HHS Public Access

Author manuscript

*Nat Rev Neurol*. Author manuscript; available in PMC 2022 June 30.

Published in final edited form as:

*Nat Rev Neurol*. 2020 April ; 16(4): 199–212. doi:10.1038/s41582-020-0333-7.

# Protein transmission in neurodegenerative disease

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## Abstract

Most neurodegenerative diseases are characterized by the intracellular or extracellular aggregation of misfolded proteins such as amyloid-β and tau in Alzheimer disease, α-synuclein in Parkinson disease, and TAR DNA-binding protein 43 in amyotrophic lateral sclerosis. Accumulating evidence from both human studies and disease models indicates that intercellular transmission and the subsequent templated amplification of these misfolded proteins are involved in the onset and progression of various neurodegenerative diseases. The misfolded proteins that are transferred between cells are referred to as 'pathological seeds'. Recent studies have made exciting progress in identifying the characteristics of different pathological seeds, particularly those isolated from diseased brains. Advances have also been made in our understanding of the molecular mechanisms that regulate the transmission process, and the influence of the host cell on the conformation and properties of pathological seeds. The aim of this Review is to summarize our current knowledge of the cell-to-cell transmission of pathological proteins and to identify key questions for future investigation.

The term 'neurodegenerative disease' encompasses a large group of conditions that are clinically and pathologically diverse, the majority of which are characterized by the accumulation of misfolded proteins into insoluble aggregates (or inclusions) in the CNS accompanied by a progressive loss of neurons in the affected regions. The protein aggregates involved vary between diseases, for example, amyloid-β (Aβ) and tau aggregates in Alzheimer disease (AD)[1,2], misfolded α-synuclein in Parkinson disease (PD)[3], TAR DNA-binding protein 43 (TDP43) and superoxide dismutase 1 (SOD1) pathology in amyotrophic lateral sclerosis[4], and mutated huntingtin (HTT) in Huntington disease[5]. Numerous studies have explored the toxic effects of these protein aggregates on the CNS and have investigated the molecular mechanisms underlying the resulting neuronal dysfunction[6–9]. The findings of these studies have highlighted the crucial role of misfolded proteins in the aetiology and pathogenesis of neurodegenerative diseases.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Interestingly, the spatial distribution of pathological proteins in diseased brains follows stereotypical patterns[10,11], which were historically attributed to differences in the vulnerability of the subtypes of neurons in different brain regions[12]. However, over the past decade, studies using post-mortem brain tissue and various animal and cell models have suggested that many neurodegenerative disease-related pathological proteins undergo cell-to-cell transmission. Following transmission to the recipient cell, pathological proteins act as templates to induce their normal endogenous counterpart protein to misfold, leading to the amplification of the pathological protein conformation[13–16], known as 'templated amplification'. The intercellular transmission and templated amplification of these 'pathological seeds' might lead to the spreading of pathological protein aggregates along neuronal networks, which could explain the stereotypical distribution of protein pathology in the brain. Multiple studies have now investigated the nature of these pathological seeds and the mechanisms that modulate the transmission process. For example, studies using post-mortem brain tissue from individuals with neurological disease have shown that different pathological seeds can have unique properties and conformations. The aim of this Review is to summarize the evidence supporting the transmission hypothesis and to discuss the latest progress in this field, particularly regarding our understanding of the cell-to-cell transmission of α-synuclein, Aβ and tau. We also identify key questions for future study.

## Evidence of protein transmission

### Stereotypical distribution of protein aggregates in diseased brains.

The distribution of pathological protein in the brains of individuals with neurodegenerative disease follows highly predictable spatiotemporal patterns. For example, in individuals with PD, α-synuclein pathology is first found in the olfactory bulb and the dorsal motor nucleus of the glossopharyngeal and vagal nerves. This pathology then spreads in a rostral direction from the brainstem to the midbrain and forebrain, eventually reaching the cerebral cortex[11,17]. In individuals with AD, pathological tau first appears in the locus coeruleus and transentorhinal cortex, and then spreads to the entorhinal and hippocampal regions, followed by the basal temporal cortex and the insular cortex. In the later stages of AD, tau pathology can be found throughout the neocortex[10,18,19]. Interestingly, the distribution of Aβ pathology in the brains of individuals with AD follows a different pattern to the distribution of tau. Aβ plaques first develop in the orbitofrontal neocortex and basal temporal cortex, and then spread throughout the neocortex before finally reaching the hippocampus, midbrain, brainstem and cerebellum[10,20,21].

Historically, the stereotypical distribution of pathological proteins in the CNS was thought to result from differences in vulnerability between brain regions[12] or the progressive spreading of pro-inflammatory cytokines[22]. For example, neurons that are more vulnerable to α-synuclein pathology in individuals with PD tend to have highly branched axons, slow tonic activity and low levels of $Ca^{2+}$-buffering proteins[12]. However, these features do not fully explain the distribution pattern of α-synuclein pathology in diseased brains. During the past decade, multiple studies, which are discussed later in this Review, have demonstrated that templated amplification and dissemination of various pathological proteins can occur

in animal and cell models. These observations support the view that this stereotypical involvement of different brain regions is the result of the spreading of pathological proteins between anatomically connected brain areas[23].

However, the transmission hypothesis has several limitations. First, the post-mortem studies that identified the stereotypical distribution of pathology are limited by a lack of longitudinal data and therefore do not provide direct evidence of sequential evolvement of different brain regions during disease progression. Nevertheless, PET imaging with ligands for specific pathological proteins has now been used to visualize changes in tau and Aβ pathology over time in the same patients, confirming the major conclusions of post-mortem studies[24,25]. Second, the stereotypical spreading pattern is not observed in all patients[12], indicating that the factors affecting the distribution of pathology can vary between individuals. Finally, some of the brain regions that are anatomically connected to areas containing pathological proteins do not develop pathology and the spreading of pathological proteins is not proportional to the strength of synaptic connections[12]. Therefore, the selective vulnerability of different neuronal populations could be a crucial modifier of the transmission process[12]. This selective vulnerability could result from differences in the release or uptake of pathological seeds or in the intracellular environment that modulates the templated amplification process.

The pathological seeds responsible for transmission might not exist in the form of mature aggregates that can be easily detected through histopathological methods. Therefore, to complement traditional histopathological studies, other studies have also been performed to map the seeding activity of pathological proteins isolated from different brain regions. In these studies, potential pathological seeds were isolated from different brain regions and their ability to seed protein aggregation was tested in reporter cell lines[26,27]. For example, in one study, preparations from brain regions free of tau deposition, including regions that are usually affected further along the Braak staging pathway, could induce tau aggregation in a reporter cell line[26]. Using similar methods, the origin of tau seeding activity was mapped to the transentorhinal and entorhinal cortices[27].

### Transplantation.

Strong evidence for the transmission hypothesis comes from studies of patients who have received transplants of human fetal brain-derived cells as a therapy for PD. In these patients, α-synuclein aggregates were observed in the grafted cells, indicating transmission from the host to the graft[28,29]. More recently, intracerebral deposition of Aβ was found in individuals with iatrogenic Creutzfeldt–Jakob disease (CJD) caused by human-derived growth hormone treatment or dura mater grafts[30–34]. Intracerebral Aβ deposition was also observed in individuals who had received neuronal grafts but who died from causes other than CJD[31,35], indicating that Aβ seeds in transplanted materials could induce Aβ pathology regardless of the existence of CJD pathology. Tau has been detected in several batches of cadaver-derived human growth hormone, but substantial amounts of pathological tau were not identified in individuals with iatrogenic CJD[36].

