP pathway, enabling its degradation. The allosteric small-molecule Hsc70 inhibitors YM-01 and YM-08 (TABLE 1) both decreased tau levels in various preclinical brain-relevant model systems, suggesting that this therapeutic strategy is promising[175].

Extracellular protein aggregation can also be attenuated by activating the UPR, which regulates proteostasis (quality control) in the ER and in downstream compartments of the secretory pathway[156,157], including the extracellular space[177]. The UPR signalling pathway consists of three arms that are activated through the ER transmembrane stress-sensor proteins IRE1, activating transcription factor 6α (ATF6α) and PERK (also known as EIF2AK3). Activation of these arms adapts ER proteostasis through both transient attenuation of new protein synthesis (downstream of PERK) and activation of stress-response transcription factors, including XBP1s (downstream of IRE1), ATF6α (a cleaved product of full-length ATF6) and ATF4 (downstream of PERK). These transcription factors upregulate components of the ER protein-folding, trafficking and degradation pathways that constitute the ER proteostasis network.

Arm-selective activation of the UPR seems to be a very promising strategy for increasing the stringency of protein quality control in the secretory pathway of human cells, by selectively reducing secretion of destabilized amyloidogenic proteins[146,147]. The hypothesis underlying this strategy is that transcriptional remodelling of the ER proteostasis network through the UPR could lead to increased partitioning of amyloidogenic mutant proteins (for example, V30M TTR) to ER degradation pathways, including ER-associated degradation (ERAD) or autophagy, while still allowing efficient secretion of the more stable WT protein (for example, WT TTR) at normal levels[146]. To date, preclinical results from this approach are encouraging. For example, selective activation of the ATF6 arm of the UPR leads to a ~40% reduction in the secretion of a destabilized A25T TTR variant, while permitting efficient secretion of WT TTR[146]. Importantly, the preferential reduction in secretion of destabilized TTR afforded by ATF6 activation corresponds to increased partitioning of the destabilized TTR variants to ER degradation pathways, indicating that the reduced secretion of destabilized TTR does not lead to the intracellular accumulation of destabilized, aggregation-prone TTR that could aggregate in the ER. The ATF6-dependent reduction in destabilized TTR secretion reduces the extracellular concentration of destabilized TTR available for concentration-dependent aggregation[146]. Both the selective degradation of an energetically compromised TTR variant and the global reduction in extracellular concentrations of destabilized TTR will reduce the TTR aggregation propensity in the extracellular space. Moreover, ATF6 activation results in the secretion of an Hsp40 chaperone, ERdj3 (also known as DNAJB11), which can further reduce aggregation of amyloidogenic proteins in the extracellular space[177]. Stress-independent activation of UPR-associated transcription factors has also recently been shown to reduce the secretion of a destabilized amyloidogenic LC, without affecting the secretion of an energetically normal LC[147].

Such a dual quality-control and protein reduction strategy is also a very attractive approach for enhancing the degradation capacity of the cytosolic proteostasis network, which is currently being investigated in patients

with degenerative diseases[178,179]. For example, the concentration of α-synuclein or tau can be reduced by diminishing the negative regulation of the proteasome[178,179]. The deubiquitylation enzymes (DUBs) are proteasome-associated enzymes that inhibit ubiquitin-mediated proteasomal degradation by removing ubiquitin from the client protein. Thus, DUB inhibitors are being developed by several companies to enhance the degradation of client proteins, including α-synuclein and tau, to treat neurodegenerative diseases[171,179]. Small molecules that inhibit the DUB ubiquitin-specific peptidase 14 (USP14) (TABLE 1) are being actively pursued to enhance clearance of aggregation-prone proteins.

Proteolysis strategy. Proteases such as the Aβ-degrading enzyme neprilysin were shown to degrade monomeric Aβ, and possibly also low-*n* oligomeric Aβ[180], an observation that may potentially be exploited therapeutically[181,182]. A concern is that Aβ amyloid fibrils or late-stage oligomers are no longer enzymatically degradable owing to their kinetic stability or slow dissociation to a monomer that neprilyin acts on. However, it is not inconceivable that depletion of the monomer pool could not only prevent aggregate formation but also promote fibril dissociation owing to the change in equilibrium concentrations (see 'Strategies that target amyloid fibrils' below). Consistent with this hypothesis, upregulation of neprilysin levels can delay Aβ deposition in APP transgenic mice[181]. Such an approach may be most useful as a preventive treatment or in the early stages of AD[183].

