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Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases

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Abstract

A common feature of many neurodegenerative diseases is the deposition of β-sheet-rich amyloid aggregates formed by proteins specific to these diseases. These protein aggregates are thought to cause neuronal dysfunction, directly or indirectly. Recent studies have strongly implicated cell-to-cell transmission of misfolded proteins as a common mechanism for the onset and progression of various neurodegenerative disorders. Emerging evidence also suggests the presence of conformationally diverse 'strains' of each type of disease protein, which may be another shared feature of amyloid aggregates, accounting for the tremendous heterogeneity within each type of neurodegenerative disease. Although there are many more questions to be answered, these studies have opened up new avenues for therapeutic interventions in neurodegenerative disorders.

Neurodegenerative diseases encompass a wide variety of age-related pathological conditions caused by progressive dysfunction and deterioration of the central nervous system (CNS). Despite their enormous diversity in clinical phenotypes, most neurodegenerative diseases share a common feature, which is the accumulation of disease- specific proteins into insoluble aggregates. This list includes β-amyloid (Aβ) in senile plaques and tau in neurofibrillary tangles (NFTs) of Alzheimer's disease[1,2], α-synuclein (α-syn) in Lewy bodies and Lewy neurites of Parkinson's disease[3], TDP-43 aggregates in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration[4], polyglutamine (polyQ)-rich huntingtin inclusions in Huntington's disease[5] and prion plaques in Creutzfeldt-Jakob disease (CJD)[6]. When viewed by electron microscopy, most of these protein aggregates consist of 8- to 20-nm wide filaments and are characterized by enriched β-pleated sheet structures ('amyloid') that can be stained by dyes such as Congo Red or thioflavin S (ThS)[7,8], with the exception of TDP-43 inclusions, in which the aggregates comprise mostly granular non-amyloid fibrils[9,10].

In the past few years, a growing number of studies have provided converging evidence for the cell-to-cell transmissibility of the diverse disease proteins that form the hallmark lesions

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of these neurodegenerative disorders (Table 1). Such lesions were traditionally thought to develop in a cell-autonomous manner in selectively vulnerable brain regions. The newly evolved 'transmission hypothesis' for non-prion neurodegenerative diseases not only provides a viable explanation for the stereotypical pathology spreading patterns that have long been observed in multiple diseases, but also offers a fresh perspective on the processes underlying the onset and progression of CNS amyloidosis[11–13]. In this review, we compare recent findings on the transmission of different amyloidogenic proteins, speculate on how intercellular spreading of misfolded proteins may be related to the pathogenesis of neurodegenerative diseases, present evidence for the existence of conformationally diverse pathological strains to account for the divergence and convergence of various diseases and, finally, discuss the therapeutic implications of these findings.

Cell-to-cell transmission of amyloid protein aggregates

Templated recruitment by existing seeds

For many years, prion diseases were thought to be a unique group of neurodegenerative disorders in which the conformationally altered prion protein PrP^{Sc} constitutes the infectious agent that corrupts normal cellular PrP (PrP^{C}) through 'seeded' fibrillization[14]. Recently, a collection of studies has provided convincing evidence that a 'prion-like' self-propagating mechanism may be applicable to a wide range of disease-associated proteins, including Aβ, tau, α-syn, huntingtin with polyQ repeats, superoxide dismutase 1 (SOD1) and TDP-43 (Table 1 and Fig. 1). For each of these proteins, aggregate-containing lysates and/or synthetic fibrils assembled from recombinant proteins were shown to act as templates or seeds that could efficiently recruit their soluble counterparts into elongating fibrils in cultured cells and/or living animals, sometimes even without overexpression of the protein of interest[15,16]. A miniscule amount of preformed fibrils was shown to be sufficient to initiate robust conversion of normal proteins, suggesting that templated recruitment is probably a self-perpetuating process that, once started, will progress relentlessly[17]. Moreover, *in vivo* administration of inoculums containing aggregated proteins not only led to induction of pathology near the inoculation site(s) but also invariably resulted in time-dependent spreading of pathology to synaptically connected distant brain regions[16,18–22]. For tau and α-syn, a trans-synaptic spreading pattern was also observed when the trans-gene expression was restricted to specific brain regions[23–25]. Taken together, these studies strongly support templated self-propagation and intercellular transmissibility as shared properties of protein aggregates involved in CNS amyloidosis.

