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Propagation of Protein Aggregation in Neurodegenerative Diseases

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amyloid, tau, prion, propagation, strain, aggregation

Abstract

Most common neurodegenerative diseases feature deposition of protein amyloids and degeneration of brain networks. Amyloids are ordered protein assemblies that can act as templates for their own replication through monomer addition. Evidence suggests that this characteristic may underlie the progression of pathology in neurodegenerative diseases. Many different amyloid proteins, including Aβ, tau, and α-synuclein, exhibit properties similar to those of infectious prion protein in experimental systems: discrete and self-replicating amyloid structures, transcellular propagation of aggregation, and transmissible neuropathology. This review discusses the contribution of prion phenomena and transcellular propagation to the progression of pathology in common neurodegenerative diseases such as Alzheimer's and Parkinson's. It reviews fundamental events such as cell entry, amplification, and transcellular movement. It also discusses amyloid strains, which produce distinct patterns of neuropathology and spread through the nervous system. These concepts may impact the development of new diagnostic and therapeutic strategies.

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INTRODUCTION

Neurodegenerative diseases are a major health problem for the world's aging population. The most common are linked to the accumulation of protein amyloids inside or outside cells. We use the term amyloid to refer to long, unbranched protein fibrils that display cross-β fiber diffraction when examined with X-rays (1, 2). Much like crystallization of small molecules or proteins, amyloids serve as templates for their own replication in vitro. Several models have been proposed to explain the mechanisms of amyloid growth. The first model proposes a nucleated polymerization event, in which a monomer is converted to a seed from which fibrils grow via monomer addition (2–8). Recent work indicates that for tau the pathogenic seed can be a single molecule (9). The second model proposes an induced fit, whereby monomers convert into aggregates that are unable to grow into ordered amyloid fibrils. Instead, these aggregates can serve as substrates for other proteins to bind and undergo a conformational change that enables subsequent amyloid growth (2, 4). In the case of both models, after fibrils begin to grow, they are subject to fragmentation and secondary nucleation events that rapidly amplify protein amyloids. This self-replication mechanism based on template formation has provided a conceptual framework for understanding the origin and progression of multiple neurodegenerative diseases. Importantly, in most cases, mutations that cause dominantly inherited neurodegenerative diseases typically alter the very same proteins that accumulate in sporadic cases, usually via structural destabilization that promotes amyloid formation, or via overproduction. Although the triggers of protein aggregation in sporadic diseases remain unknown, many groups have reported diminished protein quality control in aging organisms (10), which could conceivably play a role. In the case of the most common age-related neurodegenerative disease, Alzheimer's disease (AD), branches of the ubiquitin–proteasome system and the endosomal/lysosomal pathways are particularly important for keeping

aggregation-prone proteins under control (11). Perhaps unsurprisingly, genome-wide association studies have reported that many proteins involved in these pathways are linked to AD. Both sporadic and dominantly inherited AD show a primary pathogenic role for protein aggregation and accumulation.

Prions are infectious protein assemblies that can transmit disease between individuals by serving as templates that convert normal protein to a pathogenic conformation (12–14). The elucidation of this novel and frightening basis of neurodegenerative disease led to the Nobel Prize in Physiology or Medicine in 1997 for Stanley Prusiner, who identified the causative agent, the prion protein (PrP). However, with the predominant effort to confirm protein-based infectious propagation of pathology, in some respects the field was distracted from investigation into the molecular and cellular mechanisms of pathogenesis, in which a tiny inoculum progressively amplifies its structure within individual cells and then spreads throughout the nervous system. Importantly, most prion disease cases are sporadic, not infectious, indicating that this process can begin and progress on the basis of endogenous mechanisms.

In most respects, prion disease resembles noninfectious diseases such as AD, Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), as virtually all cases include progressive neurodegeneration, deposition of protein amyloids, and genetic as well as sporadic causes. In the last decade compelling basic research has linked prion mechanisms to common amyloid diseases (15–21). New studies make clear that parallels extend to fundamental aspects of propagation and the role of strains, which are faithfully self-replicating amyloid assemblies that produce unique patterns of disease (**Figure 1**). Elucidation of basic mechanisms involved in the cell–cell propagation of pathology promises to introduce new therapeutic and diagnostic strategies. Additionally, by learning from prion biology about the role of discrete amyloid structures in driving certain patterns of pathology, we may gain additional insights into pathogenic mechanisms. This review focuses on two aspects of prion mechanisms in the spreading pathology of disease: (*a*) transcellular propagation and disease progression and (*b*) the role of strains in dictating neuronal vulnerability and patterns of spread through the nervous system.

Prion: a structured protein assembly that self-replicates in living systems and whose conformation controls its biological activity and potential for transmission between individuals

Strain: a unique prion conformation that replicates faithfully in living systems and confers specific biological effects

Transcellular propagation: growth of a specific amyloid structure within a cell, followed by its movement to another cell and subsequent amplification based on interaction with native protein

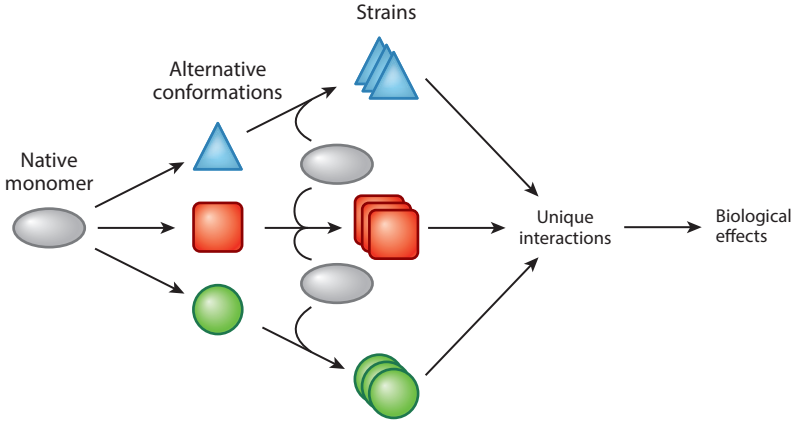

Figure 1

Prions and strains. Prion formation begins with conversion of native monomer to a form that can self-associate into ordered assemblies. These amplify through contact with free monomer, preserving their original structure by acting as templates. Strains are distinctly defined structures that lead to unique biological consequences, such as neurotoxicity, variation in the rate of spread through the nervous system, and specific patterns of cell vulnerability within brain networks. This presumably occurs because of strain-dependent variation in uptake, intracellular seeding, and contact with other cellular components.

CELL BIOLOGY OF AGGREGATE PROPAGATION

Seeding: the ability of an ordered protein or protein assembly to act as a template to trigger subsequent growth of a homotypic amyloid fibril

Studies of PrP have long implicated propagation of pathological protein aggregation by way of neural connections, as inoculation of the eye leads to spread of pathology along the visual network of the central nervous system (22–24). In 2000, Walker and colleagues (25) described the induction of plaque pathology in transgenic mice overexpressing amyloid precursor protein (APP) five months after infusion of brain homogenates containing amyloid-β (Aβ), but not homogenates of young, nondiseased brain. A follow-up study (26) described the appearance of histopathological markers in the contralateral hemisphere, suggesting spread of pathology. Subsequent work indicated that Aβ can propagate a distinct structure in vitro (27). Jucker and colleagues (18) found that inoculation of transgenic mice that overexpress APP with various preparations of Aβ fibrils can induce plaque pathology, and they observed that Aβ immunodepletion, protein denaturation, or immunization effectively abolished the seeding ability of the extracts. These pioneering studies clearly indicated that Aβ fibrils could be infectious in animal models (i.e., they could be propagated on the basis of inoculation) and suggested that template-induced aggregation might play a role outside of classical prion diseases (18).

Induction of Aβ pathology occurs via multiple routes, including direct inoculation (18), insertion into the brain of metal wires coated with Aβ fibrils (28), and even peripheral administration (29). These and other studies also highlighted the presence of distinct Aβ conformations that could be detected in human tissue (30) or could produce distinct pathological patterns in animals (31). This work followed up predictions made by Prusiner (32) in 1984 linking AD and prion disease, which were based on his observation of PrP fibril rods that were similar to Aβ fibrils seen in AD. Unlike most amyloids associated with neurodegeneration, Aβ accumulates outside cells, which is presumably where growth of fibrils occurs, and thus progressive deposition need not involve transcellular propagation.

AGGREGATE PROPAGATION INTO CELLS

In 2008, two landmark studies documented the occurrence of α-synuclein inclusions, or Lewy bodies, in cells transplanted into the striatum of patients with PD (20, 33). In an effort to ameliorate their PD symptoms, these patients had received fetal dopaminergic transplants up to 14 years before autopsy. The investigators observed α-synuclein protein inclusions in the transplanted cells, which expressed tyrosine hydroxylase, and could thus be discriminated from surrounding neurons. These observations suggested either that pathological synuclein had transferred from the host to the grafted cells, or that something toxic in the brains of patients with PD led to aggregation of synuclein in the relatively young transplanted cells. Follow-up studies by S.-J. Lee and colleagues (19) indicated that aggregated α-synuclein transfers between cultured cells, and from host to engrafted cells, and work from V. Lee and colleagues (34) indicated that α-synuclein directly transduced into cultured cells triggers accumulation of phosphorylated α-synuclein aggregates.

Investigations of tau protein provided clear evidence of induced misfolding that could transmit across membranes. Tolnay and colleagues (17) injected brain lysates containing tau aggregates into the brains of tau transgenic mice. They observed induction of local tau pathology, and apparent spread to connected cells, albeit across relatively short distances (17). Concurrently, our group (21) determined that exposure of cultured cells expressing tau protein to extracellular fibrils would directly induce intracellular amyloid formation and that induced intracellular aggregates would transfer to cocultured cells. Additionally, the Kopito laboratory (35) determined that polyglutamine aggregates driven into cultured cells expressing a wild-type form of the protein huntingtin can trigger a persistent state of aggregation that is maintained in a few cells over many cell divisions. Other groups have made similar observations with tau (36), α-synuclein (34), superoxide

Table 1 Evidence for prion mechanisms in neurodegenerative diseases

Disease	Protein	Evidence for prion-based mechanism of spread	Reference(s)
Alzheimer's disease	Microtubule-associated protein tau (tau)	Cells Rodent	21, 36, 41, 42 17, 43–45
	Amyloid-β (Aβ)	Cells Rodent Human	46, 47 18, 25, 29 48
Parkinson's disease	α-Synuclein	Cells Rodent Human	34, 36, 49, 50 49, 51, 52 20, 33
Amyotrophic lateral sclerosis	Superoxide dismutase 1 (SOD1)	Cells Rodent	37, 38, 53 54, 55
Transmissible spongiform encephalopathy	Prion protein (PrP)	Cells Rodent Human	56, 57 58, 59 60, 61
Huntington's disease	Huntingtin (Htt)	Cells Rodent Human	35, 62 63 64

dismutase 1 (SOD1) (37, 38), and other proteins associated with neurodegeneration (39) (**Table 1**). Taken together, a broad consensus has rapidly emerged regarding fundamental cellular events that could underlie transcellular propagation: Protein aggregates move between cells in culture and in vivo, and aggregates taken into cells can trigger intracellular misfolding. It remains unknown whether these mechanisms truly underlie progressive propagation of pathology in humans, and other models are still proposed (40).