Catalytic antibodies, or 'catabodies', that bind to and proteolytically degrade amyloidogenic proteins are being explored as possible biological drugs. If subsets of such catabodies could recognize a toxic and/or amyloidogenic epitope of a protein that is not exposed in the natively folded form — that is, differentiate the native form from the toxic form — then such catabodies could hold promise for specific degradation of toxic protein structures. Importantly, degradation by catabodies should not elicit an inflammatory response. A recent study reported immunoglobulin M-class catabodies that degrade both misfolded soluble and misfolded particulate forms of TTR (TABLE 1), but not the physiological form, suggesting a protective function of such catabodies against TTR amyloidogenesis[184]. The same laboratory recently engineered catabodies capable of degrading aggregated Aβ[185] (TABLE 1).

Strategies that target amyloid fibrils

In addition to therapeutic strategies that aim to prevent protein misfolding, and therefore the initiation and propagation of aggregation, approaches to remove or inactivate amyloid fibrils and other higher-order aggregates are emerging. Owing to the long presymptomatic phase of amyloid diseases, initiation of treatment may only be feasible after substantial protein deposition has occurred, especially in sporadic cases. Removal of existing aggregates is advisable, as these aggregates can themselves be toxic. Moreover, amyloid fibrils are known to dissociate[186–188]; thus, it is possible that amyloid fibril dissociation into diffusible alternative structures with higher toxicity could continue to drive pathology in some patients treated by a protein reduction, kinetic stabilization or protein quality-control strategy. Therefore, it might be necessary to combine one of the aforementioned approaches with strategies that target the amyloid fibrils themselves, such as amyloid crosslinking, remodelling, removal or disaggregation coupled to degradation. Elimination or inactivation of the existing amyloid load should also prevent the self-sustaining cascade of seeded or prion-like misfolding that is hypothesized to have an important role in driving the propagation of many neurodegenerative protein misfolding disorders (see below).

Amyloid remodelling. A recent observational report suggests that epigallocatechin-3-gallate (EGCG), the principal polyphenol present in green tea and an amyloid fibril binder and crosslinker, is effective at slowing the progression of cardiac TTR amyloidosis[189]. EGCG (TABLE 1) is able to remodel protein aggregates — including amyloid fibrils and oligomers, which comprise different amyloidogenic proteins — although the mechanistic underpinnings of this process are not entirely clear[190–197]. Ample evidence shows that EGCG amyloid remodelling activity *in vitro* is dependent on auto-oxidation of the EGCG molecule. The amyloid remodelling that occurs in response to EGCG treatment seems to be driven by hydrophobic binding of oxidized EGCG molecules to amyloid fibrils, followed by oxidized EGCG reacting with free amines in the amyloid fibrils, which crosslinks the fibrils[193,196]. Amyloid remodelling seems to stabilize the aggregates and prevent the formation of toxic oligomers, possibly explaining the reduction in proteotoxicity in the patients who have experienced clinical benefit from taking EGCG[189]. A recent study suggests that EGCG can also reduce the toxicity of α-synuclein oligomers *in vitro*, presumably by binding to the oligomers and thereby moderately reducing their toxic interaction with membranes[198]. It was also shown that EGCG is able to direct α-synuclein aggregation towards the formation of off-pathway, non-toxic oligomers, to prevent seeding of α-synuclein aggregation, and to remodel α-synuclein fibrils to form smaller non-toxic aggregates[190,191].

Amyloid removal by immunization. Another approach to remove amyloid deposits from the tissue is passive or active immunization against the amyloidogenic protein. The binding of antibodies to the monomeric protein, to oligomers and/or to fibrils can direct the degradation of these structures by phagocytic cells, resulting in their clearance. This strategy is being intensively explored in the context of AD. Indeed, in APP transgenic mice, immunization against Aβ peptides elicits an anti-Aβ immune response and is effective at removing Aβ deposits[199]. Importantly, active immunization was also effective in preventing Aβ deposition when it was initiated during the pre-depositing stage (before amyloid deposition had occurred). Additional studies confirmed these findings, found improved behavioural and cognitive performance[200,201] and suggested that this