Although fibrillar aggregates were shown to be capable of self-amplification, what triggers the initial conversion of normally soluble proteins into filamentous polymers remains enigmatic. Small misfolded protein species, such as oligomers, have been isolated as intermediates from *in vitro* fibrillization of multiple disease-associated proteins and have been suggested to be more neurotoxic than mature fibrils[26]. However, difficulties in observing these prefibrillar species in human brains have prohibited the establishment of a convincing link between these species and disease pathogenesis. Nonetheless, a few studies have reported the presence of tau oligomers in human brains, and oligomeric tau purified from Alzheimer's disease brains could induce tau pathology in mice through intracerebral

injection[27–29]. Interestingly, oligomeric PrP^{Sc} was found to be even more infectious than fibrillar PrP^{Sc} (ref. 30). To better understand the roles of soluble misfolded species in the onset and progression of neurodegenerative disorders, future studies should devote more effort to isolating these small protein species from diseased brains, conducting detailed biochemical and biophysical analyses on them and characterizing their transmission properties.

Pathophysiological relevance to human diseases

A highly predictable spatiotemporal progression of protein lesions has been well described for Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies[31–35] and, very recently, for ALS[36]. In Alzheimer's disease brains, for example, NFTs spread from the limbic areas to neocortex with advancing stages of the disease[31], whereas Aβ plaques first emerge in the neocortex before arising in the subcortex[35]. Such a stereotypical involvement of different brain regions was historically attributed to differential vulnerability of distinct brain regions to the misfolding of specific pathogenic proteins. Alternatively, neuronal injury caused by protein aggregation and protein aggregates released from dying neurons may lead to chronic activation of microglia and astrocytes, which secrete proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β and interleukin-6 and generate reactive oxygen species; these chemical mediators could in turn promote aberrant modifications and misfolding of proteins in neighboring healthy neurons[37]. This hypothesis is supported by evidence of increased neuroinflammation in various neurodegenerative diseases, although it cannot adequately account for the progression of disease-specific protein lesions over long distances.

The recent surge of studies showing the propagation of misfolded proteins themselves along defined neuronal projections offers yet another possibility (Figs. 1 and 2), which not only could explain parsimoniously the curious anatomical connections between sequentially affected brain areas, but also resolves the paradox that the same type of cells in slightly different anatomical locations (for example, cortical pyramidal neurons in layer Va compared with those in layer IV) have differential tendencies to develop pathology[38]. Importantly, *in vivo* studies showed that inoculating aggregated proteins at different sites in the brain elicited distinct patterns of pathology progression, suggesting that the initial areas of insult determine the brain areas to be subsequently affected[16,19]. Although published *in vivo* studies have focused on Aβ, tau and α-syn, it is tempting to hypothesize that the same spreading process could underlie the progression of all neurodegenerative proteinopathies. Studies are currently underway to verify how common this pathogenic mechanism is among these diseases.

One apparent discrepancy between human disease and mouse models of cell-to-cell transmission is the drastically different time course: neurodegenerative proteinopathies in humans typically occur only after decades, but protein aggregation induced by exogenously supplied misfolded proteins in mouse models happens within months, sometimes even weeks. Such inconsistency potentially raises doubts about the validity of these mouse models and the applicability of the transmission hypothesis to explain human diseases. However, a number of factors could explain such temporal discrepancy. First, mouse brains

are substantially smaller than human brains, thus the former would require much less time for protein aggregates to propagate from one brain region to the other (assuming a comparable rate of spreading across species). Second, as in other polymerization reactions, the rate-limiting step of protein aggregation is the nucleation phase[39,40], whereby small assemblies of misfolded proteins—the seeds—first form. In young, healthy neurons, misfolded proteins are efficiently removed by the cellular protein degradation machinery, whereas in aged neurons, the proteolytic machinery gradually deteriorates, thereby giving rise to the very long incubation time for seed formation. In animal models of transmission, misfolded proteins are directly delivered into the brains of animals, thereby dramatically accelerating the disease process by skipping the nucleation phase. Third, the transmission paradigm has typically been conducted in transgenic animals overexpressing the protein of interest, sometimes even carrying disease-causing mutations that further promote aggregation[18–20,22,41,42]. Given that seeded fibrillization is a concentration-dependent process, it is not surprising this process takes much longer in human brains that are not engineered to overexpress the pathogenic proteins. In fact, the use of overexpression- based mouse models in demonstrating disease protein transmissibility is unsatisfactory, because the spreading pattern of seeded pathology could be confounded by the transgene expression pattern in a particular mouse model, and—importantly—neurodegenerative diseases in humans do not usually involve upregulation of the disease-associated proteins, except in rare familial cases. Therefore, future studies will need to demonstrate robust propagation of templated pathology in nontransgenic mice to make a stronger case for the transmission hypothesis.