### Disease models.

Numerous studies have shown that pathological seeds generated from recombinant proteins, animal models or diseased brains can induce the development of protein pathology in various in vitro and animal models (TABLE 1). Injection of α-synuclein preformed fibrils (misfolded α-synuclein generated from recombinant α-synuclein monomers) or pathological α-synuclein isolated from transgenic mice or human brains, promoted the development of α-synuclein pathology in M83 transgenic mice, which express a familial PD-associated mutant form of human α-synuclein[37–39]. Importantly, α-synuclein preformed fibrils as well as pathological α-synuclein derived from diseased brains also induced α-synuclein pathology in wild-type mice[15,40] and in primary neuronal cultures derived from wild-type mice[16,41]. Most instances of α-synucleinopathy are sporadic and are not the result of a mutation or amplification of *SNCA* (the gene encoding α-synuclein). Therefore, induction of α-synuclein aggregation in non-transgenic mice is an important observation that indicates the potential for transmission of α-synuclein in individuals with sporadic PD. Moreover, α-synuclein aggregation in non-human primates was induced by the intracerebral injection of recombinant α-synuclein preformed fibrils or Lewy body-containing extracts from brains of individuals with PD, and the resulting pathology could be found far from the injection site[42,43]. In both mice and non-human primates, brain regions with α-synuclein pathology also showed neurodegeneration; pathology and neurodegeneration were particularly prominent in the dopaminergic neurons of the substantia nigra[15,42,43]. In addition to the seeding models described above, the transmission of α-synuclein has also been explored using virus-mediated selective expression of human α-synuclein in the medullary neurons of rats[44]. In this model, the exogenous α-synuclein protein produced by the medullary neurons was observed to spread in a caudal direction to brain regions such as the pons, midbrain and forebrain.

**Recombinant proteins**

Proteins that are artificially expressed in, and purified from, bacteria.

Cell-to-cell transmission of tau was first demonstrated with brain extracts obtained from transgenic mice that express the P301S mutant form of human tau and develop tau aggregation with age. Pathological tau-containing extracts from these mice were injected into the brains of mice that express human wild-type tau and do not naturally develop tau pathology. The induction of tau pathology was observed in multiple brain regions in the injected mice[45]. Synthetic tau preformed fibrils induced tau aggregation in transgenic mice that express the P301L mutant form of human tau but not in wild-type mice[46]. However, pathological tau derived from the brains of individuals with tauopathies, such as AD, induced tau pathology in wild-type mice[14,47], demonstrating that pathological tau proteins in human diseased brains have unique conformations that are not readily recapitulated by their recombinant protein counterparts[14,47–50]. The transmission of pathological tau has also been demonstrated in transgenic mice that express P301L mutant tau only in layer II of the entorhinal cortex[51]. In these mice, the tau pathology induced by the mutant form spread to neighbouring cells in the entorhinal cortex and to connected brain regions, including the hippocampus and cingulate cortex. However, the expression pattern of P301L mutant

tau in this model has been a matter of debate, as expression has also been detected in cortical regions outside of layer II of the entorhinal cortex, complicating the interpretation of the results[52]. Nevertheless, restricted expression of mutant tau in layers II and III of the entorhinal cortex of mice has been achieved using an adeno-associated virus-based technique; in these mice, pathological tau was transmitted to the dentate gyrus[53].

Injection of synthetic Aβ fibrils or extracts from brain tissue of individuals with AD or from Aβ mouse models into the brains of transgenic mice expressing human Aβ precursor protein (APP) instigated the aggregation of Aβ peptides to form senile plaques[54–56]. These Aβ plaques were found in brain regions that were far from the injection site but were anatomically connected to it, suggesting that trans-synaptic transmission of Aβ seeds occurs. Interestingly, unlike transmission of α-synuclein and tau, the transmission of Aβ seeds has not been achieved in wild-type mice, which could be a result of the sequence difference between human and mouse Aβ peptides and the low Aβ expression levels in wild-type mice.

Studies have also demonstrated the cell-to-cell transmission of other pathological proteins, including SOD1 (REF.[57]), mutant HTT[58,59] and TDP43 (REFS[60–62]). Interestingly, TDP43-containing brain extracts from individuals with frontotemporal dementia induced TDP43 aggregation in transgenic mice expressing mutant TPD43 as well as, to a lesser extent, in wild-type mice[62].

### Transmission from the peripheral nervous system to the CNS.

Some evidence supports the hypothesis that the initial misfolding of α-synuclein occurs in the enteric nervous system and spreads retrogradely to the brainstem. For example, α-synuclein pathology has been detected in the enteric nervous system[63–65] and in the submandibular glands[66] of individuals with PD. Furthermore, vagotomy or appendix removal has been reported to reduce the risk of PD (evidence suggests the appendix contains a considerable amount of misfolded α-synuclein)[65,67]. Transmission of pathological seeds from the periphery to the brain has been observed in various animal models. For example, intraperitoneal, intramuscular, intraglossal or intravenous infusion of α-synuclein preformed fibrils into M83 A53T mutant α-synuclein transgenic mice facilitated the development of α-synuclein pathology in the CNS with varying degrees of efficiency[68–70]. More recently, injection of α-synuclein preformed fibrils into the gastric wall of wild-type mice was shown to induce α-synuclein pathology in the CNS[71].

Aβ peptides can be produced in peripheral tissues[72–75] and are capable of crossing the blood–brain barrier[76,77], suggesting that peripherally derived Aβ contributes to Aβ pathology in the brain. In favour of this hypothesis, Aβ pathology was found in the brains of patients who had received human-derived growth hormone treatment or dura mater grafts, suggesting peripheral-to-CNS transmission of pathological Aβ[30–34]. Oral, intravenous, intraocular and intranasal administration of Aβ-containing brain extracts to young APP transgenic mice (which do not yet show age-related Aβ pathology) failed to induce cerebral Aβ amyloidosis; however, intraperitoneal administration of the extracts did promote Aβ deposition in the brains of these mice[78,79]. A study that performed parabiosis between APPPS1 transgenic mice and wild-type mice identified human Aβ in the brains of the wild-type mice, suggesting that Aβ seeds circulating in blood can enter the CNS[80].

Evidence supporting periphery-to-brain spreading of tau seeds is not as strong as that for Aβ and α-synuclein; however, intraperitoneal injection of brain extracts containing pathological tau did induce tau aggregation in the brains of P301S tau transgenic mice[6].

**Parabiosis**

The anatomical joining of two individuals.

## The transmission process

### Intracellular transportation.

The transmission process starts with the formation and amplification of the initial pathological seeds in the donor cell. These seeds are then transported intracellularly to the site of release (FIG. 1). Studies using microfluidic chambers have shown that pathogenic forms of tau, α-synuclein, Aβ and HTT can all be transported along axons in primary neuronal cultures. Both anterograde and retrograde transport of these proteins was observed, although the pathological HTT protein showed a preference for retrograde transmission[81–83]. Interestingly, Aβ fibrils were transported an estimated ten times more efficiently than pathological forms of α-synuclein and HTT[82].