approach was suitable for removal of Aβ deposits in such mice[32]. Unfortunately, a clinical trial investigating active immunization against Aβ in patients with late-stage AD showed serious adverse events — specifically, aseptic meningoencephalitis — in ~6% of the patients, forcing Élan Corporation to halt the trial in 2002 (REF. 202). Post-mortem analysis of the brains of patients who participated in this trial confirmed that the Aβ load was reduced and provided evidence that amyloid fibrils were removed by immunotherapy, showing that the biological outcome was met[203,204]. Closer examination suggests that extracellular plaques are cleared first and that vascular Aβ deposits remain longer, or may even increase owing to clearance of solubilized Aβ along perivascular drainage pathways[205,206]. Several companies (Elan, Janssen, Novartis, Pfizer and others) have explored modified active immunization approaches to improve safety: for example, with shorter Aβ peptides. However, no major disease modification has been observed as yet.

Passive immunization trials with monoclonal antibodies against Aβ also demonstrate that the Aβ plaque burden in mice is cleared[123,207]. Hopes remain high that the passive immunization approach will be a safe alternative, as it does not elicit an uncontrolled autoimmune response (reviewed in REF. 208). Although the AD passive immunization trials completed so far have demonstrated the removal of Aβ deposits, significant improvement of cognitive performance, slower progression of the disorder and improved survival were not observed in these patients with later-stage AD[204,208]. However, recent data from a Phase Ib study has shown that aducanumab (Biogen) treatment resulted in statistically significant reductions in amyloid load and a reduction in cognitive decline in patients with prodromal or mild AD, providing hope for the use of this strategy. Several other Aβ-specific antibodies continue to be explored in clinical trials; for example, solanezumab (Eli Lilly) is currently in Phase III trials and has been reported to be well tolerated and to show a small benefit in patient subpopulations with mild AD[307]. Thus, new passive immunization trials are focusing on early treatment of patients carrying AD risk genes[209].

Similar immunization strategies are also being developed for intracellular amyloid disorders such as tauopathies and synucleinopathies. Promising results were obtained in transgenic mouse models, and these therapies are progressing towards clinical trials[210–215]. An exploratory Phase I study targeting tau by active immunotherapy (AADvac1; Axon Neuroscience SE) has recently been completed. The safety profile seems promising, and a follow-up study continues to observe patients who have completed the Phase I trial. With regard to α-synuclein immunization, AFFiRiS conducted a small Phase I trial and reported in a recent press release that the immunization regimen (vaccine PD01A for Parkinson disease) seems to be well tolerated and safe, and that most participants developed α-synuclein-specific antibodies. As the development of immunization strategies against tau and α-synuclein is at an early stage, it is currently unknown whether there is a disease-modifying effect in humans.

Disaggregation of misassembled proteins. Enzymatic disaggregation of misfolded and misassembled proteins linked to degradation may be a viable strategy to clear amyloid deposits from tissue. The proteostasis networks of yeast and bacteria have established disaggregases: that is, Hsp104 and ClpB, respectively[216]. Despite intensive efforts, the mammalian equivalent of these disaggregases has not yet been identified and may not exist. However, it may still be possible to engineer yeast Hsp104 or bacterial ClpB to disaggregate certain amyloids and thus use these variants as injectable protein drugs. For example, Jackrel *et al.* showed that 'potentiated' variants of Hsp104 (TABLE 1) that were discovered by screening were able to reduce proteotoxicity of TDP43, FUS and α-synuclein in model organisms[217,218].

It was recently demonstrated that α-synuclein aggregates could be disassembled by the Hsp70–Hsp40–nucleotide exchange factor pathway; however, the more kinetically stable Aβ amyloid fibrils could not be disassembled[219]. It may be the case that multicellular organisms abandoned a disaggregation strategy as the primary detoxifier, instead using autophagy or other pathways ending in lysosomal degradation to purge aggregates because of the risk of dissociating amyloid fibrils to more toxic structures. Strategies to use autophagy enhancement therapeutically are currently being explored but are currently limited by our incomplete understanding of this process[220,221].