UPTAKE AND RELEASE MECHANISMS

Several aggregate internalization mechanisms for Aβ, α-synuclein, and tau have been proposed (**Figure 2**). Evidence for endocytosis of α-synuclein stems from original observations of a reduction in uptake and toxicity of fibrils exogenously added to neurons expressing GTPase-deficient Rab5A, which mediates both clathrin-dependent and clathrin-independent endocytosis (65). Internalization of α-synuclein via clathrin-mediated endocytosis is supported by proteomic evidence that identifies clathrin as a necessary component for microglial activation following uptake of α-synuclein fibrils (66). A recent report proposed lymphocyte-activation gene 3 as the receptor that mediates internalization of α-synuclein (67). Exogenous Aβ has been proposed to be internalized via heparan sulfate proteoglycans (HSPGs) (68); via endocytosis mediated by multiple factors, including dynamin, caveolin-1, and GM1 (69); and via receptor-mediated endocytosis involving lipoprotein receptor-related protein 1/apolipoprotein E, sortilin, and alpha-7-nicotinic acetylcholine receptors (70, 71), NMDA receptors (72–75), and the cellular form of PrP (76). Finally, a mechanism involving tunneling nanotubes has been suggested for internalization of Aβ (77). Given the uncertainty about where Aβ seeding might occur, the relevance of its uptake to aggregate growth must be studied further.

Macropinocytosis is the best-characterized uptake mechanism for tau fibrils in cells and animals (78). This involves binding and uptake of free protein aggregates. Our laboratory initially observed that tau aggregates are taken up via fluid-phase endocytosis (21). Subsequent work determined that tau monomer and aggregates bind directly to HSPGs on the cell surface (78) on

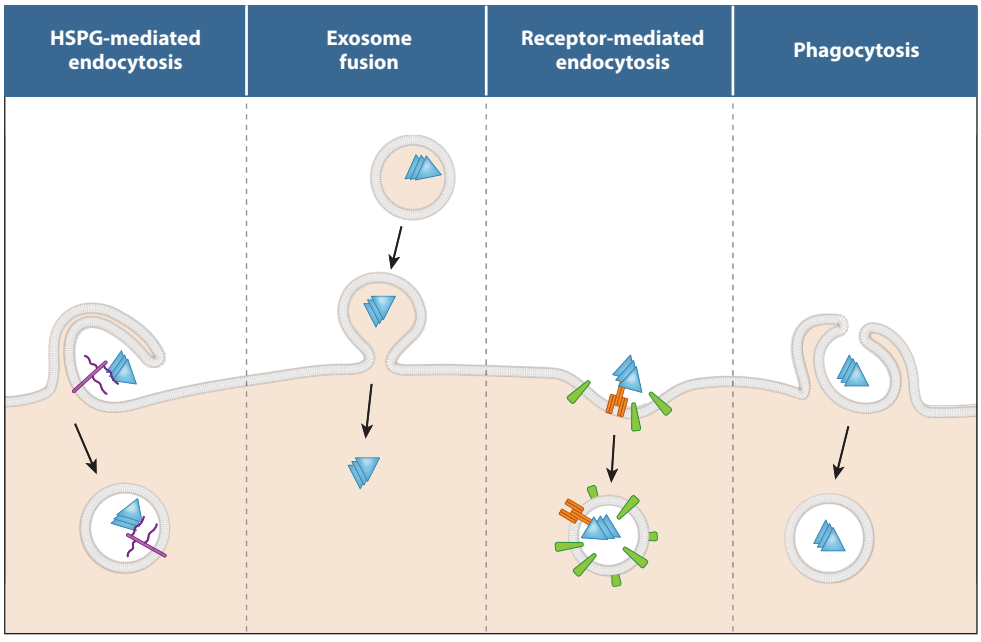

Figure 2

Mechanisms of uptake. Multiple mechanisms of uptake for pathogenic amyloid seeds have been proposed. Macropinocytosis has been clearly linked to tau and α-synuclein aggregate uptake and seeding in diverse cells, including neurons. This is based on binding to heparan sulfate proteoglycans (HSPGs) on the cell surface, which triggers the formation of large endocytic vesicles (macropinosomes) that bring aggregates into the cell. Although not clearly defined, receptor-mediated endocytosis, based on binding of aggregates to specific proteins at the cell surface, could occur. Exosome fusion and phagocytosis might also play a role.

the basis of specific sulfation patterns (79). HSPGs are transmembrane and glycolipid-anchored proteins that are heavily glycosylated and sulfated during their maturation. They coat all cells and mediate interactions with other cells, signaling molecules, and the extracellular matrix (80). Tau uptake into cells and neurons is blocked by genetic disruption of HSPG synthesis, enzymatic cleavage of heparan moieties, or interference with proper HSPG sulfation (78, 79). Finally, glycosaminoglycan mimetics such as heparin mask the HSPG binding site on tau, which blocks its binding to the cell surface, uptake, and seeding. We found that a synthetic heparin-like compound termed F6 binds tau and prevents its uptake into cells and neurons in mouse brain (78). Elucidation of this molecular mechanism of cell uptake requires further study, including development of more effective compounds that directly bind tau, and potentially the identification of HSPG-related synthetic genes that might be targeted to block uptake in neurons. Interestingly, we observed that α-synuclein aggregates utilize HSPG-mediated uptake in a manner similar (78), but not identical (79), to that of tau. Other studies have implicated release of aggregates in ectosomes (81) and exosomes (82), which could mediate uptake upon fusion to secondary cell membranes.

The precise size of aggregates that mediates transcellular propagation of neuronal pathology in vivo is unknown. One study reported that low-molecular-weight aggregates and short fibrils, but not monomers or long fibrils, are competent to seed into cells, although precise sizes of the species were not defined (83). We have detected several soluble tau aggregates in the brains of patients with AD (84). Fractionation of recombinant protein indicated that three units of tau are the minimal size of an aggregate that is sufficient to trigger uptake and seeding into HEK293 cell and primary neurons (84). Remarkably, this is the same size of PrP assembly determined almost

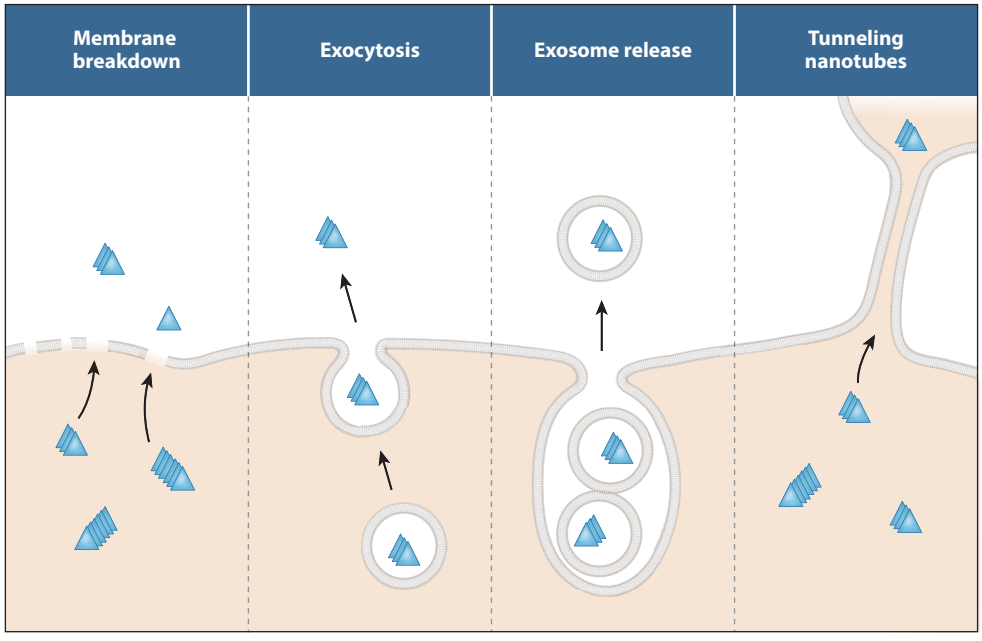

Figure 3

Mechanisms of release. Multiple mechanisms of aggregate release have been proposed. Membrane breakdown could allow passive, transient efflux of aggregates from the cell. Alternatively, aggregates could be packaged into vesicles and released via exocytosis. Ectosome formation also could occur, in which aggregates are packaged in smaller vesicles and subsequently released by fusion of a larger multivesicular body. Finally, aggregates might transfer via tunneling nanotubes that physically link nearby cells.

30 years ago to be the minimal unit of infectivity (85). Others have also implicated tau trimers as toxic agents (86). We observed no clear upper limit of assembly size for seeding into cells (84). These observations directly bear on other cellular studies of tau secretion, which have quantified the release of tau monomer from cultured cells into the medium or into interstitial fluid or cerebrospinal fluid (CSF) (87–93). The secretion of tau monomer may have a physiologic role. However, our studies of aggregate uptake and seeding indicate that tau monomer is unlikely to mediate transcellular propagation of aggregation.

Multiple studies have now also studied aggregate uptake and movement in primary neurons (83). Microfluidic systems have enabled the study of axonal trafficking and have documented both anterograde and retrograde transport of aggregates, with uptake by secondary cells (94). In mouse models there is now clear evidence for transneuronal movement of tau protein (43, 45, 94), although it is unclear whether this is mediated by aggregates. Many studies have evaluated release of α-synuclein from cells and have implicated a variety of release mechanisms that include direct membrane penetration, exosomes, and exocytosis (95) (**Figure 3**). Recent work also suggests that synaptic activity might play a role in stimulating tau release (91, 96). It is unknown whether pathological aggregates can be released in vesicles or whether aggregates enter the extracellular space without an enclosing membrane. Studies of cell–cell propagation must also take into account the fact that aggregates will move even between nonneural cells (21). It may be that a synapse, from the point of view of an aggregate, is merely a place where two plasma membranes come into close approximation. This idea is supported by the findings of Moechars and colleagues (97), who observed that creation of an artificial synapse through expression of neuroligin 1 and leucine-rich repeat transmembrane protein 2 on aggregate donor cells, which facilitated a connection to

primary neurons, increased induction of tau aggregation in the neurons. Negative control expression of N-cadherin, which also increased cell contact without creating a synapse-like structure, did not facilitate propagation (97). Direct aggregate release, or loss of integrity of even a small component of the cell membrane, could lead to extracellular aggregates that subsequently bind the surface of a connected or neighboring cell and trigger their own uptake. Although neuronal signaling may enhance tau aggregate propagation (97) or release in vitro (98), the observation of transcellular movement in the absence of synapses implies a mechanism distinct from a classical transmission process linked to synaptic vesicle release. With this in mind, atypical release via ectosomes could play a role (81). While it is unknown whether distinct release mechanisms will apply to each amyloid protein, we favor a unifying model involving release of free aggregates into the extracellular space, especially given the effectiveness of antitau antibodies to modulate cell uptake in vitro (99) and toxicity in vivo (100, 101) and the conspicuous absence of tau from the proteome of synaptic vesicles (102).