**Microfluidic chambers**

Cell culture chambers that enable the isolation of the axonal or dendritic component from cell bodies.

### Release.

The molecular mechanisms responsible for the secretion of pathological proteins by donor cells have been investigated in multiple studies. Even though α-synuclein lacks a secretory signal peptide sequence, the protein can be detected in the plasma and cerebrospinal fluid (CSF) of individuals with PD[84,85], supporting the view that α-synuclein is secreted into the extracellular space. Similarly, tau has been detected in the CSF of individuals with AD[86] and in the brain interstitial fluid of wild-type mice[87]. Protein secretion into the extracellular space can occur via multiple routes, including diffusion, classical secretion and unconventional secretion such as pore-mediated translocation, ABC transporter-based secretion, membrane-bound organelle-based secretion and the Golgi bypass pathway.

Exosome-based secretion, one of the unconventional secretion pathways, is by far the most extensively studied pathway for the secretion of pathological proteins. Exosomes are membrane-bound extracellular vesicles that are formed as internal vesicles of a multivesicular body and released to the extracellular space by the fusion of the multivesicular body with the plasma membrane. α-Synuclein has been found in exosomes isolated from cell cultures, and human CSF and plasma[88–96], suggesting that exosomes could mediate the release of α-synuclein from donor neurons. In support of this hypothesis, levels of α-synuclein were higher in exosomes isolated from the plasma of individuals with PD than in exosomes from healthy controls[90]. Furthermore, exosomes isolated from

individuals with Dementia with Lewy bodies induced α-synuclein aggregation when injected into the brains of wild-type mice[97]. Similarly, phosphorylated tau (tau aggregates are hyperphosphorylated) was detected in exosomes isolated from the CSF of individuals with AD or from primary neuronal cultures[98,99]. The transmission of tau-containing exosomes was thought to occur through trans-synaptic connections, as studies using microfluidic devices suggested that synaptic connectivity is required for exosomes to mediate the transfer of tau[99].

APP, Aβ and secretases that cleave APP to produce Aβ have been detected in exosomes isolated from cultured cells expressing human APP or the brains of APP transgenic mice[100–102]. Exosomes isolated from the serum and plasma of individuals with AD contain higher levels of phosphorylated tau and Aβ42 (a major component of Aβ plaques) than exosomes from healthy controls[103]. Intracerebral injection of Aβ42-containing exosomes facilitated Aβ plaque formation in the brains of 5XFAD transgenic mice, which express AD-associated mutant forms of human APP and presenilin 1 (REF.[104]). Conversely, some evidence suggests that exosomes deliver proteolytically active enzymes to assist in degrading extracellular Aβ and might therefore also inhibit Aβ pathology[105]. Despite these findings, the role of exosomes in the secretion of pathological proteins is not yet clear; for example, two studies using two different neuronal cell lines detected only a small fraction of secreted α-synuclein in exosomes[106,107]. In another study, when tau was expressed at physiological levels in neurons derived from human induced pluripotent stem cells, the protein could not be detected in exosomes[108].

Some evidence suggests that transmission of pathological proteins occurs via methods other than exosome-based secretion; for example, an anti-tau antibody blocked the transfer of tau between cultured cells, suggesting that tau was released into the medium as free protein[109]. If tau had been packaged in exosomes, the protein–antibody interaction would have been prevented by the lipid membrane. In support of this hypothesis, only a very small portion of secreted tau was located in the isolated vesicle fraction[108,110]. Some evidence suggests that tau could be released through ectosomes[111] or by direct translocation[110]. Another possibility is that pathological seeds are released to the extracellular space after cell death and diffuse into the surrounding area; however, no direct evidence exists that this is the case in diseased brains. Regardless of the route of secretion, diffusion could have a particularly important role in the spreading of Aβ pathology as Aβ aggregates are extracellular. The existence of cerebral amyloid angiopathy in various lines of APP transgenic mice also suggests that diffusion of pathological Aβ from neurons into blood vessels occurs[112–115]. Further studies are needed to evaluate the relative contributions of different secretion pathways to the release of pathological seeds.

**Ectosomes**

Vesicles (0.1–1 μm in diameter) that are budded and released directly from the plasma membrane.

**Direct translocation**

Pore-mediated translocation across the plasma membrane.

**Uptake.**

The internalization of pathological seeds by recipient cells is the next step in the transmission process and multiple mechanisms for this uptake have been proposed. Evidence suggests that misfolded tau and α-synuclein are internalized at the somatodendritic compartment as well as at the axon and presynaptic terminals[16,81–83]. Heparin sulfate proteoglycan-mediated macropinocytosis was identified as a key mechanism for the uptake of both tau and α-synuclein, which could be blocked by heparin[116,117]. Wheat germ agglutinin, a drug that facilitates adsorptive endocytosis, promoted the uptake of tau in cultured cells, suggesting that adsorptive endocytosis is involved in the internalization process[13]. α-Synuclein fibrils are internalized through endocytosis and degraded in lysosomes[118]; however, α-synuclein monomers can be internalized through direct translocation[119]. One study found that α-synuclein fibrils bind to the extracellular immunoglobulin-like domains of the transmembrane protein LAG3 (REF.[120]). Genetic deletion of LAG3 or treatment with anti-LAG3 antibodies reduced the uptake of α-synuclein fibrils and the subsequent induction of α-synuclein pathology in primary neurons in vivo. Irrespective of the specific mechanisms of entry, most studies indicate that endocytosis is the predominant pathway for the internalization of pathological seeds.

Once inside the recipient neuron, pathological seeds need to exit the endosomal vesicle in order to access cytosolic proteins and begin templated amplification. α-Synuclein, tau and mutant HTT fibrils are all able to induce vesicle rupture[121,122], which could enable the pathological seeds to access the cytosol[123]. Finally, the amplification of the transmitted seeds requires the existence of a ‘substrate’, that is, the normal counterparts of pathological proteins in the cytoplasm. The expression level of these substrates could contribute to the selective vulnerability of different neuronal populations[7].

In addition to the release–uptake hypothesis described above, tunnelling nanotubes might mediate the direct intercellular transport of pathological proteins. In support of the tunnelling nanotube hypothesis, misfolded tau, α-synuclein and mutant HTT can all be observed in nanotube structures[124–126]. Interestingly, the addition of aggregated α-synuclein, mutant HTT and tau to cell cultures increased the number of tunnelling nanotubes[124–126], which might facilitate the transmission process. Cell-to-cell transmission of α-synuclein via tunnelling nanotubes also occurred in cultured astrocytes and pericytes[127,128], indicating that this kind of transmission is not limited to neurons.

**Tunnelling nanotubes**

Protrusions that extend from the plasma membrane and enable the communication of cell contents between two cells.