Inhibition of seeded and aggregate cell-to-cell spreading. For several amyloidogenic proteins, the transition from their native structures to amyloid seems to follow a nucleation-dependent polymerization process[61,65,222]. In this mechanistic paradigm, the formation of the nucleus (a putative β-sheet-rich species) is energetically unfavourable and is the rate-limiting step for efficient aggregation (FIG. 2a). The formation of such a nucleus may not occur during the lifetime of a healthy individual; if nucleus formation does occur, it should typically be identified and neutralized via one of the mechanisms used by the intracellular or extracellular proteostasis networks[148]. However, if the amyloid nucleus escapes neutralization or degradation in one rogue cell or one extracellular location within the body, it can act as a template for the further aggregation of soluble homotypic proteins[223] (FIG. 2a). The resulting higher-order aggregates are referred to as 'seeds' because their addition to a monomeric solution immediately initiates aggregation of the homotypic protein without a requirement for nucleus formation. This seeded, or templated, aggregation is relatively efficient and relentlessly progressive, and initiates a fatal cascade of progressive aggregation that also seems to drive proteotoxicity and tissue degeneration (FIG. 4).

This concept of seeded aggregation was first hypothesized to explain the pathogenesis of prion diseases[62,224]. It is now widely accepted that prion disorders arise from misfolded and misassembled prion protein (PrP^{Sc}), which is sufficient to act as a seed, thus promoting sustained aggregation of the normal, cellular prion protein (PrP^{C})[224]. In sporadic prion disorders — for example, Creutzfeldt–Jakob disease in humans — PrP^{Sc} nucleus