The transcellular movement of cytosolic protein aggregates

Misfolded cytosolic protein aggregates are found in both cell bodies and processes of the asymmetric neurons. To accomplish neuron-to- neuron transmission, they must have means to travel within a neuron to reach potential sites for interneuronal transfer, exit the originating neuron and enter the recipient neuron. Studies using microfluidic chambers have shown that both tau and α-syn aggregates can move anterogradely as well as retrogradely within a neuron, possibly by axonal transport[15,43,44]. Perhaps the most perplexing aspect of interneuronal transmission lies in the release and uptake of pathological aggregates (Fig. 1).

Recent evidence suggests that tau and α-syn may be released from neurons. The presence of these two proteins in human cerebrospinal fluid has been well documented[45–47], and two microdialysis studies in healthy mice detected appreciable concentrations of tau and α-syn in the interstitial fluid, suggesting that these proteins are, for unknown reasons, constitutively released from healthy neurons[48,49]. Other cell culture studies have shown that non-fibrillar (monomeric or oligomeric) α-syn and tau can be secreted into the cell medium via exosomes[50–52], which are cell-derived extracellular vesicles that are also implicated in the propagation of PrP^{Sc} (ref. 53), and this process may be modulated by neuronal activity[54]. Although further studies are needed to determine whether a similar mechanism mediates the efflux of fibrillar protein aggregates, one study suggested that tau aggregates are released into the extracellular space as free-floating fibrils without any membrane association[55].

Different mechanisms of internalization have been proposed for various neurodegenerative disease proteins. PolyQ aggregates seem to be able to penetrate cell membranes directly without going through endocytic compartments[56]. SOD1 aggregates were shown to enter cells through lipid raft–dependent macropinocytosis[57], an endocytic pathway mediating nonselective fluid-phase uptake. Tau fibrils were similarly shown to be taken up partially through fluid-phase endo-cytosis or, more specifically, macropinocytosis[43,58,59]. Furthermore, adsorptive endocytosis (an intermediate between receptor-mediated endocytosis and fluid-phase endocytosis) enhanced the uptake of both tau and α-syn fibrils[15,17]. Several studies have shown that α-syn fibrils enter cells via receptor-mediated endocytosis that is dynamin dependent[60,61]. However, considering the size of fibrillar aggregates, receptor-mediated endocytosis, which requires specific interactions between ligands and cell-surface receptors, seems unlikely to be a major mode of fibril internalization. Irrespective of the precise mode of entry, if endocytosis is the predominant pathway for fibril uptake, an additional hurdle for these endocytosed protein aggregates is to exit vesicles to access soluble cytosolic proteins. So far, no study has specifically addressed this issue, but the successful induction of cytoplasmic aggregates that are not bound to vesicular structures, as shown by electron microscopy[15,17,62], indirectly demonstrates that internalized misfolded seeds must be able to escape into the cytosol, possibly through physical disruption of the vesicle membrane.

In addition to the mechanisms discussed above, several others could potentially mediate the transcellular movement of cytosolic protein aggregates. First, nanotubes, which are tunnel-like structures connecting two cells, have been demonstrated to be involved in the spreading of PrP^{Sc} (ref. 63). These structures would provide a convenient conduit for misfolded proteins, obviating the need to cross plasma membranes. Second, because studies of aggregate inoculation in mice have consistently demonstrated spreading of pathology along neuronal connections, it would be worthwhile to investigate whether synaptic transmission is somehow hijacked by pathological protein aggregates to facilitate interneuronal transfer. Compounds that modulate neuronal activity could be employed to assess the potential roles of synaptic transmission in pathology propagation. Third, a single injection of rabies virus or adeno-associated virus into the mouse brain can result in widespread distribution of viral particles to distant brain regions, and the spreading efficiency depends heavily on sequence variations of the viral capsids[64–66]. The intriguing similarities between the transmission patterns of viruses and protein aggregates *in vivo* raise the possibility that similar cellular machineries are being exploited to achieve trans-synaptic movement. Although mechanisms facilitating interneuronal propagation of viruses are also not well understood, the internalization of viruses is known to be mediated by cell-surface receptors, such as membrane-associated heparan sulfate proteoglycans (HSPGs), for adeno-associated virus, and neural cell adhesion molecule, for rabies virus[67,68]. Intriguingly, HSPGs were recently shown to mediate the uptake of both tau and α-syn fibrils[59].