## Molecular nature of the seeds

Understanding the molecular nature of pathological seeds in diseased brains will be crucial for the elucidation of transmission mechanisms and the development of targeted therapeutic interventions. However, tools that can track the behaviour of pathological seeds and analyse their properties in human brains are lacking. One way to identify potential seeds is to investigate the seeding ability of different misfolded protein species isolated from diseased brains. The underlying hypo thesis of this kind of investigation is that aggregates with a higher seeding ability are likely to have a greater role in the spreading of pathology. After ultracentrifugation of brain extracts from APP transgenic mice, Langer et al. identified a small fraction of soluble Aβ (<0.05% of total Aβ) that was more proteinase K sensitive, meaning that it forms a more open and less aggregated structure, than insoluble Aβ. However, this soluble Aβ induced greater Aβ pathology than insoluble Aβ when injected into young APP transgenic mice, suggesting that this form of soluble Aβ is a more potent seed than insoluble Aβ for the transmission of Aβ pathology[129]. Potent soluble Aβ seeds were also identified in brain tissue but not CSF from individuals with AD[130]. A more detailed analysis found that insoluble Aβ from intracellular membrane fractions had a stronger seeding ability when injected into APP transgenic mice than insoluble Aβ from a general brain homogenate[131]. This finding suggests that membrane-associated Aβ could be a source of the seeds that contribute to the spreading of pathology.

For intracellular aggregates such as α-synuclein and tau, the pathological seeds must be able to undergo transport between cells while maintaining the ability to induce templated amplification. Very mature aggregates are unlikely to be pathological seeds because of their large size, which would hinder cell-to-cell transmission. Therefore, smaller species that can be readily released and internalized by cells are the more promising candidates. In one study, soluble high-molecular-weight tau was derived from brain interstitial fluid and cortical extracts taken from tau transgenic mice or individuals with AD. This tau species was taken up by cells in primary neuronal cultures, transported intracellularly and passed to connected neurons, suggesting that it could be a pathological seed. Interestingly, the uptake process seems to require phosphorylated tau[132].

Mirbaha et al. used size exclusion chromatography to isolate tau repeat domain assemblies ranging from 1 to >100 tau units, and only species containing more than 3 tau units were internalized and induced aggregation in HEK293 cells[117]. This size threshold was the same for tau assemblies isolated from the brains of individuals with AD, suggesting that trimers are the minimal unit of tau pathological seeds. However, Jackson et al. used a sucrose gradient to isolate tau aggregates from P301S mice and found that only assemblies containing more than 10 tau units could induce tau aggregation in HEK293 cells[133]. These inconsistent findings are very likely to be the result of the two studies using different systems to evaluate the seeding ability of tau assemblies: Mirbaha et al. used a cell line that expresses the P301S mutant repeat domain of tau, whereas Jackson et al. used a cell line that expresses the full form of P301S mutant tau. A more recent study observed that even the tau monomer, which is traditionally considered to be unstructured, can exist in different conformations[134]. The monomer derived from sonicated tau fibrils was able to trigger tau aggregation in HEK293 cells, and this observation was replicated using tau monomers

isolated from the brains of individuals with AD, suggesting that the pathological tau seed could even be a monomer with a pathological conformation. The difference between this study and the study of tau trimers by Mirbaha et al.[117] is that lipofectamine was used in the former to facilitate the transduction of tau into cells.

In an experimental setting, the seeding of α-synuclein pathology by α-synuclein preformed fibrils required the breakdown of fibrils by sonication, suggesting that very long α-synuclein fibrils are not efficient seeds[118], likely as a result of inefficient uptake. However, sonication generates a heterogeneous population of short fibrils and potentially also oligomers[135], any of which could be responsible for seeding. α-Synuclein oligomers generated from recombinant proteins are soluble and have shown seeding ability in primary neurons; therefore, oligomers are potential pathological seeds[136,137]. However, the definition of oligomers encompasses a spectrum of α-synuclein aggregates that are all smaller than fibrils but are structurally diverse and have very heterogeneous seeding properties. For example, low-molecular-weight oligomers are less efficient seeds than the larger and more stable oligomers[138]. One specific α-synuclein oligomer (4-hydroxy-2-nonenal induced) did not show any seeding activity in vivo[139], indicating that only oligomers with certain conformations are pathological seeds.

Despite the long list of potential pathological seeds, identifying which candidates are responsible for the spread of protein pathology in individuals with neurological disease will be extremely challenging unless new technologies are developed to track and isolate individual species of misfolded proteins in diseased brains. Another possibility is that the seeds are not pathological proteins and that protein misfolding in neurons is induced by other factors.

### Conformational diversity.

Accumulating evidence indicates that many pathological proteins, including prions, α-synuclein[41,140–144], Aβ[145–148], tau[47,48,149], SOD1 (REF.[150]) and mutant HTT[58], exist in multiple different conformations[41,47,48,140–145,148,149,151] (TABLE 2). Different conformations of the same pathological protein have the potential to show dramatically different seeding capacities and spreading behaviours, which in turn could contri bute to the pathological and clinical diversity of neurodegenerative diseases. For example, prion protein exists in many different conformations, known as strains, which contributes to the diversity of prion diseases[152].

Although conformational variants of many pathological proteins have now been identified, how these different strains are generated remains unclear. One study demonstrated that the different intracellular environments of oligodendrocytes and neurons could lead to the generation of different α-synuclein strains, which highlights the effect of the local environment on the misfolding process[41] (FIG. 2). Studies using artificially generated strains of recombinant proteins have also provided important information about how different strains could develop in diseased brains. For example, repeated seeding of α-synuclein preformed fibrils led to the development of a new strain that could also induce tau pathology in primary neurons[140], which suggests that continuous transmission and

templated amplification of pathological α-synuclein in diseased brains might also lead to the development of new strains.

The different neurodegenerative disease-related proteins have distinct properties; however, the formation of β-sheet-enriched structures is a shared feature and a crucial step for the formation and amplification of pathological protein conformations. Many studies have tried to illustrate and model the structural basis of the conversion into a pathological conformation[153–159]. For example, using a seven-amino acid peptide that could form either an α-helix or a β-strand, two studies showed that a pre-formed β-strand can promote the unfolding of an α-helix to form another β-strand[155,157]. The results of a detailed study of the aggregation process of SOD1 suggested the existence of several distinct steps, including dimer dissociation, metal loss and oligomer formation[156]. Recently, cryogenic electron microscopy was used to analyse the structure of pathological proteins purified from diseased brains[160–164], providing crucial information on the structural basis of the misfolding and aggregation process in diseased brains, including the arrangement and composition of the core of the protein aggregates.

### Conformation and potency.

Different conformational variants of pathological seeds can have dramatically different seeding abilities. For example, pathological α-synuclein isolated from oligodendrocytes was conformationally distinct from pathological α-synuclein isolated from neurons, with the oligodendrocyte-derived form having a ~1,000-fold greater seeding ability than the neuron-derived form[41]. Similarly, pathological tau isolated from brains of individuals with progressive supranuclear palsy (PSP) was conformationally distinct from, and had greater seeding ability than, pathological tau isolated from brains of individuals with AD[14]. Pathological TDP43 protein isolated from patients with frontotemporal lobar degeneration (FTLD) with a Granulin mutation had a greater seeding ability than those isolated from individuals with sporadic FTLD[62]. In addition, distinct conformational strains of recombinant α-synuclein have been shown to have different seeding abilties[140–142].