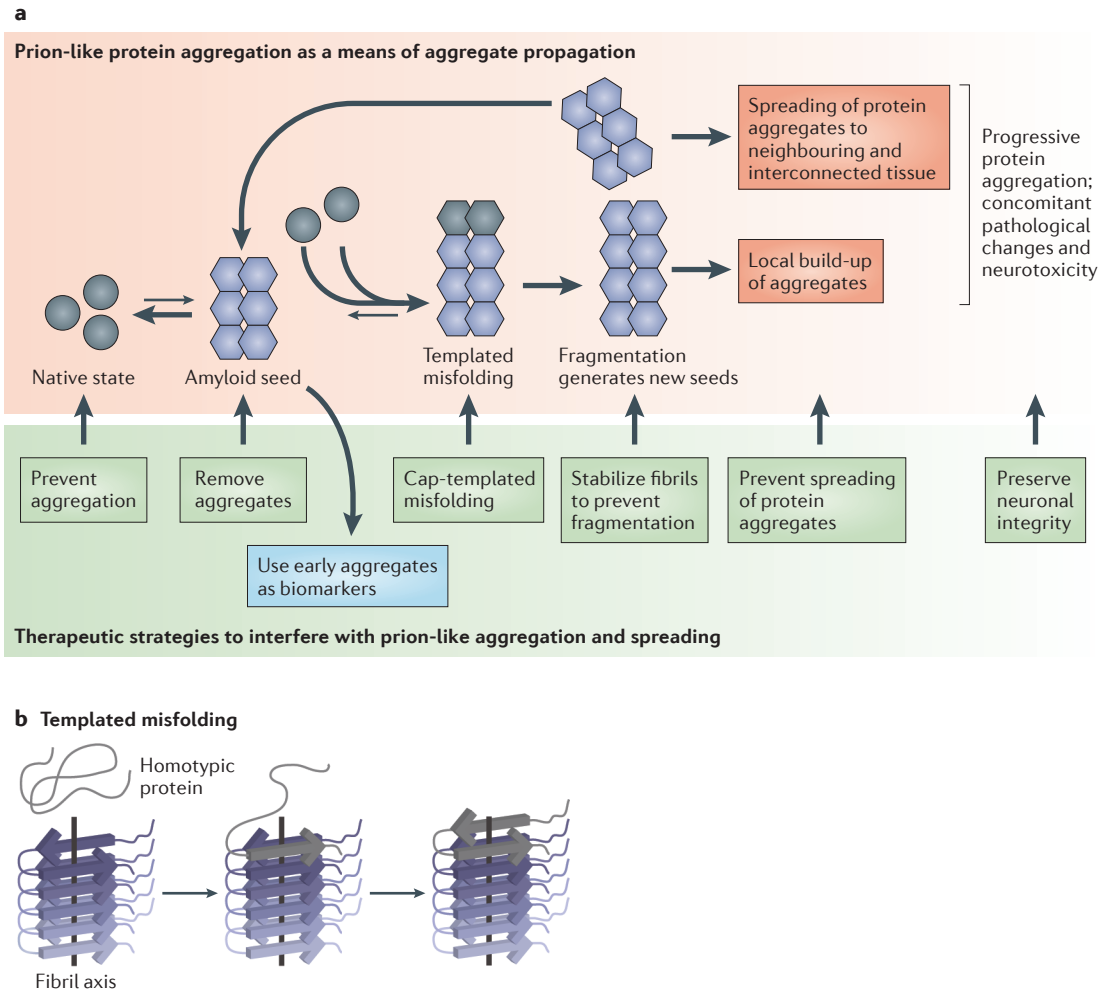


Figure 4 | **Prion-like protein aggregate spreading: disease initiation, progression and therapeutic strategies.** **a** | Recent advances have shown a prion-like spreading (also referred to as templated misfolding or seeded aggregation) of protein aggregation for several amyloidogenic proteins. In this cascade of events, the amyloidogenic protein, once misfolded and aggregated, catalyses the misfolding and incorporation of the native, homotypic protein into the growing aggregate. Fragmentation generates new seeds, and the vicious cycle of templated misfolding and aggregation leads to the spreading of aggregation to neighbouring and interconnected tissue, assisted by cellular aggregate uptake and aggregate secretion. This mechanistic paradigm argues for an early intervention or preventive treatment before the aggregation spreading cascade progresses out of control. Several therapeutic strategies are possible (shown in the green boxes), including preventing or stopping active aggregation, removing aggregates, inhibiting templated misfolding, stabilizing aggregates against fragmentation and/or altering their structure, preventing the cellular uptake and secretion of aggregates, and preserving neuronal integrity. **b** | Amyloid fibrils often adopt an in-register cross-β-sheet arrangement, wherein the strands are oriented perpendicular to the fibril axis. The amyloid fibril can serve as a template to incorporate the homotypic monomer into the growing fibril. The same protein can adopt distinct amyloid structures or strains, which can be reliably propagated by templated misfolding, and which seem to be associated with different pathogenic phenotypes. Part **b** is adapted with permission from REF. 223, Wiley.

and subsequent aggregate (seed) formation occurs spontaneously. This is followed by aggregate spreading by a seeding mechanism. Prion seeds (of PrP^{Sc}) are also known to be transmissible between individuals: for example, as seen in very rare instances of surgical instrument contamination that occurred in the past, before adequate testing and precautions were implemented; by funerary cannibalism, as in kuru; and, more commonly, by uptake of prions from the environment, as in bovine spongiform encephalopathy (mad cow disease) and in scrapie in sheep[224].

Several human amyloid diseases are now hypothesized to spread within the affected tissue by a seeded aggregation or prion-like mechanism; however, there is currently no evidence for transmissability between humans, in contrast to the very rare transmissible prion disorders[225]. The evidence for seeded spreading of Aβ, tau and α-synuclein aggregates comes mainly from *in vitro* studies and animal models. However, the pathology observed during the course of the disease in the human brain provides additional support for this hypothesis, as pathology seems to spread via neurons known to be connected to one another.