SEEDING ASSAYS

The detection of PrP prions based on in vitro seeding assays has advanced to extremely high sensitivity (103, 104). In 2017, researchers described an in vitro detection system (real-time quaking-induced conversion, or RT-QuIC) for biological fluids that detects three-repeat tau seeding activity in patient-derived CSF using minute amounts of material (105). RT-QuIC is similar to previously described methods (106, 107), but the sensitivity appears higher. Other researchers have exploited similar techniques with high sensitivity and specificity to detect Aβ oligomers in CSF from patients with AD (108) and α-synuclein (109) in patients with PD and dementia with Lewy bodies (**Figure 4**).

To study the biology of transcellular propagation of tau and α-synuclein pathology, and that of other propagating amyloid disorders, researchers required new tools to identify and quantify seeding activity in biological samples. Consequently, we developed a biosensor cell line based on stable expression of tau repeat domain containing a single disease-associated mutation (P301S) fused to cyan and yellow fluorescent proteins (RD-CFPs/YFPs) (110). Upon aggregation, quenching of CFP by YFP leads to fluorescence resonance energy transfer (FRET). This can be quantified most accurately by flow cytometry (110, 111) (**Figure 4**). This assay is highly sensitive and specific, detects tau seeds to the level of ~300 femtomolar (monomer equivalent), and can be applied to fresh frozen or fixed brain material (112). When used to study brain tissue from a transgenic mouse model (PS19) that expresses full-length human tau containing the P301S mutation, this assay detected tau seeding activity at 6 weeks of age, several months before the earliest neuropathology could be detected at approximately 16 weeks (110). The biosensor assay has proved useful for monitoring tau seeding activity in localized brain tissue in patients and in transgenic mouse models of propagating pathology (113); it also works with formalin-fixed tissue (112). Recent work based on this assay indicates that seeding activity in human AD samples anticipates the development of classical neurofibrillary pathology, as it occurs widely throughout the brain at relatively early Braak stages, whereas AT8-positive (phospho-tau) inclusions become apparent only at later stages (114). The introduction of a rapid, quantitative metric to monitor pathology could have important implications for tracking disease progression in mouse models and patients.

MOUSE MODELS OF SPREADING PATHOLOGY

Many mouse models exist for the major neurodegenerative diseases (**Table 1**). Transgenic mice typically feature abnormal protein expression throughout the brain, making it difficult to monitor

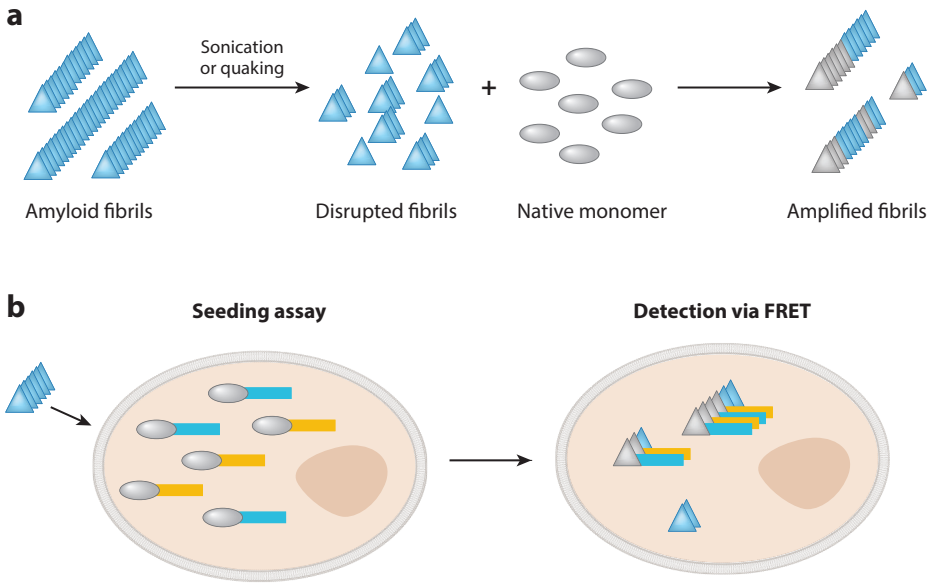

Figure 4

Seeding assays. Two distinct approaches quantify seeding activity. (*a*) In the first approach, a sample containing seeds is exposed to recombinant cognate monomer. A cycle of incubation, quaking (agitation), or sonication is repeated to encourage fibril breakage and exponential growth. Large assemblies can be detected by a variety of means, typically through incorporation of a fluorescent dye (e.g., thioflavin). (*b*) In the second, cell-based approach, a biosensor line is created with stable expression of cognate protein fused to cyan and yellow fluorescent proteins, or a similarly complementary fluorescent pair. Upon exposure to a sample containing seeds, often with a reagent such as a cationic liposome to increase cell uptake, seeds are brought into the cell, where they interact with the labeled protein and trigger intracellular aggregation. This is quantified in a defined population of cells either by fluorescence microscopy or FRET flow cytometry. Abbreviation: FRET, fluorescence resonance energy transfer.

spreading pathology without specific control of gene expression. One conditional model uses the Tet-Off factor driven by the neuropsin promoter to activate mutant tau expression in one brain region. At early ages, this activates gene expression predominantly in layer II of the entorhinal cortex (43, 45). Tau pathology subsequently becomes apparent in hippocampal cells that receive input from the entorhinal cortex. Results from two independent laboratories were consistent with the spreading model of neurodegeneration, as they described the movement of pathological tau protein from one region to another. It is less clear that this movement actually represents true transcellular propagation of pathology, in which a seed moves between cells and then corrupts native protein to amplify an aggregated state. Two reports cast doubt on propagation in this mouse model. The first study found that activity of the neuropsin promoter, which was used to drive the Tet-Off factor, may not be anatomically restricted to the entorhinal cortex and that it expresses tau more widely over time, especially within the hippocampus (115). Thus, during the long incubation periods used for the studies, mutant tau may have been expressed at low levels in secondary cells and may not have moved there. In a second study, propagation of entorhinal tau was observed in a tau knockout background (116). In the absence of endogenous tau, it is technically impossible to propagate pathology, but nonetheless progressive tau pathology was observed. These results suggest that earlier propagation may simply have represented transcellular movement of pathological

proteins. Similar results have been obtained with virus-mediated expression of mutant tau, which also transfers between neurons in vivo (94).

Other mouse models have used inoculation of virus or proteopathic seeds directly into the brain. Inoculation of synuclein fibrils derived from either patients or recombinant sources leads to progressive pathology (51). In fact, even wild-type mice inoculated with α-synuclein fibrils develop progressive disease (52). Many studies of tau indicate that progressive pathology develops from local inoculation of seeds (15, 17). In all such studies it is important to rule out simple movement of inoculum from one cell to another, as this does not represent propagation. Our laboratory has observed that distinct tau aggregate structures, or strains, replicate and propagate throughout the brain at different rates. This is independent of the seeding activity of each strain and thus appears to depend on tau prion structure (113). Other laboratories have used virus-mediated expression of full-length, mutant tau to drive tauopathy in different brain regions (94). In most cases, documentation of the spreading pathology, but not extraction and documentation of fibrillar material or tau seeding activity, has been based on immunostains for hyperphosphorylated tau (17, 43, 94). Consequently, it is difficult to know at this time whether the presence of tau or α-synuclein seeding activity directly correlates with standard histopathological markers. Taken together, multiple useful mouse models exist to study propagation of pathology. We do not yet know their validity for studying basic cellular mechanisms of propagation. But it is clear that these models can be used to study transport of protein monomer and aggregates and seeded induction of protein aggregation.

THERAPEUTIC MECHANISMS

As we learn more about the cellular mechanisms by which aggregates bind the cell surface, trigger uptake, seed intracellular aggregation, and move between cells, we anticipate this will facilitate the development of mechanism-based therapies to slow down or stop disease progression (**Figure 5**).

Blocking Release

Blocking the release of pathological aggregates from the cell could in theory prevent the transcellular propagation of pathology. It is unknown how release of pathological seeds occurs in patients. On the one hand, this process could be initiated upon cell death or local membrane breakdown, in which case it seems unlikely that any biological target will emerge. On the other hand, if unconventional secretion of protein aggregates forms the basis of cell release, this could theoretically be a viable therapeutic mechanism. Katsinelos et al. (117) reported that tau is released via an unconventional pathway that involves binding to the inner leaflet plasma membrane followed by direct translocation mediated by interaction with sulfated proteoglycans. Although intriguing, it remains to be understood how this mode of secretion may enable neuronal activity–dependent release of tau as reported previously (91). Currently, we do not know enough about fundamental release mechanisms to design specific therapeutic inhibitors.

Immunotherapy

Immunotherapy is a very near-term therapeutic option, as clinical trials are under way to evaluate antibodies against tau and Aβ (NCT02494024, NCT02760602, NCT02353598, NCT02051608, NCT01998841). Since the initial reports of the effectiveness of immunotherapy against the Aβ protein (118), this approach has been explored to treat myriad neurodegenerative diseases. Initial attempts to vaccinate mouse models against α-synuclein were successful (119). At that time the

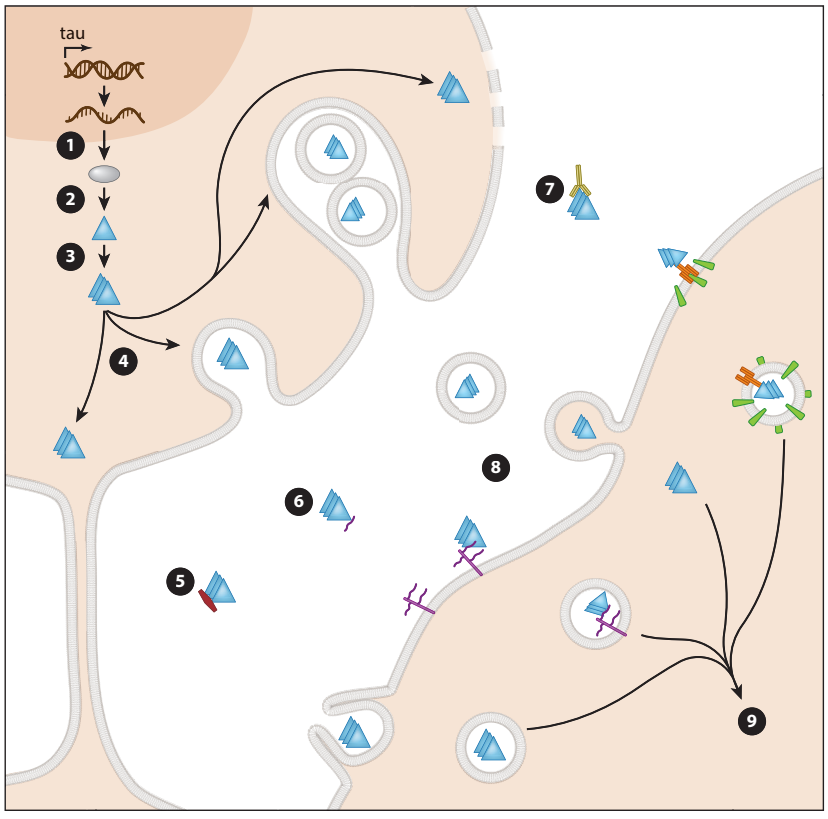

Figure 5

Therapeutic mechanisms. Given the knowledge about propagation of amyloid pathology, several mechanisms could be exploited to block this process. (❶) Reduction in expression of tau could be mediated by antisense oligonucleotides or shRNAs. (❷) Prevention of monomer conversion could be effected by compounds that bind and stabilize native tau or by prevention of events that lead to its conformational change. (❸) Blocking aggregate formation could be achieved by small molecules to prevent self-assembly. (❹) Blocking aggregate release might prevent transcellular propagation. (❺) Small-molecule inhibitors of tau binding and uptake could be used to prevent transcellular propagation. (❻) Heparin mimetics to block HSPG binding could prevent transcellular propagation. (❼) Antibodies could be used to target extracellular tau, promoting clearance or preventing uptake. (❽) Blocking uptake mechanisms could prevent subsequent intracellular seeding. (❾) Blocking aggregate amplification could block propagation. Abbreviations: HSPG, heparan sulfate proteoglycan; shRNA, small hairpin RNA.