Insights from comparing different amyloidogenic proteins

Transmission, infectiousness and biosafety

Although templated recruitment and cell-to-cell transmission may be shared properties of amyloid protein aggregates, one important distinction between prion diseases and other neurodegenerative diseases is that the former are infectious (as defined by their ability to transmit between individuals via natural routes, such as ingestion), perhaps owing to the exceptional protease resistance of PrP^{Sc} (ref. 69). So far, there is no evidence to support the idea that non-prion neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, can spread from one person to another. A retrospective, postmortem study of recipients of cadaver- derived human growth hormone (hGH) found no reported incidence of Alzheimer's disease or Parkinson's disease, although the donors of pituitary glands used for hGH preparation (in the United States alone, more than 1.4 million donors between 1963 and 1985) probably included people with Alzheimer's disease or Parkinson's disease, and immunohistochemical examination revealed frequent accumulations of pathological Aβ, tau and α-syn in the postmortem pituitary glands of people with Alzheimer's disease or Parkinson's disease[70]. In contrast, hGH administration has resulted in worldwide outbreaks of iatrogenic CJD[71] caused, presumably, by PrP^{Sc} contamination of the hGH preparation, despite the extremely low rate of CJD incidence in the population. Therefore, existing human data suggest that the potential infectiousness of non-prion protein aggregates is sufficiently low that normal contact with afflicted individuals would not increase one's lifetime risk for the respective diseases. Nevertheless, in research laboratories where large quantities of aggregated proteins are used experimentally, precautionary measures should be taken to minimize potential occupational risk, such as requiring proper laboratory attire, designating separate work space for handling misfolded proteins and adopting enclosed sonication systems to prevent aerosol transmission.

Spreading propensity of different amyloid aggregates

Peripheral administration of inoculums containing aggregated Aβ or prions was shown to induce pathology in the cerebral cortex of injected animals[72–74], suggesting that these extracellularly located protein aggregates can propagate from the peripheral tissues to the brain while preserving their seeding-competent conformation. These experimental results are not surprising for prions, given the transmission routes of acquired prion diseases in humans[75], but the pathophysiological relevance of periphery-to-central spreading of Aβ is not yet clear, as Aβ plaques are found only in the brains of patients with Alzheimer's disease.

Cerebral induction of pathology through peripheral injection has not been demonstrated for intracellular protein aggregates, which conceivably would encounter more obstacles to long-distance spreading than would extracellular plaques. However, recent studies suggest α-syn pathology in Parkinson's disease may actually initiate from the gut (the enteric nervous system) and progressively ascend to the brain[76]. Indeed, injection studies in mice showed that pathological α-syn is more transmissible than pathological tau, whereby seeded α-syn pathology spreads to much wider brain regions than seeded tau tangles under the same inoculation paradigm[19,20]. α-Syn may be privileged in transmission owing to its high local

concentration at the presynaptic terminals[77], which could be a crucial site for initiating templated recruitment and interneuronal propagation.