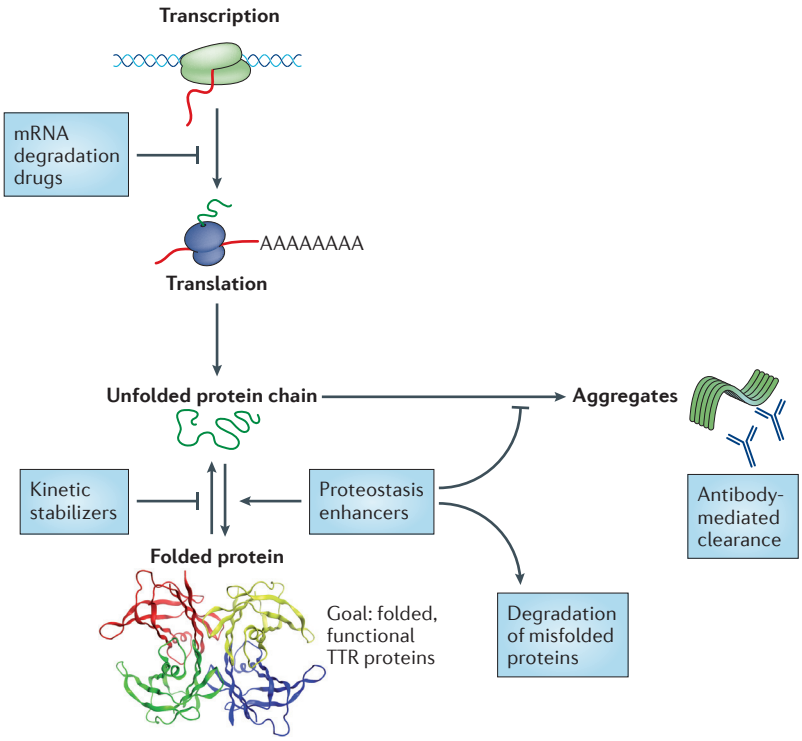


Figure 5 | **Combining therapeutic strategies to ameliorate protein misfolding and aggregation diseases.** We envision that, in the future, amyloid diseases featuring degenerative phenotypes will be treated using combinations of drugs exhibiting distinct mechanisms of action. For example, we anticipate treating the transthyretin (TTR) amyloidoses with kinetic stabilizers (such as tafamidis, which is already in clinical use) and *TTR* mRNA degradation drugs (currently in clinical trials), or by using a kinetic stabilizer in combination with drugs that enhance the capacity of the proteostasis network (currently being developed) to achieve proteome maintenance. Given the myriad proteins that lead to aggregation-associated degenerative diseases, strategies such as proteostasis network adaptation that could be useful for multiple maladies are particularly appealing. The goal is to achieve a fully functional proteome without pathogenic amyloidogenicity.

Multiple research groups have now demonstrated that injecting a tissue homogenate from a human or mouse harbouring an amyloid burden into an amyloid-free mouse can dramatically accelerate amyloid deposition of the homotypic protein in the recipient mouse. This seeding phenomenon for non-prion diseases was first shown *in vivo* in animal models of AA amyloidosis[226]. More recently, this approach of injecting homotypic aggregates has been shown to accelerate Aβ[227,228], tau[229] and α-synuclein[20,230] aggregation. The idea that aggregation can begin in one rogue cell or extracellular area and then spread within an organism is also under discussion for several other amyloid diseases[231,232].

The experimental induction of amyloidogenesis by injection of seeds is first evident within the area of the brain into which seeds are injected; however, the induced pathology subsequently spreads to neighbouring and interconnected regions along neuronal pathways with increasing incubation time[233–237]. This is especially interesting and important because spreading of amyloid seeds along neuroanatomical routes would also explain why and how neurodegenerative diseases target neuronal networks[238]. Although the mechanisms of cellular release and uptake of seeds, as well as of intracellular and extracellular spreading, are poorly understood, it seems that seeded active aggregation on cell membranes can compromise membrane integrity, rendering the cells permeable (even for fibrillar aggregates)[239–241]. Recent studies also show that injection of amyloid into the periphery—that is, intraperitoneal injections of brain extracts containing Aβ or tau aggregates — can induce progressive aggregation in the brains of transgenic mice[17,242,243].

The aggregation-inducing seed is almost certainly the aggregated protein itself (for example, Aβ) in a conformation generated most effectively in the living brain[228,244]. However, amyloid seeds can also be generated *in vitro* from synthetic or recombinant proteins[236,237,245–248]. Strains, or specific misassembled conformations of a protein, were first defined in prion disorders and were found to correlate with distinct biochemical, biophysical and pathological characteristics[249–253]. Recent studies show similar strain-like phenomena for several amyloidogenic proteins, such as Aβ[254–256], tau[257,258] and α-synuclein[259,260].

The availability of the soluble homotypic protein for incorporation into the growing aggregate as well as seed concentration are important in determining the efficiency of propagation of protein aggregation[228,235,242]. Small soluble protein aggregates are the most potent inducers of cerebral Aβ amyloidosis; however, seeds range in size and also comprise large, insoluble amyloid fibrils[261,262]. Importantly, seeded protein aggregation in these animal models induces the typical pathological signature that also arises from spontaneous aggregation of the respective protein in animals and humans. This suggests that the process of seeded spreading initiates the typical ensemble of aggregate structures, which range from relatively disordered to cross-β-sheet amyloid fibrils. This process of aggregation generates proteotoxicity[240] and induces the typical tissue response, including inflammation and eventually tissue dysfunction and degeneration.