effectiveness of the vaccine was not understood in the context of spreading protein pathology. Since then, multiple studies have reported vaccine therapies against α-synuclein and tau (100, 120–122). These have included active (121) and passive (100, 120) vaccination strategies. In our experience, peripheral administration typically has more modest effects on transgenic mouse models (122), whereas central administration of antibodies has been more effective (100). The mechanisms of immunotherapy are not well defined. Antibodies could promote the clearance of pathogenic proteins from the interstitial space to the periphery (122). Alternatively, antibodies may directly affect brain clearance. Intraneuronal mechanisms of action for therapeutic antibodies have even been proposed (123, 124). We found that two antibodies directed against the amino terminus of the tau protein promoted uptake into microglia-like cells in vitro, while another antibody that

targeted the repeat domain of tau directly inhibited uptake of protein aggregates into neurons but did not affect their uptake into microglia-like cells (99). Overall, we observed multiple mechanisms for different antitau antibodies, including differential effects based on aggregate size (99). Immunotherapy could thus have multiple mechanisms of action that depend on particular epitopes as well as the size of the targeted aggregate.

Cell Uptake

Several modes of tau uptake into neurons have been proposed, but data are limited for most mechanisms with the exception of macropinocytosis, also termed bulk or fluid phase endocytosis. Cell uptake mediated by HSPGs has been clearly defined in vitro and is required for efficient seeding. We have previously observed that HSPGs mediate the binding and uptake of both tau and α-synuclein seeds (78). Targeting the HSPG pathway could involve small-molecule inhibitors of HSPG synthesis or molecules such as F6 that bind tau and inhibit binding to the cell surface. Functional HSPGs require enzyme-mediated maturation within the secretory pathway. This includes sulfation and glycosylation patterns that lend specificity to ligand binding. Recently, we and others have reported that tau and α-synuclein require specific but distinct sulfation patterns of heparan sulfate chains to bind glycans and enter cells (79, 125). However, this pathway has not been studied extensively enough for tau or other propagating amyloids to know whether it holds viable therapeutic enzyme candidates. Clathrin-mediated endocytosis has also been implicated for α-synuclein (126), possibly suggesting an alternative therapeutic approach.

Intracellular Clearance

Increased clearance of protein aggregates has long been a therapeutic goal (127, 128). The knowledge that small molecules can upregulate protein clearance has raised the hope of shifting the cellular balance from aggregate accumulation to degradation (129–133). We do not yet know the effects of chronic administration of compounds such as rapamycin, which upregulate autophagy, to patients with neurodegenerative diseases. However, it may be possible to avoid chronic administration through drug holidays, whereby patients receive brief courses of therapy designed to promote clearance of protein aggregates, followed by gaps in treatment. In theory, such an approach could allow recovery of injured neurons and reset their protein aggregate load. We are still learning about distinct pathways of protein aggregate degradation, and as these data emerge, along with the development of compounds that activate them specifically, it may be possible to better direct aggregate clearance.

Antisense oligonucleotides (ASOs) and RNA interference also represent important approaches to reduce intracellular toxic protein expression. Clinical trials of intrathecally administered ASOs are now under way to evaluate the efficacy of SOD1 knockdown in familial forms of ALS (134) (NCT01041222, NCT02623699). An ASO targeting tau in mouse models of tauopathy has also reduced pathology and seeding activity (135), and another is now in early clinical trials (NCT03186989). The use of ASOs could revolutionize therapy of myriad neurodegenerative diseases linked to intracellular amyloid accumulation, as a common mode of therapy (ASO) could be used against many genetic targets.

INHIBITION OF INTRACELLULAR SEEDING

Upon cell entry, pathological aggregates act as templates for conversion of native protein. This has sparked many attempts to develop therapies based on preventing fibril growth (101). So far

none have been approved for clinical use. With more potent compounds it may be possible to achieve greater efficacy, although most still must function stoichiometrically. In theory, approaches to modulate aggregation directly could employ multiple mechanisms, including stabilization of monomer; inhibition of fibril growth; acceleration of aggregation to form larger, less toxic assemblies; or reduction of fibril fragmentation (136). Indeed, some groups are exploring small-molecule and antibody approaches to inhibit primary and secondary nucleation steps of amyloid aggregation (3, 137, 138).

New approaches may also come from better elucidation of the mechanisms of intracellular aggregation. In contrast to seeded polymerization of amyloids in vitro, within a cell the complexity of the environment and dedicated degradation systems would appear to mitigate against spontaneous fibril assembly following uptake of a limited number of seeds, suggesting a role for a replication machinery. For example, the heat shock protein 70/40 (Hsp70/40) chaperone system has been proposed to fragment fibrils and thus enable subsequent growth (139). Other cellular chaperones, however, inhibit microscopic steps in the process of Aβ aggregation, such as secondary nucleation, and could thus be important therapeutic targets (140). We do not yet know which of the many subtypes of chaperones could be therapeutic targets for preventing amyloid formation of other proteins. Functional genomics will play a key role in identifying these and other regulators of aggregation.

THE ROLE OF PRION STRAINS AND NETWORKS IN NEURODEGENERATIVE DISEASES

Prion strains are pathological assemblies of unique conformation that faithfully replicate in living systems and produce predictable patterns of neuropathology. We discriminate true strains from amyloid conformers that can be produced in vitro because only certain conformations replicate stably in living systems, account for specific biological activity, and thus have clinical significance. Tau strains clearly have unique effects on cells and are linked to specific neuropathological syndromes (15). Work from our laboratory also indicates that strain conformation alone is sufficient to account for enormous variation in neuropathology (113). Understanding strains in neurodegenerative diseases will help us account for neuronal vulnerability, rates of progression, responses to future therapies, and more accurate diagnoses.

YEAST PRION STRAINS

Yeast prions were identified in the 1960s as mediators of epigenetic inheritance (141). Their study has greatly informed our knowledge of mammalian PrP prions. It was not clear initially how yeast prions served the organism, but they now appear to play a fundamental role in regulating metabolism (142–145). Using computational and functional approaches, investigators have now identified multiple proteins within the yeast proteome that are putative prions (142). The best-studied yeast prion, Sup35, normally functions as a translation termination factor in the setting of nonsense mutations (146). Under certain circumstances, Sup35 transitions to an amyloid structure that recruits and depletes free monomer from the cytosol, permitting read-through of genetic errors. This is presumably beneficial during organismal stress. In this sense Sup35 functions as a binary switch. However, multiple strains of Sup35 have been identified that aggregate to variable degrees. How much they leave free monomer to function in the cell is inversely proportional to their strength as a prion. Sup35 and fusion proteins created from its prion domain have enabled the development of synthetic phenotypes in yeast cells to dissect regulatory factors. Principal among these is the chaperone Hsp104, which plays an essential

role in prion replication (147) and helps break amyloid fibrils (148). At levels optimal for prion replication, Hsp104 enables efficient fibril severing to create new amyloid seeds. These seeds are efficiently inherited by dividing yeast cells, maintaining mother-to-daughter transmission of the prion state. Somewhat paradoxically, either under- or overexpression of Hsp104 blocks prion maintenance. Low levels lead to diminished fibril severing with inefficient inheritance, whereas high levels lead to fibril degradation that exceeds growth. This has led to a model of yeast prion strain maintenance that takes into account fibril growth rate versus stability (149) and may be applicable to mammalian prions (150). There is no known mammalian homolog of Hsp104, but other factors, such as Hsp70 and Hsp40, may play similar roles (139). Given the evolutionary conservation of yeast signaling mechanisms in metazoans, it seems likely that functional prion strains will have widespread importance in cell and organismal biology (16).

Aβ, TAU, AND α-SYNUCLEIN STRAINS AND STRAIN-LIKE BEHAVIOR

Walker and colleagues (18, 31) first described the effects of distinct amyloid conformations on neuropathology following inoculation in mice. These studies were highly reminiscent of prior studies of PrP strains that had spanned decades (151, 152). The investigators found that both the source of Aβ fibrils and the strains of mice into which they were injected dictated unique patterns of neuropathology (18). Because Aβ accumulates predominantly in the extracellular space, there was no study of transcellular propagation. However, these investigators were the first to observe unique pathologies arising from amyloid proteins other than PrP prion strains. Subsequent studies of Aβ propagation from animal to animal have reported consistent strain-like protein behavior (153, 154). Although Aβ conformers have not been demonstrated to propagate indefinitely in vivo, it is clear that distinct amyloid structures produce unique patterns of neuropathology upon inoculation, which is consistent with Aβ strains. This idea is supported by recent reports that patients infected with pathogenic prions also developed unique Aβ deposition patterns, consistent with the idea that Aβ transmits pathology in humans under the right circumstances (48) and that unique Aβ conformers are associated with AD subtypes (155).

Multiple reports now additionally describe distinct tau and α-synuclein aggregate conformers in experimental systems (156–158), and reports indicate that different strains may exist in patients (15, 30, 159, 160). The clearest evidence to date is the elucidation of the cryo–electron microscopy structure of ultrastructurally distinct tau fibrils from a tauopathy patient (160). In 2013, Lee and colleagues (157) created uniquely structured synuclein fibrils in vitro that triggered distinct patterns of tau pathology in cultured neurons and upon inoculation into mice. In this study, α-synuclein was iteratively fibrillized with successive seeding reactions. The investigators noticed that the conformation of the α-synuclein fibril preparations changed over successive reactions, and fibrils created after 10 generations of seeding had biological effects different from those of the initial preparation (157). Although not meeting the criteria of strains as defined above, this investigation clearly showed variance in the biological activities of amyloids of distinct structure.

In other studies of α-synuclein, Melki and colleagues (156) created two distinct types of fibril conformations based on different fibrillization conditions. When inoculated into cells, the two types replicated the biochemical characteristics of the original fibrils (156). A subsequent publication described inoculation of distinct synuclein fibril conformers into animals, which produced unique patterns of neural pathology (161). Prusiner and colleagues (159, 162) have also studied α-synuclein and have observed differential seeding from human synucleinopathy brains into cultured cell and animal models. In both cases, they found that PD brain had no detectable seeding activity, whereas multiple system atrophy (MSA) brain exhibited seeding activity in cells and animals.