One interesting observation is that pathological proteins isolated from diseased brains generally have a greater seeding ability than aggregates generated with recombinant proteins, suggesting that the environment in diseased brains leads to the formation of unique protein conformations that are different from those generated in vitro. For example, pathological tau isolated from tauopathy brain samples induced more tau pathology than synthetic tau fibrils when injected into the brains of wild-type mice[14,47]. Similarly, the induction of TDP43 aggregation in wild-type mice has only been achieved with pathological TDP43 isolated from brains of individuals with FTLD[62]. The seeding activity of Aβ aggregates purified from brains of individuals with AD is much higher than the seeding activity of synthetic Aβ aggregates[56,130]. The seeding ability of α-synuclein derived from diseased brains has not yet been compared with the seeding ability of α-synuclein preformed fibrils. However, in one study, pathological α-synuclein derived from transgenic mouse brains had a greater seeding ability than a pathological form of recombinant α-synuclein[37]. The extracts from diseased brains that were used in these studies contained other proteins and lipids, in addition to the pathological proteins of interest. Therefore, the high potency of

brain-derived seeds could be the result of co-factors in the extracts that promote the seeding process. The development of new technologies to generate highly purified pathological proteins from diseased brains will be needed to exclude the contribution of contaminating proteins to seeding activity.

### Conformation and glial cell pathology.

Different conformations of pathological proteins might induce pathology in specific cell types. For example, the injection of pathological tau derived from brains of individuals with AD into tau transgenic or wild-type mice only induced tau pathology in neurons, whereas tau derived from the brains of individuals with cortical basal degeneration (CBD) or PSP induced pathology in neurons, astrocytes and oligodendrocytes[47,48,149]. Pathology in these cell types is a pathological feature of CBD and PSP. In contrast, the cell type specificity of α-synuclein spreading in the experimental setting does not seem to correlate with clinical features. In individuals with multiple system atrophy, the vast majority of α-synuclein aggregation is in oligodendrocytes; however, the injection of pathological α-synuclein isolated from multiple system atrophy brains into wild-type mice only induced α-synuclein aggregation in neurons[41].

### Conformation and spreading pattern.

Some evidence suggests that the conformation of pathological seeds modulates the transmission pattern of these pathological proteins along the neuron network. For example, different pathological α-synuclein strains show different spreading patterns after intracerebral injection into wild-type mice[41]. Similarly, the results of a PET imaging study suggested that the spreading of tau aggregates in individuals with AD is mainly determined by neuronal connectivity, whereas tau aggregates in individuals with PSP spread into brain areas with a high metabolic demand and a lack of trophic support[165].

## Modifiers of the transmission process

### Neuronal activity.

Of the factors that could influence the transmission of pathological seeds, neuronal activity is one of the most well studied and has been repeatedly shown to promote the propagation of various pathological proteins[99,166–172] (FIG. 1). For example, in a microdialysis study, the concentration of Aβ in the brain interstitial fluid of mice could be modulated by neuronal activity and was correlated with the concentration of lactate, which is a marker of neuronal activity[169]. Using a similar technique, another study showed that an increase in neuronal activity can rapidly increase the level of extracellular tau in the brain[168]. In a more recent study, which used a virus-mediated method to overexpress human tau in primary neurons and mice, elevated neuronal activity caused an increase in tau secretion in vitro and in tau transmission in vivo[170]. Furthermore, a study that used optogenetic and chemogenetic approaches to modulate neuronal activity demonstrated that higher neuronal activity leads to increased tau pathology in mice expressing a mutant form of human tau[167]. Elevated neuronal activity also caused a rapid increase in extracellular levels of α-synuclein in both primary neuronal cultures and mice, and reduced neuronal activity caused a decrease in extracellular α-synuclein in both experimental models[166].

Consistent with a role for neuronal activity in pathological protein transmission, extracellular tau and Aβ levels change according to the sleep–wake cycle, and chronic sleep deprivation, which causes an increase in neuronal activity, can facilitate the secretion and propagation of Aβ and tau[172,173]. Mechanistically, the increased secretion of tau induced by neuronal activity could be mediated by increased Golgi dynamics[174]. Given these highly consistent findings, which come from multiple studies using various different models, abnormal neuronal activity, such as the seizure-like activity seen in individuals with AD[175] and sleep disorders in individuals with PD[176], is likely to facilitate or modulate the spreading of pathological proteins.

### Glial cells.

The activation of microglia and astrocytes has been observed in various neurodegenerative diseases; however, the role of these glial cells in the disease process is complicated. Evidence suggests that activated glial cells facilitate the clearance of pathological proteins from the extracellular space and thus inhibit the spreading of pathological seeds from cell to cell. For example, microglial cells can phagocytose both soluble and insoluble forms of tau[175,177]. Astrocytes can take up tau fibrils in vitro and can also reduce tau pathology in mutant tau transgenic mice[178]. Microglia and astrocytes are also involved in multiple mechanisms of Aβ clearance. First, glial cells produce Aβ-degrading enzymes, such as matrix metalloproteinases[179], tissue plasminogen activator[180] and metalloendopeptidases[181], which help to clear Aβ peptides. Second, microglia and astrocytes can directly phagocytose fibrillary Aβ[176,182].

A specific population of microglial cells associated with neurodegenerative diseases, known as disease-associated microglia, have been identified by single-cell sequencing[183]. Disease-associated microglia have been detected near Aβ plaques and Aβ particles, suggestiing that this microglial population might be involved in the clearance of Aβ aggregates[183]. Similarly, evidence suggests that pathological α-synuclein is phagocytosed and cleared by astrocytes and microglia[184–186]. The overexpression of IL-6 activates microglia and astrocytes and attenuates the spread of pathology induced by α-synuclein preformed fibrils in mice[187]. Interestingly, in one study, monomeric α-synuclein enhanced microglial phagocytosis, whereas aggregated α-synuclein inhibited this process, suggesting a mechanism by which aggregated α-synuclein can avoid degradation by microglial cells[188].

The results of more recent studies suggest that microglia and astrocytes actually facilitate the spreading of pathological proteins and promote disease progression. For example, pharmacological depletion of microglia dramatically suppressed the propagation of tau pathology in an adeno-associated virus-based tau mouse model, and inhibiting exosome secretion by microglia also significantly reduced the spreading of pathological tau both in vitro and in vivo[53]. These findings suggest that tau can be taken up by microglia and secreted in exosomes. Similarly, tau aggregates develop in astrocytes and oligodendrocytes in the brains of individuals with CBD or PSP[189,190], indicating that transmission of tau can occur between neurons and glial cells, or between glial cells[191]. Microglia might also promote the transmission of Aβ pathology. Apoptosis-associated speck-like protein containing a CARD (ASC) specks secreted by microglia have been shown to bind to and

promote the aggregation of Aβ[192]. In addition, ASC deficiency blocked the seeding of Aβ pathology in APPSwePSEN1dE9 transgenic mice by brain homogenates from aged APPSwePSEN1dE9 mice.