These exciting findings raise many questions, perhaps one of the most important being: in addition to amyloid fibrils, which other types of aggregates are formed? The fact that the field cannot answer this question reflects how much remains to be learned. We need tools to discern whether other aggregate structures are present in these mice and in human patients in addition to cross-β-sheet amyloid structures that seem to be acting as seeds. A related question that also needs to be answered by the field is, if there are non-cross-β-sheet aggregates present, how are they formed? Is seeded growth also possible for non-cross-β-sheet aggregates, or are they formed in parallel with cross-β-sheets? It is possible that incomplete degradation of amyloid fibrils or fibril fragmentation could yield the diffusible aggregate structures. A complete inventory of aggregates formed as a function of ageing in seeded and non-seeded animal models of amyloid disease, as well as in patients with amyloidosis, should provide much-needed knowledge of the structure–proteotoxicity relationships that are required to understand how the process of aggregation causes postmitotic tissue loss.

Taken together, the findings of seeded propagation of aggregates and pathology suggest therapeutic opportunities for the treatment and the prevention of these degenerative diseases (FIG. 4a). As the formation of seeds is a self-sustaining process, neutralizing or removing existing seeds should represent promising therapeutic strategies. Because seeded protein aggregation is concentration dependent, the formation of seeds could be prevented by reducing the concentration of the native amyloidogenic protein by the various protein reduction strategies discussed above. For well-folded category 1 proteins, the protein stabilizer strategy should prevent seed formation as well as the progression of aggregation by reducing the concentration of the aggregation-competent protein. The 'amyloid removal' strategies should reduce the efficiency of seeded spreading and, if applied early, potentially reduce the neurotoxic effects of protein aggregates. Immunotherapy seems to be especially promising for removing extracellular protein aggregates and was shown to be effective in reducing seeded spreading of Aβ and tau in animal models[10,228,242]. In addition, stabilizing or remodelling fibrils to prevent fragmentation and/or templated misfolding may slow the progression of prion-like spreading. Progression of the disease should be further prevented or attenuated by inhibiting aggregate spreading within and between cells and tissues. An effective approach towards this end will probably require a better understanding of how aggregates enter cells, how they move along axons and how they are secreted from one cell and taken up by others. All of these strategies will probably be most effective if applied early, before widespread protein aggregation and tissue damage has occurred: this highlights the importance of early disease detection, which is currently difficult.

Perspective

Although the exact mechanism (or mechanisms) by which protein aggregation causes loss of postmitotic tissues remains unclear, the genetic and pharmacological evidence summarized above makes a compelling case for the hypothesis that the process of protein aggregation drives degenerative disease pathology.

Of the therapeutic strategies discussed above, the one which will be most appropriate, most easily applicable and most effective will probably depend on the nature of the target protein: that is, whether it is intrinsically disordered or natively folded, whether it forms extracellular or intracellular deposits and whether it undergoes nucleation-dependent polymerization or downhill polymerization. The disease stage is also an important factor in discerning which strategy will be most effective. We envision that drugs functioning to reduce aggregation through distinct mechanisms of action will be used together in the future as combination therapies to treat amyloid diseases more effectively (FIG. 5). For example, we envision that small-molecule TTR kinetic stabilizers[130,131] will be used in combination with drugs that enhance the capacity of the proteostasis network to achieve proteome maintenance[148–150], or with drugs that reduce the concentration of the amyloidogenic protein of interest[125,126,152,159,179,263] (FIG. 5).

A major challenge in assessing successful therapeutic strategies for the amelioration of human amyloid disease is the lack of biomarkers for early diagnosis and for monitoring the response to candidate therapies in human clinical trials. We envision that these biomarkers will be identified through basic research efforts that are focused on detecting and quantifying all the aggregate structures present before and after clinical symptom presentation. We also expect such biomarkers to emerge from much-needed natural history studies on individual amyloid diseases. Natural history or disease progression studies facilitate the design of better clinical trials, ideally examining patients with early-stage pathology.

Currently, we also lack an understanding of how protein aggregates induce cell toxicity and tissue degeneration, the prevention of which remains the ultimate goal of any single-agent strategy or combination strategy. Understanding the structures of the aggregates mediating proteotoxicity and elucidating their mechanism (or mechanisms) of tissue degeneration will undoubtedly provide new insights into therapeutic strategies to ameliorate these maladies, which as a group represent a considerable economic burden to modern society and which are arguably the most urgent unmet medical need.

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Competing interests statement

The authors declare competing interests: see Web version for details.

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