Moreover, they recently reported that familial PD mutations abolish the ability of MSA prions to replicate (163). They interpreted these data to mean that distinct α-synuclein prion strains exist in the two diseases. Taken together, these studies are consistent with the idea of α-synuclein prion strains, but they have not conclusively demonstrated their existence through isolation and characterization of synuclein prion assemblies of distinct structure to fulfill Koch's postulates for transmission.

Our laboratory (15) studied tau prion strains using a reductionist system based on expression of the tau repeat domain containing two disease-associated mutations (P301L, V337M). This protein was fused to YFP to enable visualization of intracellular inclusions. We exposed cells expressing this protein to recombinant fibrils and measured the retention of inclusions over time by visual inspection. We observed a rapid reduction of cells with inclusions, but even after 50 days in culture the cells that had been exposed to an inoculum maintained approximately 1–2% of the population with inclusions (15). This finding was similar to a prior report of maintenance of polyglutamine inclusions (35). We hypothesized that the maintenance of inclusions came from mother-to-daughter aggregate transmission during mitosis. We tested this hypothesis by deriving multiple monoclonal cell lines that stably propagated aggregates, and observed two dominant intracellular inclusion morphologies associated with distinct patterns of seeding activity, detergent solubility, and protease sensitivity. Remarkably, aggregation patterns were transferable by extraction of tau from one cell line and seeding into a naïve line. Thus, in a cell culture system, tau met the criteria of a prion: It produced aggregates of distinct morphology and biological effects and had a structure that was maintained indefinitely through mother-to-daughter transmission or through successive cellular seeding reactions. We extended these studies to mice and observed that lysates from these distinct cell lines produced unique patterns of neuropathology. This work involved creation of tau strains from recombinant protein and faithful propagation through living systems, with the induction of conformation-dependent pathology. Thus, tau satisfies the criteria of a bona fide prion in most respects, save for spontaneous transmission between individuals.

Finally, we evaluated human tauopathies to test for distinct strain composition. We isolated several hundred monoclonal lines that stably propagated human tau prions. A blind analysis of these lines indicated that different syndromes had clearly distinct strain compositions, often with multiple strains in a single individual (15). Altogether, we observed a surprising fidelity of tau strain propagation in cells and animals and, further, that human tauopathies appear to be comprised of clouds of prion strains, as has been previously described for PrP prions (151).

Although individuals with tauopathy can manifest multiple strains, the basis of variation in clinical and neuropathological findings observed in tauopathy patients has remained unclear. We addressed this problem by isolating and characterizing 18 individual tau strains passaged in cultured cell lines, each with a unique seeding, toxicity, and proteolytic digestion pattern (113). We inoculated extracts from each line individually into a single mouse model that expresses full-length tau containing a disease-associated mutation (P301S). Each strain produced a completely distinct neuropathological syndrome. This included unique patterns of intracellular pathology, differential rates of propagation of pathology throughout the brain, and distinct patterns of regional pathology. We directly tested for regional vulnerability by inoculating a subset of strains into multiple brain regions and observed striking differences. Some strains were promiscuous and would induce pathology in any region to which they were introduced. Other strains were highly restricted in their tropism to specific brain regions. Taken together with the preceding studies, this work indicates that strain identity alone, independent of genetic background, is sufficient to account for tremendous phenotypic diversity (113) (**Figure 6**). Clearly, genetic and environmental factors could also influence presentation, but these data indicate that study of isolated strains might

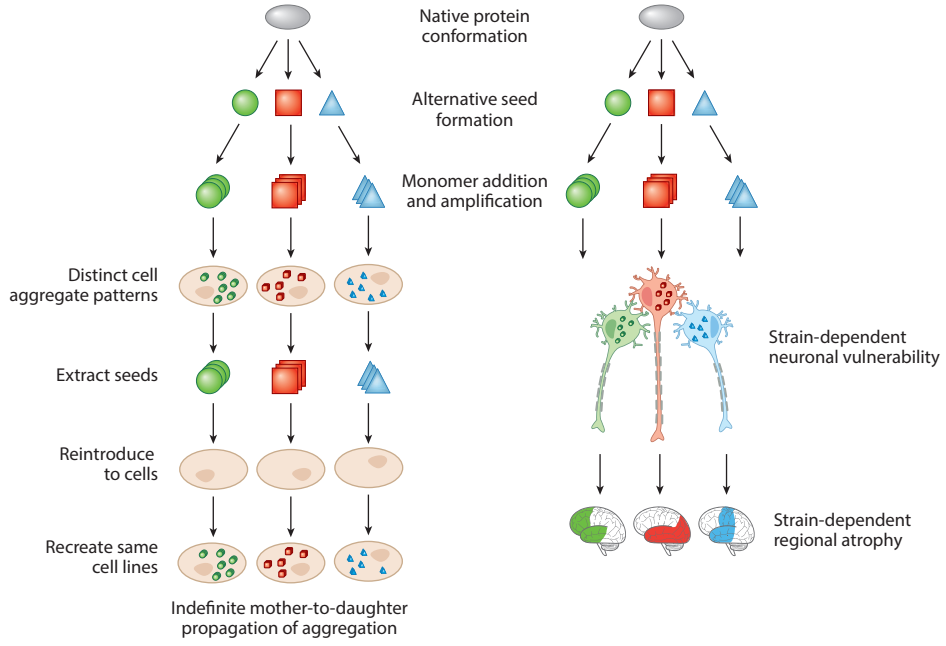

Figure 6

Strains and patterns of neuronal degeneration. Strains for amyloid proteins such as tau have been studied in depth. Distinct strains of tau will replicate a defined structure when introduced into a previously unexposed cell line. In many cases, this structure is maintained indefinitely in cultured cells by mother-to-daughter transmission. If tau aggregates are extracted from a cell line and reintroduced to a naïve cell line, they will recreate the same structure. When inoculated into animals, cell lysates containing the tau strains produce defined pathology that can be transmitted from animal to animal, and even back into cultured cells. Each distinct strain produces a unique pattern of cellular pathology, rate of progression, and involvement of brain networks. Strains thus can account for enormous variation in the presentation of neurodegenerative diseases due to amyloid accumulation.

provide molecular tools to understand the principles of specific neuronal vulnerability and progression rates in neurodegenerative diseases.

NETWORKS IN PROPAGATION

The association of neural networks with neurodegeneration syndromes has been recognized for some time. In experimental models of prion disease, inoculation into the eye leads to progressive degeneration that follows the optic tracts, which is consistent with a transneuronal propagation mechanism (86). In noninfectious human disorders such as ALS, there is combined degeneration of the upper and lower motor neurons that comprise a network (164). ALS progression can involve local spread within either the cortex or the spinal cord (165). Braak & Braak (166) carried out meticulous studies of hundreds of patients with AD to create a staging system to characterize AD progression. Their system groups patients into six distinct stages that are consistent with a progressive pattern of tau deposition in regions known to share synaptic connectivity, such as the entorhinal cortex, transentorhinal cortex, hippocampus, and neocortex (166–168).

Human pathological studies are by necessity cross-sectional, but imaging of living patients offers new insights into progression within individuals (169). Human brain imaging has advanced considerably in the last decade, especially in the realm of positron emission tomography (PET)

and functional connectivity magnetic resonance imaging (fcMRI). fcMRI uses activity-dependent variations in blood flow patterns to infer connectivity among groups of neurons, although the timescale in which blood flow varies (seconds) is much slower than neuronal signaling (milliseconds). Nonetheless, fcMRI has largely recapitulated known local networks and has suggested the existence of many distributed networks in the brain (170–172). A series of important studies have compared networks defined by fcMRI to patterns of progressive atrophy in patients with neurodegenerative diseases. Networks were first defined in normal patients. Progressive atrophy was then determined on the basis of sequential structural MRI scans that analyzed brain volume. When the atrophy patterns were superimposed on known networks, the investigators found a significant correlation, with distinct syndromes tracking to particular networks (173). Subsequent work tested the likelihood of neurodegeneration that follows atrophy in one region of brain against network predictions (174, 175). Putatively connected regions tended to degenerate together, independent of geographical distance between them. Coupled with the original pathological studies of Braak & Braak, these imaging studies have strongly supported the idea that neuronal connections underlie patterns of neurodegeneration.

THE PRION MODEL ADVANCES THERAPEUTIC AND DIAGNOSTIC OPPORTUNITIES

Fortunately, we have not yet encountered cases in which direct exposure of tau, α-synuclein, or other proteins that account for common, sporadic neurodegenerative diseases clearly causes a neurodegeneration syndrome in the manner of infectious prions. Nonetheless, a recent study documented highly unusual patterns of Aβ pathology in cases of transmitted Creutzfeldt-Jakob disease (CJD) that could indicate infection by this protein (because patients died of CJD, it is unknown whether they would ever have become symptomatic from the putative Aβ exposure) (48, 176). Newer reports independently confirmed an increase in coincident Aβ pathology with iatrogenic CJD (177) and implicated transplanted dura mater as a source of infectious Aβ (178). We still do not know whether transcellular propagation underlies the progression of common neurodegenerative diseases. Yet the transcellular propagation model has many important implications. As cellular mechanisms of propagation are defined, specific interventions designed to interrupt key steps such as cell entry or intracellular seeding will provide critical tests of this hypothesis in animal models and, if successful, in patients. The use of therapeutic antibodies against tau, which are currently in early clinical trials, provides an excellent example.

This concept of prion strains has important implications for categorization of amyloid diseases. Strains can explain many aspects of phenotypic diversity and network involvement. Small molecules or antibodies designed to bind tau or inhibit specific aspects of replication of tau or α-synuclein may have strain specificity. Thus, it may be crucial to define the composition of strains within a given patient to predict efficacy of a certain drug. At a minimum, an ability to classify strain composition in patients may provide more accurate correlation of clinical and neuropathological phenotypes, which can be divergent. The existence of strains makes it risky to treat all proteinopathies equivalently, and we must recognize the potential diversity of pathogenic targets. From the standpoint of diagnosis, it is then critical to discriminate distinct amyloid structures in patients. This could be done through biochemical approaches to define seed structure or through novel PET imaging that exploits conformational differences in protein amyloids. In this light, it is remarkable that multiple tau imaging agents have been developed and used in patients, but there is little uniformity across syndromes with regard to PET ligand binding (179). Clearly, more work is required to understand why one agent binds tau in some syndromes but not in others. We anticipate that the prion model will ultimately help lead the field to more effective therapy and diagnosis for neurodegenerative diseases.

SUMMARY POINTS

1. Multiple proteins now exhibit many characteristics of prions, except for spontaneous transmission of pathology between individuals.
2. Multiple mechanisms have been proposed for cell uptake and cell release.
3. Although the best evidence suggests macropinocytosis as a primary mechanism of uptake, no studies in vivo have directly tested this or any uptake or release mechanism.
4. The ability of antibody therapies to reduce pathology in animal models strongly suggests that free extracellular aggregates play some role in pathogenesis.