Pathological α-synuclein can be transmitted between neurons and astrocytes[193] and, as mentioned earlier, α-synuclein might also be transmitted between astrocytes via tunnelling nanotubes[127,128]. Using a mouse model that expresses human α-synuclein specifically in oligodendrocytes, we showed that pathological α-synuclein can undergo transmission between different oligodendrocytes, a process that is independent of α-synuclein pathology in neurons[41].

Finally, glial cells could also modulate the transmission process by influencing the conformation of the pathological seeds. As discussed earlier, the intracellular environment of oligodendrocytes can modify the conformation of the pathological seeds in a different way to the intracellular environment of neurons, which leads to differences in seeding ability[41]. Microglia and astrocytes also have a key role in the neurodegenerative process, which has been extensively reviewed elsewhere[194–196].

### Genetic risk factors.

Evidence suggests that some genetic risk factors for neurodegenerative diseases contribute to disease development and progression by modulating the transmission of pathological proteins. For example, the gene encoding amphiphysin 2 (also known as BIN1) is the second most prevalent risk locus for late-onset AD[197]. In one study, amphiphysin 2 inhibited tau propagation in vitro by decreasing the endocytosis of pathological tau by primary neurons[122]. Mutations in the gene encoding leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2), which usually increase the activity of the kinase[198–202], are the most common cause of hereditary PD[199]. LRRK2 activity promoted the propagation of pathological α-synuclein in vitro and in vivo via the phosphorylation of RAB35 (REF.[203]), which is a small GTPase involved in vesicle trafficking. The effect of LRRK2 activity on the seeding ability of α-synuclein preformed fibrils has also been studied in both cell and animal models[204–206]. The apolipoprotein E type 4 allele is the strongest genetic risk factor for late-onset AD[207]. One study used PET imaging to compare the distribution of Aβ and tau in the brains of healthy adults with regional gene expression values from the Allen Human Brain Atlas. The propagation patterns of both Aβ and tau were associated with a lipid metabolism-related genetic profile in which apolipoprotein E has an important role[208].

### Interaction between pathological proteins.

The co-existence of multiple pathological proteins in diseased brains is common in various neurodegenerative diseases. For example, ~50% of individuals with AD have α-synuclein pathology in the brain in addition to the characteristic Aβ and tau pathology[209]. Furthermore, ~50% of post-mortem brains from individuals with PDD have sufficient AD co-pathology to warrant a second diagnosis of AD[210]. The co-existence of different pathologies suggests that one pathological protein could promote the spreading of another.

Histopathological and genetic data suggest that Aβ plaques drive the spreading of tau pathology out of the medial temporal lobe[211–215], and this suggestion is supported by studies

using various disease models. For example, brain extracts from APP transgenic mice as well as synthetic Aβ fibrils can promote tau aggregation in mutant tau transgenic mice[216–219] and synthetic Aβ aggregates can induce tau fibrillization in cells as well as in test tubes[217]. Furthermore, transgenic mice expressing mutant forms of human APP and tau had greater tau pathology than mice expressing the mutant form of tau only[216,220]. In a more recent study, pathological tau isolated from the brain tissue of individuals with AD was injected into the brains of mice that had Aβ pathology. In these animals, tau fibrilization was promoted in the dystrophic neurites surrounding Aβ plaque cores[221]. A similar observation was made in transgenic mice that express the four-repeat domain of human tau as well as a mutant form of human APP[185].

The interaction of pathological α-synuclein with Aβ and tau has also been extensively studied. For example, co-incubation of α-synuclein and tau promoted the fibrilization of both proteins[222]. α-Synuclein preformed fibrils inhibited tau-promoted microtubule assembly and induced tau aggregation in cells overexpressing tau[223]. Increased phosphorylation of tau has been observed in mice that overexpress a PD-associated mutant form of human α-synuclein[224,225]. Importantly, one study compared two conformationally distinct α-synuclein strains and found that only one of the strains was able to induce tau aggregation in wild-type primary neurons and in tau transgenic mice[140].

The interaction between α-synuclein and Aβ is more complicated than the interaction between α-synuclein and tau. Cross-seeding between α-synuclein and Aβ has been observed using recombinant proteins in vitro[226]. A transgenic mouse model of tau, Aβ and α-synuclein pathology showed enhancement of all three types of pathology when compared with mouse models of the individual pathologies[227]. Similarly, expression of the Aβ peptide promoted the formation of α-synuclein pathology in α-synuclein transgenic mice and in mice that had been injected with misfolded α-synuclein[228,229]. However, in one study, injection of α-synuclein preformed fibrils or brain homogenates from mice expressing mutant human α-synuclein into APP transgenic mice failed to induce Aβ aggregation. In the same mouse line, expression of the A30P form of mutant α-synuclein even reduced the Aβ plaque load[230]. Therefore, more studies are needed to clarify the interaction between α-synuclein and Aβ pathology.

## Therapeutic implications

Studying the transmission and amplification process of the pathological proteins associated with neurodegenerative disease has important therapeutic applications. Cell-based therapies, which aim to replace degenerating cells with healthy ones, have been tested in clinical trials but might not provide long-term benefit because of pathological protein transmission from the patient to the transplanted cells. For example, as discussed earlier, α-synuclein aggregation was found in transplanted fetal ventral mesencephalic neurons in individuals with PD[28,29]. Therefore, therapies that target the transmission process could slow down disease progression and might improve the outcome of cell therapy.

Currently, passive immunotherapy using antibodies targeting various pathological proteins is being investigated as a potential treatment for neurodegenerative disease. For example,

one study systemically administered antibodies against the proximal C-terminal amino acids (91–99) of α-synuclein to mice with lentivirus-mediated overexpression of α-synuclein. In these mice, the antibodies blocked the transportation and aggregation of α-synuclein in axons and reduced axonal degeneration[231]. In two other studies, antibodies against the C-terminus of α-synuclein were shown to promote α-synuclein clearance and reduce behavioural deficits in α-synuclein transgenic mice[232,233]. Furthermore, antibodies against misfolded α-synuclein reduced α-synuclein preformed fibril-induced pathology in both primary neurons and wild-type mice[234]. These anti bodies also ameliorated dopaminergic neuron degeneration and improved motor deficits in mice. Antibodies targeting N-terminal and mid-domain regions of tau prevented the uptake and propagation of pathological tau in cell cultures[235]. Similarly, antibodies targeting Aβ reduced Aβ levels in animal models and individuals with AD[236].

The protective effect of these antibody therapies was thought to be mediated by lysosomal[232] or microglial-dependent degradation of pathological proteins[237]. However, the conformational diversity of the pathological seeds might complicate the development of immunotherapies because antibodies efficient for one pathological strain might not be as effective for another strain. As with any therapy that targets the CNS, developing strategies that enable antibodies to cross the blood–brain barrier will dramatically improve the efficiency of passive immunotherapy for neurodegenerative diseases.