FUTURE ISSUES

1. Clinical trials are planned or underway for multiple vaccine and genetic therapies.
2. Direct targeting of aggregate formation or growth is a promising therapeutic strategy.
3. Better knowledge of cell biology and biochemistry of aggregate formation and growth may yield new enzymatic targets.
4. Experimental data suggest that distinct amyloid structures target specific cells and networks, providing a framework for understanding diversity of neurodegeneration syndromes.
5. Identification of strains in living patients may improve diagnostic accuracy within syndromes. This might bridge the gaps between clinical and neuropathological diagnoses.
6. Better classification of protein amyloid diseases according to strain composition may lead to improved decision-making for therapies.

DISCLOSURE STATEMENT

M.I.D. is the coinventor of a therapeutic antibody that was licensed from Washington University in St. Louis by C2N Diagnostics and is in clinical trials.

LITERATURE CITED

1. Eisenberg DS, Sawaya MR. 2017. Structural studies of amyloid proteins at the molecular level. *Annu. Rev. Biochem.* 86:69–95
2. Knowles TPJ, Vendruscolo M, Dobson CM. 2014. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* 15(6):384–96
3. Dobson CM. 2017. The amyloid phenomenon and its links with human disease. *Cold Spring Harb. Perspect. Biol.* 9(6):a023648–15
4. Chiti F, Dobson CM. 2017. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. *Annu. Rev. Biochem.* 86:27–68
5. Jarrett JT, Lansbury PT Jr. 1993. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* 73:1055–58
6. Harper JD, Lansbury PT Jr. 1997. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu. Rev. Biochem.* 66:385–407

7. Collins SR, Douglass A, Vale RD, Weissman JS. 2004. Mechanism of prion propagation: amyloid growth occurs by monomer addition. *PLOS Biol.* 2(10):e321–29
8. Powers ET, Powers DL. 2006. The kinetics of nucleated polymerizations at high concentrations: amyloid fibril formation near and above the "supercritical concentration." *Biophys. J.* 91:122–32
9. Mirbaha H, Chen D, Morazova OA, Ruff KM, Sharma AM, et al. 2018. Inert and seed-competent tau monomers suggest structural origins of aggregation. *eLife* 7:e36584
10. Labbadia J, Morimoto RI. 2015. The biology of proteostasis in aging and disease. *Annu. Rev. Biochem.* 84:435–64
11. Kundra R, Ciryam P, Morimoto RI, Dobson CM, Vendruscolo M. 2017. Protein homeostasis of a metastable subproteome associated with Alzheimer's disease. *PNAS* 114(28):E5703–11
12. Prusiner SB. 1982. Novel proteinaceous infectious particles cause scrapie. *Science* 216(4542):136–44
13. Bolton D, McKinley M, Prusiner S. 1982. Identification of a protein that purifies with the scrapie prion. *Science* 218(4579):1309–11
14. Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, et al. 1993. Conversion of α-helices into, β-sheets features in the formation of the scrapie prion proteins. *PNAS* 90:10962–66
15. Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, et al. 2014. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron* 82(6):1271–88
16. Sanders DW, Kaufman SK, Holmes BB, Diamond MI. 2016. Prions and protein assemblies that convey biological information in health and disease. *Neuron* 89(3):433–48
17. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, et al. 2009. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* 11(7):909–13
18. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, et al. 2006. Exogenous induction of cerebral β-amyloidogenesis is governed by agent and host. *Science* 313(5794):1781–84
19. Desplats P, Lee H-J, Bae E-J, Patrick C, Rockenstein E, et al. 2009. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *PNAS* 106(31):13010–15
20. Li J-Y, Englund E, Holton JL, Soulet D, Hagell P, et al. 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* 14(5):501–3
21. Frost B, Jacks RL, Diamond MI. 2009. Propagation of tau misfolding from the outside to the inside of a cell. *J. Biol. Chem.* 284(19):12845–52
22. Fraser H, Dickinson AG. 1985. Targeting of scrapie lesions and spread of agent via the retino-tectal projection. *Brain Res.* 346:32–41
23. Scott JR, Foster JD, Fraser H. 1993. Conjunctival instillation of scrapie in mice can produce disease. *Vet. Microbiol.* 34(4):305–9
24. Brandner S, Raeber A, Sailer A, Blattler T, Fischer M, et al. 1996. Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. *PNAS* 93(23):13148–51
25. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, et al. 2000. Evidence for seeding of β-amyloid by intracerebral infusion of Alzheimer brain extracts in β-amyloid precursor protein-transgenic mice. *J. Neurosci.* 20(10):3606–11
26. Walker LC, Callahan MJ, Bian F, Durham RA, Roher AE, Lipinski WJ. 2002. Exogenous induction of cerebral β-amyloidosis in βAPP-transgenic mice. *Peptides* 23(7):1241–47
27. Petkova AT, Leapman RD, Guo Z, Yau W-M, Mattson MP, Tycko R. 2005. Self-propagating, molecular-level polymorphism in Alzheimer's β-amyloid fibrils. *Science* 307(5707):262–65
28. Eisele YS, Bolmont T, Heikenwalder M, Langer F, Jacobson LH, et al. 2009. Induction of cerebral β-amyloidosis: intracerebral versus systemic Aβ inoculation. *PNAS* 106(31):12926–31
29. Eisele YS, Obermüller U, Heilbronner G, Baumann F, Kaeser SA, et al. 2010. Peripherally applied Aβ-containing inoculates induce cerebral β-amyloidosis. *Science* 330(6006):980–82
30. Lu J-X, Qiang W, Yau W-M, Schwieters CD, Meredith SC, Tycko R. 2013. Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. *Cell* 154(6):1257–68
31. Heilbronner G, Eisele YS, Langer F, Kaeser SA, Novotny R, et al. 2013. Seeded strain-like transmission of β-amyloid morphotypes in APP transgenic mice. *EMBO Rep.* 14(11):1017–22
32. Prusiner SB. 1984. Some speculations about prions, amyloid, and Alzheimer's disease. *N. Engl. J. Med.* 310(10):661–63

33. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. 2008. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat. Med.* 14(5):504–6
34. Luk KC, Song C, O'Brien P, Stieber A, Branch JR, et al. 2009. Exogenous α-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. *PNAS* 106(47):20051–56
35. Ren P-H, Lauckner JE, Kachirskaia I, Heuser JE, Melki R, Kopito RR. 2009. Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat. Cell Biol.* 11(2):219–25
36. Nonaka T, Watanabe ST, Iwatsubo T, Hasegawa M. 2010. Seeded aggregation and toxicity of α-synuclein and tau: cellular models of neurodegenerative diseases. *J. Biol. Chem.* 285(45):34885–98
37. Münch C, O'Brien J, Bertolotti A. 2011. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. *PNAS* 108(9):3548–53
38. Grad LI, Guest WC, Yanai A, Pokrishevsky E, O'Neill MA, et al. 2011. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. *PNAS* 108(39):16398–403
39. Nonaka T, Masuda-Suzukake M, Arai T, Hasegawa Y, Akatsu H, et al. 2013. Prion-like properties of pathological TDP-43 aggregates from diseased brains. *Cell Rep*. 4(1):124–34
40. Walsh DM, Selkoe DJ. 2016. A critical appraisal of the pathogenic protein spread hypothesis of neurodegeneration. *Nat. Rev. Neurosci.* 17(4):251–60
41. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. 2012. Trans-cellular propagation of Tau aggregation by fibrillar species. *J. Biol. Chem.* 287(23):19440–51
42. Guo JL, Lee VM-Y. 2011. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *J. Biol. Chem.* 286(17):15317–31
43. de Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, et al. 2012. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* 73(4):685–97
44. Iba M, Guo JL, McBride JD, Zhang B, Trojanowski JQ, Lee VM-Y. 2013. Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J. Neurosci.* 33(3):1024–37
45. Liu L, Drouet V, Wu JW, Witter MP, Small SA, et al. 2012. Trans-synaptic spread of tau pathology in vivo. *PLOS ONE* 7(2):e31302
46. Bahr BA, Hoffman KB, Yang AJ, Hess US, Glabe CG, Lynch G. 1998. Amyloid β protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. *J. Comp. Neurol.* 397:139–47
47. Nath S, Agholme L, Kurudenkandy FR, Granseth B, Marcusson J, Hallbeck M. 2012. Spreading of neurodegenerative pathology via neuron-to-neuron transmission of β-amyloid. *J. Neurosci.* 32(26):8767–77
48. Jaunmuktane Z, Mead S, Ellis M, Wadsworth JDF, Nicoll AJ, et al. 2015. Evidence for human transmission of amyloid-β pathology and cerebral amyloid angiopathy. *Nature* 525(7568):247–50
49. Hansen C, Angot E, Bergström A-L, Steiner JA, Pieri L, et al. 2011. α-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J. Clin. Investig.* 121(2):715–25
50. Freundt EC, Maynard N, Clancy EK, Roy S, Bousset L, et al. 2012. Neuron-to-neuron transmission of α-synuclein fibrils through axonal transport. *Ann. Neurol.* 72(4):517–24
51. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM-Y. 2012. Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *J. Exp. Med.* 209(5):975–86
52. Luk KC, Kehm V, Carroll J, Zhang Bin, O'Brien P, et al. 2012. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science* 338(6109):949–53
53. Pokrishevsky E, Grad LI, Cashman NR. 2016. TDP-43 or FUS-induced misfolded human wild-type SOD1 can propagate intercellularly in a prion-like fashion. *Sci. Rep.* 6(1):22155
54. Ayers JI, Fromholt S, Koch M, DeBosier A, McMahon B, et al. 2014. Experimental transmissibility of mutant SOD1 motor neuron disease. *Acta Neuropathol*. 128(6):791–803
55. Ayers JI, Fromholt SE, O'Neal VM, Diamond JH, Borchelt DR. 2016. Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways. *Acta Neuropathol.* 131(1):103–14

56. Kanu N, Imokawa Y, Drechsel DN, Williamson RA, Birkett CR, et al. 2002. Transfer of scrapie prion infectivity by cell contact in culture. *Curr. Biol.* 12(7):523–30
57. Magalhães AC, Baron GS, Lee KS, Steele-Mortimer O, Dorward D, et al. 2005. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells. *J. Neurosci.* 25(21):5207–16
58. Chandler RL. 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. *Lancet* 1(7191):1378–79
59. Makarava N, Kovacs GG, Bocharova O, Savtchenko R, Alexeeva I, et al. 2010. Recombinant prion protein induces a new transmissible prion disease in wild-type animals. *Acta Neuropathol.* 119(2):177–87
60. Duffy P, Wolf J, Collins G, DeVoe AG, Streeten B, Cowen D. 1974. Letter: possible person-to-person transmission of Creutzfeldt-Jakob disease. *N. Engl. J. Med.* 290(12):692–93
61. Bernoulli C, Siegfried J, Baumgartner G, Regli F, Rabinowicz T, et al. 1977. Danger of accidental person-to-person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet* 1(8009):478–79
62. Trevino RS, Lauckner JE, Sourigues Y, Pearce MM, Bousset L, et al. 2012. Fibrillar structure and charge determine the interaction of polyglutamine protein aggregates with the cell surface. *J. Biol. Chem.* 287(35):29722–28
63. Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, et al. 2016. Human-to-mouse prion-like propagation of mutant huntingtin protein. *Acta Neuropathol.* 132(4):577–92
64. Cicchetti F, Lacroix S, Cisbani G, Vallières N, Saint-Pierre M, et al. 2014. Mutant huntingtin is present in neuronal grafts in Huntington disease patients. *Ann. Neurol.* 76(1):31–42
65. Sung JY, Kim J, Paik SR, Park JH, Ahn YS, Chung KC. 2001. Induction of neuronal cell death by Rab5A-dependent endocytosis of α-synuclein. *J. Biol. Chem.* 276(29):27441–48
66. Liu J, Zhou Y, Wang Y, Fong H, Murray TM, Zhang J. 2007. Identification of proteins involved in microglial endocytosis of α-synuclein. *J. Proteome Res.* 6(9):3614–27
67. Mao X, Ou MT, Karuppagounder SS, Kam TI, Yin X, et al. 2016. Pathological-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science* 353(6307):aah3374
68. Kanekiyo T, Zhang J, Liu Q, Liu CC, Zhang L, Bu G. 2011. Heparan sulphate proteoglycan and the low-density lipoprotein receptor-related protein 1 constitute major pathways for neuronal amyloid-β uptake. *J. Neurosci.* 31(5):1644–51
69. Omtri RS, Davidson MW, Arumugam B, Poduslo JF, Kandimalla KK. 2012. Differences in the cellular uptake and intracellular itineraries of amyloid beta proteins 40 and 42: ramifications for the Alzheimer's drug discovery. *Mol. Pharm.* 9(7):1887–97
70. Clifford PM, Siu G, Kosciuk M, Levin EC, Venkataraman V, et al. 2008. α7 nicotinic acetylcholine receptor expression by vascular smooth muscle cells facilitates the deposition of Aβ peptides and promotes cerebrovascular amyloid angiopathy. *Brain Res.* 1234:158–71
71. Lilja AM, Porras O, Storelli E, Nordberg A, Marutle A. 2011. Functional interactions of fibrillar and oligomeric amyloid-β with alpha7 nicotinic receptors in Alzheimer's disease. *J. Alzheimers Dis.* 23(2):335–47
72. Bi X, Gall CM, Zhou J, Lynch G. 2002. Uptake and pathogenic effects of amyloid beta peptide 1–42 are enhanced by integrin antagonists and blocked by NMDA receptor antagonists. *Neuroscience* 112(4):827–40
73. Fuentealba RA, Liu Q, Zhang J, Kanekiyo T, Hu X, et al. 2010. Low-density lipoprotein receptor-related protein 1 (LRP1) mediates neuronal Aβ42 uptake and lysosomal trafficking. *PLOS ONE* 5(7):e11884
74. LaFerla FM, Troncoso JC, Strickland DK, Kawas CH, Jay G. 1997. Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular Aβ stabilization. *J. Clin. Investig.* 100(2):310–20
75. Takamura A, Sato Y, Watabe D, Okamoto Y, Nakata T, et al. 2012. Sortilin is required for toxic action of Aβ oligomers (AβOs): Extracellular AβOs trigger apoptosis, and intraneuronal AβOs impair degradation pathways. *Life Sci.* 91(23–24):1177–86
76. Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM. 2009. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. *Nature* 457:1128–32
77. Wang Y, Cui J, Sun X, Zhang Y. 2011. Tunneling-nanotube development in astrocytes depends on p53 activation. *Cell Death Differ.* 18(4):732–42

78. Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, et al. 2013. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *PNAS* 110(33):E3138–47
79. Stopschinski BE, Holmes BB, Miller GM, Manon VA, Vaquer-Alicea J, et al. 2018. Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau versus α-synuclein and β-amyloid aggregates. *J. Biol. Chem.* 293:10826–40
80. Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, et al. 1999. Functions of cell surface heparan sulfate proteoglycans. *Annu. Rev. Biochem.* 68:729–77
81. Dujardin S, Bégard S, Caillierez R, Lachaud C, Delattre L, et al. 2014. Ectosomes: a new mechanism for non-exosomal secretion of tau protein. *PLOS ONE* 9(6):e100760
82. Saman S, Kim W, Raya M, Visnick Y, Miro S, et al. 2012. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J. Biol. Chem.* 287(6):3842–49
83. Wu JW, Herman M, Liu L, Simoes S, Acker CM, et al. 2013. Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J. Biol. Chem.* 288(3):1856–70
84. Mirbaha H, Holmes BB, Sanders DW, Bieschke J, Diamond MI. 2015. Tau trimers are the minimal propagation unit spontaneously internalized to seed intracellular aggregation. *J. Biol. Chem.* 290(24):14893–903
85. Bellinger-Kawahara CG, Kempner E, Groth D, Gabizon R, Prusiner SB. 1988. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. *Virology* 164(2):537–41
86. Scott JR, Davies D, Fraser H. 1992. Scrapie in the central nervous system: neuroanatomical spread of infection and Sinc control of pathogenesis. *J. Gen. Virol.* 73(7):1637–44
87. Lee S, Kim W, Li Z, Hall GF. 2012. Accumulation of vesicle-associated human tau in distal dendrites drives degeneration and tau secretion in an in situ cellular tauopathy model. *Int. J. Alzheimers Dis.* 2012:172837
88. Karch CM, Jeng AT, Goate AM. 2012. Extracellular Tau levels are influenced by variability in Tau that is associated with tauopathies. *J. Biol. Chem.* 287(51):42751–62
89. Michel CH, Kumar S, Pinotsi D, Tunnacliffe A, St George-Hyslop P, et al. 2014. Extracellular monomeric tau protein is sufficient to initiate the spread of tau protein pathology. *J. Biol. Chem.* 289(2):956–67
90. Chai X, Dage JL, Citron M. 2012. Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol. Dis.* 48(3):356–66
91. Pooler AM, Phillips EC, Lau DHW, Noble W, Hanger DP. 2013. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* 14(4):389–94
92. Kim W, Lee S, Jung C, Ahmed A, Lee G, Hall GF. 2010. Interneuronal transfer of human tau between Lamprey central neurons in situ. *J. Alzheimers Dis.* 19(2):647–64
93. Plouffe V, Mohamed N-V, Rivest-McGraw J, Bertrand J, Lauzon M, Leclerc N. 2012. Hyperphosphorylation and cleavage at D421 enhance tau secretion. *PLOS ONE* 7(5):e36873
94. Dujardin S, Lécolle K, Caillierez R, Bégard S, Zommer N, et al. 2014. Neuron-to-neuron wild-type Tau protein transfer through a trans-synaptic mechanism: relevance to sporadic tauopathies. *Acta Neuropathol. Commun.* 2:14
95. Tyson T, Steiner JA, Brundin P. 2016. Sorting out release, uptake and processing of alpha-synuclein during prion-like spread of pathology. *J. Neurochem.* 139(Suppl. 1):275–89
96. Yamada K, Holth JK, Liao F, Stewart FR, Mahan TE, et al. 2014. Neuronal activity regulates extracellular tau in vivo. *J. Exp. Med.* 211(3):387–93
97. Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, et al. 2015. Synaptic contacts enhance cell-to-cell tau pathology propagation. *Cell Rep.* 11(8):1176–83
98. Wu JW, Hussaini SA, Bastille IM, Rodriguez GA, Mrejeru A, et al. 2016. Neuronal activity enhances tau propagation and tau pathology in vivo. *Nat. Neurosci.* 19(8):1085–92
99. Funk KE, Mirbaha H, Jiang H, Holtzman DM, Diamond MI. 2015. Distinct therapeutic mechanisms of tau antibodies: promoting microglial clearance versus blocking neuronal uptake. *J. Biol. Chem.* 290(35):21652–62

100. Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, et al. 2013. Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. *Neuron* 80(2):402–14
101. Panza F, Solfrizzi V, Seripa D, Imbimbo BP, Lozupone M, et al. 2016. Tau-based therapeutics for Alzheimer's disease: active and passive immunotherapy. *Immunotherapy* 8(9):1119–34
102. Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, et al. 2006. Molecular anatomy of a trafficking organelle. *Cell* 127(4):831–46
103. Zanusso G, Monaco S, Pocchiari M, Caughey B. 2016. Advanced tests for early and accurate diagnosis of Creutzfeldt-Jakob disease. *Nat. Rev. Neurol.* 12(6):325–33
104. Orrú CD, Bongianni M, Tonoli G, Ferrari S, Hughson AG, et al. 2014. A test for Creutzfeldt-Jakob disease using nasal brushings. *N. Engl. J. Med.* 371(6):519–29
105. Saijo E, Ghetti B, Zanusso G, Oblak A, Furman JL, et al. 2017. Ultrasensitive and selective detection of 3-repeat tau seeding activity in Pick disease brain and cerebrospinal fluid. *Acta Neuropathol.* 133(5):751–65
106. Morozova OA, March ZM, Robinson AS, Colby DW. 2013. Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein. *Biochemistry* 52(40):6960–67
107. Meyer V, Dinkel PD, Hager ER, Margittai M. 2014. Amplification of tau fibrils from minute quantities of seeds. *Biochemistry* 53(36):5804–9
108. Salvadores N, Shahnawaz M, Scarpini E, Tagliavini F, Soto C. 2014. Detection of misfolded Aβ oligomers for sensitive biochemical diagnosis of Alzheimer's disease. *Cell Rep.* 7(1):261–68
109. Fairfoul G, McGuire LI, Pal S, Ironside JW, Neumann J, et al. 2016. Alpha-synuclein RT-QuIC in the CSF of patients with alpha-synucleinopathies. *Ann. Clin. Transl. Neurol.* 3(10):812–18
110. Holmes BB, Furman JL, Mahan TE, Yamasaki TR, Mirbaha H, et al. 2014. Proteopathic tau seeding predicts tauopathy in vivo. *PNAS* 111(41):E4376–85
111. Furman JL, Holmes BB, Diamond MI. 2015. Sensitive detection of proteopathic seeding activity with FRET flow cytometry. *J. Vis. Exp.* (106):e53205
112. Kaufman SK, Thomas TL, Del Tredici K, Braak H, Diamond MI. 2017. Characterization of tau prion seeding activity and strains from formaldehyde-fixed tissue. *Acta Neuropathol. Commun.* 5:41
113. Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, et al. 2016. Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability in vivo. *Neuron* 92(4):796–812
114. Furman JL, Vaquer-Alicea J, White CL, Cairns NJ, Nelson PT, Diamond MI. 2017. Widespread tau seeding activity at early Braak stages. *Acta Neuropathol.* 133(1):91–100
115. Yetman MJ, Lillehaug S, Bjaalie JG, Leergaard TB, Jankowsky JL. 2016. Transgene expression in the Nop-tTA driver line is not inherently restricted to the entorhinal cortex. *Brain Struct. Funct.* 221(4):2231–49
116. Wegmann S, Maury EA, Kirk MJ, Saqran L, Roe A, et al. 2015. Removing endogenous tau does not prevent tau propagation yet reduces its neurotoxicity. *EMBO J.* 34(24):3028–41
117. Katsinelos T, Zeitler M, Dimou E, Karakatsani A, Müller H-M, et al. 2018. Unconventional secretion mediates the trans-cellular spreading of tau. *Cell Rep.* 23(7):2039–55
118. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, et al. 1999. Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400(6740):173–77
119. Masliah E, Rockenstein E, Adame A, Alford M, Crews L, et al. 2005. Effects of α-synuclein immunization in a mouse model of Parkinson's disease. *Neuron* 46(6):857–68
120. Tran HT, Chung CH-Y, Iba M, Zhang B, Trojanowski JQ, et al. 2014. α-Synuclein immunotherapy blocks uptake and templated propagation of misfolded α-synuclein and neurodegeneration. *Cell Rep.* 7(6):2054–65
121. Troquier L, Caillierez R, Burnouf S, J Fernandez-Gomez FJ, Grosjean M-E, et al. 2012. Targeting phospho-Ser422 by active tau immunotherapy in the THYTau22 mouse model: a suitable therapeutic approach. *Curr. Alzheimers Res.* 9(4):397–405