Active immunization has also been explored as a potential therapy for neurodegenerative diseases. For example, immunization with α-synuclein proteins or peptides reduced α-synuclein accumulation and behavioural deficits in various α-synuclein transgenic models[238–240]. As internalization of pathological seeds is a key process for transmission, researchers are also investigating the potential of therapies that target the molecules involved in the uptake of pathological protein. For example, synthetic heparin mimics blocked the heparin sulfate proteoglycan-mediated uptake of pathological tau and α-synuclein in vivo and in vitro[116], and antibodies targeting LAG3 blocked the transmission of pathological α-synuclein[120]. In addition to targeting the pathological seeds, reducing the concentration of the substrate, that is, the normal protein counterparts, using approaches such as antisense oligonucleotides is another strategy to inhibit the transmission process[241]. Finally, analysing pathological proteins in the CSF or even the blood of patients could facilitate the early and accurate diagnosis of various neurodegenerative diseases. For example, protein misfolding cyclic amplification has been used to detect pathological α-synuclein in the CSF as a diagnostic strategy for PD[242]. A similar technique was also used to detect aggregated tau in brains of individuals with AD[243].

**Protein misfolding cyclic amplification**

The amplification of misfolded protein by repeated incubation with corresponding monomers.

## Conclusions and future directions

During the past 5 years, the focus of neurodegenerative research has shifted from testing the transmission hypothesis to exploring the underlying molecular mechanisms of the transmission process. Given the complexity and therapeutic significance of the mechanisms underlying the transmission of pathological proteins in neurological diseases, this topic is likely to continue being the focus of the field for some time.

One challenge for the field is that the vast majority of studies were performed using preformed fibrils generated from recombinant proteins. However, accumulating evidence demonstrates crucial conformational and biological differences between preformed fibrils and pathological aggregates isolated from diseased brains[14,62]. In the future, analysing the cell-to-cell transmission of pathological proteins from diseased brains will be essential. However, these kinds of studies are currently constrained by the limited availability of brain tissue and the small amounts of pathological proteins that can be isolated from diseased brains. Therefore, developing improved methods to purify and amplify these pathological seeds from diseased brains will be extremely beneficial for the field.

Another major challenge to understanding the molecular mechanisms underlying transmission is the conformational diversity of the pathological proteins. As different strains of an increasing number of pathological proteins have been identified, conformational diversity now seems to be a phenomenon that is common to the majority of neurodegenerative disease-associated proteins, and many more strains are likely to have not yet been discovered. Analysing the distribution and interaction of different protein strains in diseased brains is extremely challenging. However, the recent development of conformation-specific antibodies[244,245] could facilitate the discovery and analysis of different strains in brains affected by neurodegenerative disease.

Despite the progress made, several key gaps in our knowledge remain. First, we do not yet know how pathological seeds form in diseased brains nor do we understand the molecular nature of these seeds. Second, whether pathological seeds cause neuronal and/or glial cell toxicity is unclear. Finally, although different strains of various pathological proteins have now been identified, how these different strains are generated remains unknown. The development of new technologies to track, isolate and analyse pathological proteins in diseased brains will be essential in addressing these important questions.

## Acknowledgements

The authors thank the Michael J. Fox Foundation.

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**Key points**

- Cell-to-cell transmission and the subsequent amplification of pathological proteins is emerging as a common mechanism for the progression of various neurodegenerative diseases.
- Transmission within the CNS as well as from the peripheral nervous system to the CNS has been reported for multiple pathological proteins.
- Multiple molecular mechanisms involved in the secretion, uptake and transport of pathological seeds have been identified.
- Neurodegenerative disease-related pathological proteins are conformationally diverse.
- Various factors can modulate the transmission process, including neuronal activity, glial cells, genetic risk factors and interactions with other pathological proteins.
- Antibodies against pathological seeds, which are designed to block the transmission process, are currently in clinical trials.

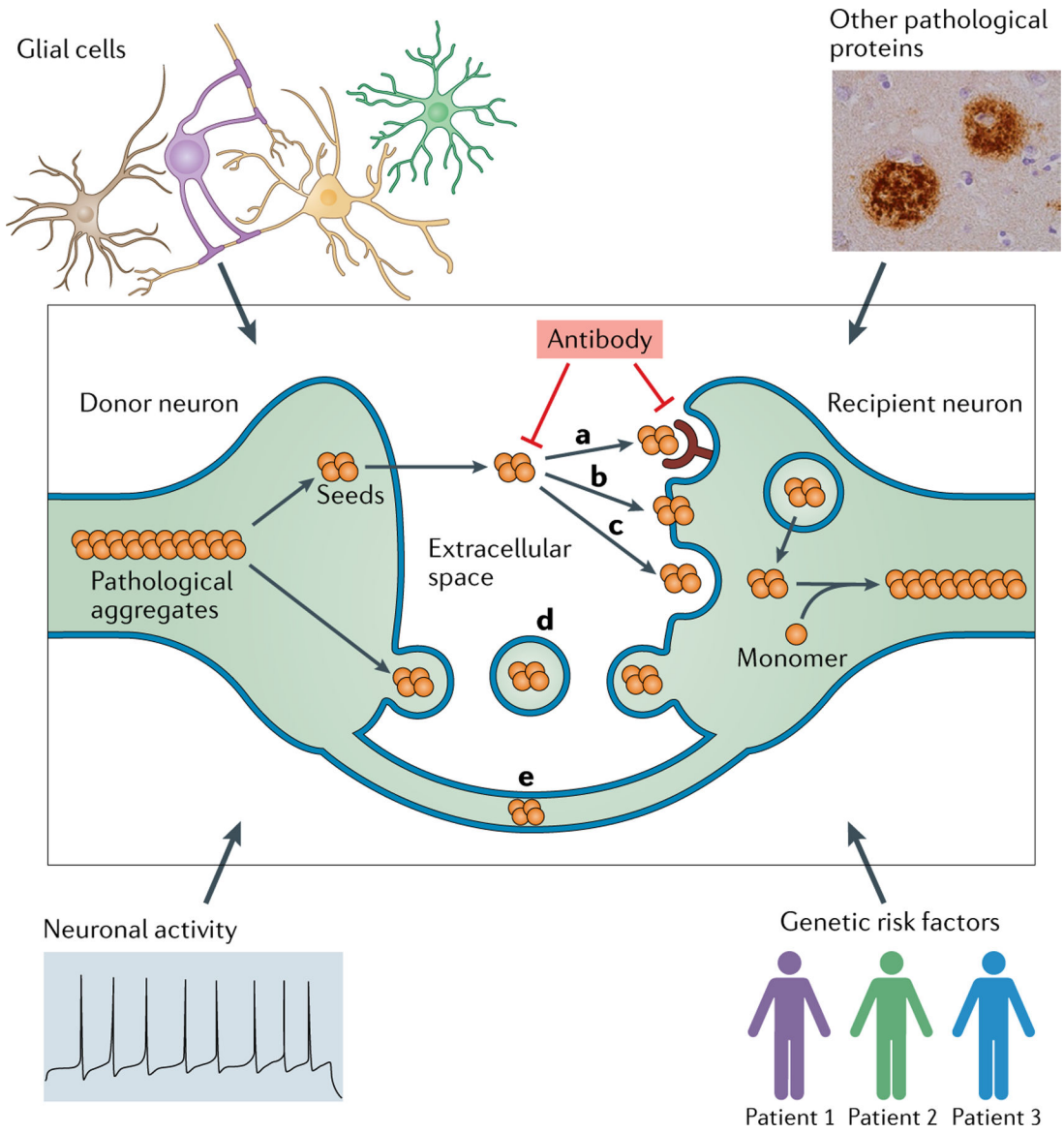


**Fig. 1 |. Mechanisms for the transmission of pathological proteins between cells.**

Pathological proteins, or 'seeds', are released from donor neurons and enter the extracellular space either as naked protein or in vesicles such as exosomes. Naked protein might be taken up by recipient neurons through receptor-mediated endocytosis (**a**), direct penetration of the plasma membrane (**b**) or fluid-phase endocytosis (**c**). Seeds in vesicles could be internalized through the fusion of vesicles with the plasma membrane (**d**). Seeds could also be transferred from a donor neuron to a recipient neuron via tunnelling nanotubes that directly connect the two cells (**e**). The transmission process can be modulated by multiple factors, including the clearance of pathological proteins by glial cells, neuronal activity, genetic risk factors and interaction of the seeds with other pathological proteins. Potential therapeutic interventions include antibodies that target the pathological seeds or the transmission machinery. Adapted from REF.[23], Springer Nature Limited.