122. Yanamandra K, Jiang H, Mahan TE, Maloney SE, Wozniak DF, et al. 2015. Anti-tau antibody reduces insoluble tau and decreases brain atrophy. *Ann. Clin. Transl. Neurol.* 2(3):278–88
123. Congdon EE, Gu J, Sait HBR, Sigurdsson EM. 2013. Antibody uptake into neurons occurs primarily via clathrin-dependent Fcγ receptor endocytosis and is a prerequisite for acute tau protein clearance. *J. Biol. Chem.* 288(49):35452–65
124. Gu J, Congdon EE, Sigurdsson EM. 2013. Two novel Tau antibodies targeting the 396/404 region are primarily taken up by neurons and reduce Tau protein pathology. *J. Biol. Chem.* 288(46):33081–95
125. Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, et al. 2018. Tau internalization is regulated by 6-O sulfation on heparan sulfate proteoglycans (HSPGs). *Sci. Rep.* 8(1):6382
126. Oh SH, Kim HN, Park HJ, Shin JY, Bae E-J, et al. 2016. Mesenchymal stem cells inhibit transmission of α-synuclein by modulating clathrin-mediated endocytosis in a Parkinsonian model. *Cell Rep*. 14(4):835–49
127. Rubinsztein DC, Bento CF, Deretic V. 2015. Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. *J. Exp. Med.* 212(7):979–90
128. Sarkar S. 2013. Chemical screening platforms for autophagy drug discovery to identify therapeutic candidates for Huntington's disease and other neurodegenerative disorders. *Drug Discov. Today Technol.* 10(1):e137–44
129. Renna M, Jimenez-Sanchez M, Sarkar S, Rubinsztein DC. 2010. Chemical inducers of autophagy that enhance the clearance of mutant proteins in neurodegenerative diseases. *J. Biol. Chem*. 285(15):11061–67
130. Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, et al. 2008. Lithium delays progression of amyotrophic lateral sclerosis. *PNAS* 105(6):2052–57
131. Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, et al. 2006. Rapamycin alleviates toxicity of different aggregate-prone proteins. *Hum. Mol. Genet.* 15(3):433–42
132. Aguib Y, Heiseke A, Gilch S, Riemer C, Baier M, et al. 2009. Autophagy induction by trehalose counteracts cellular prion-infection. *Autophagy* 5(3):361–69
133. Heiseke A, Aguib Y, Riemer C, Baier M, Schätzl HM. 2009. Lithium induces clearance of protease resistant prion protein in prion-infected cells by induction of autophagy. *J. Neurochem.* 109(1):25–34
134. Miller TM, Pestronk A, David W, Rothstein J, Simpson E, et al. 2013. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. *Lancet Neurol.* 12(5):435–42
135. DeVos SL, Miller RL, Schoch KM, Holmes BB, Kebodeaux CS, et al. 2017. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. *Sci. Transl. Med.* 9(374):eaag0481
136. Doig AJ, Derreumaux P. 2015. Inhibition of protein aggregation and amyloid formation by small molecules. *Curr. Opin. Struct. Biol.* 30:50–56
137. Aprile FA, Sormanni P, Perni M, Arosio P, Linse S, et al. 2017. Selective targeting of primary and secondary nucleation pathways in Aβ42 aggregation using a rational antibody scanning method. *Sci. Adv.* 3(6):e1700488
138. Habchi J, Chia S, Limbocker R, Mannini B, Ahn M, et al. 2017. Systematic development of small molecules to inhibit specific microscopic steps of Aβ42 aggregation in Alzheimer's disease. *PNAS* 114(2):E200–8
139. Shorter J. 2011. The mammalian disaggregase machinery: Hsp110 synergizes with Hsp70 and Hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLOS ONE* 6(10):e26319
140. Cohen SIA, Arosio P, Presto J, Kurudenkandy FR, Biverstal H, et al. 2015. A molecular chaperone breaks the catalytic cycle that generates toxic Aβ oligomers. *Nat. Struct. Mol. Biol.* 22(3):207–13
141. Wickner RB. 2016. Yeast and fungal prions. *Cold Spring Harb. Perspect. Biol.* 8(9):a023531
142. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. 2009. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. *Cell* 137(1):146–58
143. Halfmann R, Jarosz DF, Jones SK, Chang A, Lancaster AK, Lindquist S. 2012. Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 482(7385):363–68
144. True HL, Lindquist SL. 2000. A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 407(6803):477–83

145. True HL, Berlin I, Lindquist SL. 2004. Epigenetic regulation of translation reveals hidden genetic variation to produce complex traits. *Nature* 431(7005):184–87
146. Nizhnikov AA, Antonets KS, Inge-Vechtomov SG, Derkatch IL. 2014. Modulation of efficiency of translation termination in *Saccharomyces cerevisiae*. *Prion* 8(3):247–60
147. Sweeny EA, Shorter J. 2016. Mechanistic and structural insights into the prion-disaggregase activity of Hsp104. *J. Mol. Biol.* 428(9):1870–85
148. DeSantis ME, Leung EH, Sweeny EA, Jackrel ME, Cushman-Nick M, et al. 2012. Operational plasticity enables Hsp104 to disaggregate diverse amyloid and nonamyloid clients. *Cell* 151(4):778–93
149. Tanaka M, Collins SR, Toyama BH, Weissman JS. 2006. The physical basis of how prion conformations determine strain phenotypes. *Nature* 442(7102):585–89
150. Legname G, Nguyen H-OB, Peretz D, Cohen FE, DeArmond SJ, Prusiner SB. 2006. Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. *PNAS* 103(50):19105–10
151. Collinge J, Clarke AR. 2007. A general model of prion strains and their pathogenicity. *Science* 318(5852):930–36
152. Dickinson AG, Meikle VMH. 1969. A comparison of some biological characteristics of the mouse-passaged scrapie agents, 22A and ME7. *Genet. Res.* 13(2):213–25
153. Stöhr J, Condello C, Watts JC, Bloch L, Oehler A, et al. 2014. Distinct synthetic Aβ prion strains producing different amyloid deposits in bigenic mice. *PNAS* 111(28):10329–34
154. Watts JC, Condello C, Stöhr J, Oehler A, Lee J, et al. 2014. Serial propagation of distinct strains of Aβ prions from Alzheimer's disease patients. *PNAS* 111(28):10323–28
155. Qiang W, Yau W-M, Lu J-X, Collinge J, Tycko R. 2017. Structural variation in amyloid-β fibrils from Alzheimer's disease clinical subtypes. *Nature* 541(7636):217–21
156. Bousset L, Pieri L, Ruiz-Arlandis G, Gath J, Jensen PH, et al. 2013. Structural and functional characterization of two alpha-synuclein strains. *Nat. Commun.* 4:2575
157. Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, et al. 2013. Distinct α-synuclein strains differentially promote tau inclusions in neurons. *Cell* 154(1):103–17
158. Boluda S, Iba M, Zhang B, Raible KM, Lee VM-Y, Trojanowski JQ. 2015. Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. *Acta Neuropathol.* 129(2):221–37
159. Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, et al. 2015. Evidence for α-synuclein prions causing multiple system atrophy in humans with Parkinsonism. *PNAS* 112(38):E5308–17
160. Fitzpatrick A, Falcon B, He S, Murzin AG, Murshudov G, et al. 2017. Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature* 547(7662):185–90
161. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, et al. 2015. α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. *Nature* 522(7556):340–44
162. Woerman AL, Stöhr J, Aoyagi A, Rampersaud R, Krejciova Z, et al. 2015. Propagation of prions causing synucleinopathies in cultured cells. *PNAS* 112(35):E4949–58
163. Woerman AL, Kazmi SA, Patel S, Aoyagi A, Oehler A, et al. 2018. Familial Parkinson's point mutation abolishes multiple system atrophy prion replication. *PNAS* 115(2):409–14
164. Swinnen B, Robberecht W. 2014. The phenotypic variability of amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 10(11):661–70
165. Ravits J, Paul P, Jorg C. 2007. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. *Neurology* 68(19):1571–75
166. Braak H, Braak E. 1991. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82(4):239–59
167. Braak H, Braak E. 1995. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* 16(3):271–78
168. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. 2006. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112(4):389–404

169. Agosta F, Weiler M, Filippi M. 2015. Propagation of pathology through brain networks in neurodegenerative diseases: from molecules to clinical phenotypes. *CNS Neurosci. Ther.* 21(10):754–67
170. Greicius MD, Supekar K, Menon V, Dougherty RF. 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb. Cortex* 19(1):72–78
171. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, et al. 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27(9):2349–56
172. Vincent JL, Patel GH, Fox MD, Snyder AZ, Baker JT, et al. 2007. Intrinsic functional architecture in the anaesthetized monkey brain. *Nature* 447(7140):83–86
173. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. 2009. Neurodegenerative diseases target large-scale human brain networks. *Neuron* 62(1):42–52
174. Zhou J, Gennatas ED, Kramer JH, Miller BL, Seeley WW. 2012. Predicting regional neurodegeneration from the healthy brain functional connectome. *Neuron* 73(6):1216–27
175. Raj A, Kuceyeski A, Weiner M. 2012. A network diffusion model of disease progression in dementia. *Neuron* 73(6):1204–15
176. Collinge J. 2016. Mammalian prions and their wider relevance in neurodegenerative diseases. *Nature* 539(7628):217–26
177. Cali I, Cohen ML, Haik S, Parchi P, Giaccone G, et al. 2018. Iatrogenic Creutzfeldt-Jakob disease with amyloid-β pathology: an international study. *Acta Neuropathol. Commun.* 6:5
178. Jaunmuktane Z, Quaegebeur A, Taipa R, Viana-Baptista M, Barbosa R, et al. 2018. Evidence of amyloid-β cerebral amyloid angiopathy transmission through neurosurgery. *Acta Neuropathol.* 135(5):671–79
179. Marquié M, Normandin MD, Vanderburg CR, Costantino IM, Bien EA, et al. 2015. Validating novel tau positron emission tomography tracer [F-18]-AV-1451 (T807) on postmortem brain tissue. *Ann. Neurol.* 78(5):787–800