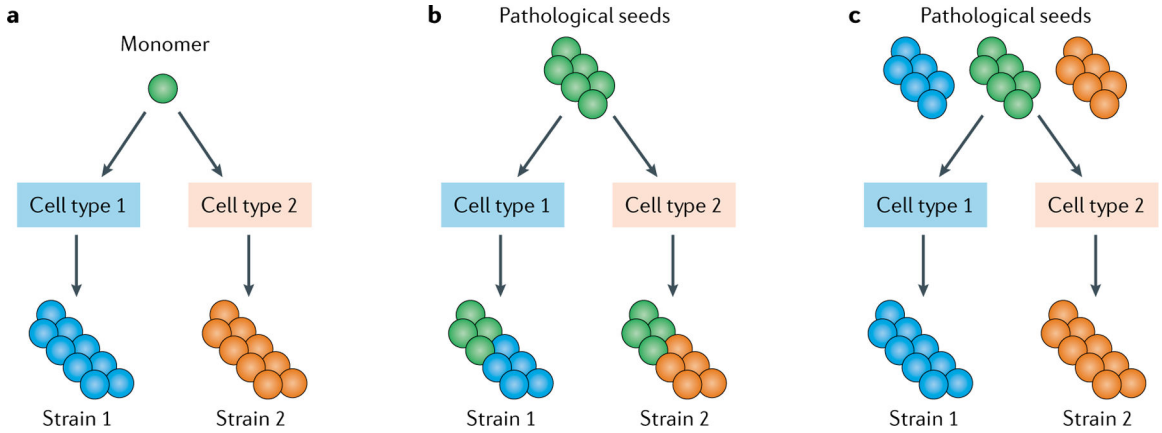


**Fig. 2 |. Generation of different pathological protein strains.**

Different intracellular environments can result in different pathological protein strains and several potential mechanisms for this differentiation have been suggested. **a** | Different intracellular environments could affect the initial protein misfolding process. **b** | Different intracellular environments could affect the templated amplification process. **c** | If pathological seeds were a mixture of different conformations or strains, different intracellular environments could lead to the selection and amplification of a specific conformation from the mixture.

**Table 1 |**

The transmissibility of non-prion protein aggregates

| Protein | Evidence of transmission in humans | Evidence of transmission in experimental models | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Type of seed | In vitro | | In vivo | | | |
| | | | Non-neuronal cells | WT neurons | Intracerebral injection in transgenic mouse models of disease | Intracerebral injection in WT mice | Transmission to glial cells following intracerebral injection in WT mice | Peripheral injection in transgenic mouse models of disease and WT mice |
| α-Synuclein | Fetal mesencephalic cell transplantation[28,29] | Synthetic fibrils | Yes[246] | Yes[16] | Yes[37] | Yes[15] | No[41] | Yes[69] |
| | | Mouse brain lysates | Not tested | Not tested | Yes[37] | Not tested | Not tested | Not tested |
| | | Human brain lysates | Yes[143] | Yes[41] | Yes[39] | Yes[40] | No[41] | Not tested |
| Tau | Human-derived growth hormone treatment[36] | Synthetic fibrils | Yes[13,247] | Yes[14] | Yes[248] | No[14] | No[14] | Not tested |
| | | Mouse brain lysates | Not tested | Not tested | Yes[45] | Not tested | Not tested | Yes[6] |
| | | Human brain lysates | Yes[249] | Yes[14] | Yes[48] | Yes[14,48] | Yes[47] | Not tested |
| Amyloid-β | Human-derived growth hormone treatment or dura mater grafts[30,31,32,33,34,35] | Synthetic fibrils | Yes[8] | Not tested | Yes[56] | Not tested | Not tested | Not tested |
| | | Mouse brain lysates | Yes[8] | Not tested | Yes[55] | Not tested | Not tested | Yes[79,250] |
| | | Human brain lysates | Yes[8] | Not tested | Yes[54,55] | No[54] | Not tested | Yes[251] |
| TDP43 | None | Synthetic fibrils | Yes[60] | Not tested | Not tested | Not tested | Not tested | Not tested |
| | | Mouse brain lysate | Not tested | Not tested | Not tested | Not tested | Not tested | Not tested |
| | | Human brain lysates | Yes[61,62] | Not tested | Yes[62] | Yes[62] | No | Not tested |

TDP43, TAR DNA-binding protein 43; WT, wild-type.

**Table 2 |**

Strains of non-prion protein aggregates

| Protein | Type of seed | Identification of different conformational strains | Strain differences in seeding activity | | | | |
|---|---|---|---|---|---|---|---|
| | | | Transmission to glial cells in WT mice | Spreading pattern | Potency | Morphology of aggregates | The ability to seed other proteins |
| α-Synuclein | Synthetic fibrils | Yes[140,141,142] | No[140] | Not tested | Yes[140,141,142] | No[140,141,142] | Yes[140] |
| | Mouse brain lysates | Not tested | Not tested | Not tested | Not tested | Not tested | Not tested |
| | Human brain lysates | Yes[41,140] | No[41] | Yes[41] | Yes[41] | No[41] | Not tested |
| Tau | Cell lysates | Yes[151,252] | No[151,252] | Yes[151,252] | No[151,252] | Yes[151,252] | Not tested |
| | Mouse brain lysates | Not tested | Not tested | Not tested | Not tested | Not tested | Not tested |
| | Human brain lysates | Yes[14,47,160,161,162,253] | Yes[47,48,149] | No[47,48,149] | Yes[47] | Yes[47] | Not tested |
| Amyloid-β | Synthetic fibrils | Yes[147] | Not tested | Not tested | Not tested | Not tested | Not tested |
| | Mouse brain lysates | Not tested | Not tested | Not tested | Not tested | Yes[146] | Not tested |
| | Human brain lysates | Yes[145] | Not tested | Not tested | Not tested | Yes[148] | Not tested |
| TDP43 | Synthetic fibrils | Not tested | Not tested | Not tested | Not tested | Not tested | Not tested |
| | Mouse brain lysates | Not tested | Not tested | Not tested | Not tested | Not tested | Not tested |
| | Human brain lysates | Not tested | No[62] | No[62] | Yes[9,62] | No[62] | Not tested |

TDP43, TAR DNA-binding protein 43; WT, wild-type.