Transmissibility of synthetic fibrils and requirement of cofactors

The 'protein-only' hypothesis of prion propagation postulates that the infectious agent in prion diseases is composed solely of PrP in the abnormal conformation[78]. This stringent criterion turns out to be difficult to attain both for prions and for non-prion pathogenic protein aggregates. Synthetic PrP^{Sc} fibrils assembled from recombinant protein are not as infectious as brain-derived PrP^{Sc} unless RNA and lipid molecules are added as cofactors[79–81]. Although both Aβ and α-syn synthetic fibrils assembled without any cofactors were shown to be fully capable of inducing substantial pathology in mice[16,20,22], the specific seeding activity of synthetic Aβ aggregates is significantly lower than that of Aβ aggregates purified from transgenic mouse brains. Similarly, brain lysates from symptomatic mice containing small amounts of aggregated α-syn propagated pathology with the same potency as high doses of synthetic α-syn fibrils, suggesting that pathological α-syn generated *in vivo* has enhanced templating efficiency[20]. No side-by-side comparisons for synthetic and *in vivo*–generated tau aggregates have yet been reported, but all synthetic tau fibrils shown to transmit in both cells and mice have, so far, been generated in the presence of heparin, a polyanionic cofactor that promotes tau aggregation *in vitro*[82]. Thus, it remains unproven whether fibrillar tau is wholly sufficient to template the conversion of normal tau, with heparin merely catalyzing the fibrillization of tau, or whether heparin is intrinsically required to generate a seeding-competent tau conformer. Future studies are needed to explore the exact roles of cofactors in facilitating the transmissibility of disease-associated protein aggregates and their pathological relevance to human diseases.

Transmissible species versus toxic species

Although fibrillar protein aggregates are the most conspicuous hallmarks of various neurodegenerative diseases and were found to be transmissible through self-amplification, they are not necessarily the toxic species that directly cause neurodegeneration. In mouse models of prion diseases, neurodegeneration sometimes occurs without overt accumulation of PrP^{Sc} (refs. 83,84). By contrast, some mice with PrP^{Sc} titers as high as those of terminally sick mice did not show obvious abnormalities[85], and PrP^{Sc} itself did not cause toxicity in PrP^{C} knockout mice[86]. Using a more accurate scrapie cell assay to measure PrP^{Sc} titers, a study showed recently that the levels of infective PrP^{Sc} reached a plateau long before the onset of symptoms, indicating a clear dissociation between transmissibility and toxicity[87]. Interestingly, the time of delay between the acquisition of maximal infectivity and the onset of symptoms is inversely proportional to PrP^{C} concentration, suggesting that PrP^{Sc} may catalyze the formation of unidentified toxic species— possibly oligomers—in a PrP^{C}-dependent manner.

In patients with Alzheimer's disease, tau tangles correlate with disease severity, but Aβ plaque burden does not[88]. It has been suggested that tau aggregation may be the downstream effector of Aβ-induced toxicity and thus a more proximal cause of neuronal dysfunction[89,90]. Alternatively, smaller assemblies of Aβ, such as oligomers, which are more difficult to detect, may be the real toxic entities. In fact, soluble Aβ oligomers, but not

Aβ fibrils, were shown to be toxic to cultured neurons[91,92] and to impair long-term potentiation both in cultured hippocampal slices and *in vivo*[93,94]. However, Aβ fibrils may not be entirely inert, as a recent study showed that in the presence of Aβ fibrils, the predominant pathway of toxic oligomer formation is actually mediated by a secondary nucleation event catalyzed by mature fibrils rather than by direct nucleation from monomers[95].

Similarly, annular or pore-like α-syn oligomers described by *in vitro* studies have been proposed to be neurotoxic by disrupting membrane integrity[96,97], and artificially designed α-syn mutants that have a higher tendency to form oligomers were shown to be more toxic *in vivo*[98,99]. Although the existence of oligomeric α-syn species in human diseases remains to be established, transmission studies in cultured cells and mice have consistently demonstrated that substantial toxicity accompanies templated α-syn fibrillization[15,16,20], indicating that filamentous α-syn aggregates have deleterious effects. The large Lewy body–like α-syn inclusions induced in cells not only resist clearance by protein degradation machinery but also impair autophagy functions[100].

In contrast, induction of NFT-like tau aggregates in cultured cells or tau transgenic mice has not resulted in obvious cellular toxicity[17–19,62]. One possible reason is that prefibrillar tau, rather than mature tangles, is the truly toxic species. Exogenously supplied misfolded seeds that readily recruit soluble tau into filamentous aggregates may result in a complete bypass of the slow oligomerization phase that normally occurs in uninjected transgenic mice before the onset of overt pathology. This hypothesis could potentially explain the curious paradox in which neuron loss is observed in aged tau transgenic mice bearing ThS-negative tau inclusions but not in fibril-injected transgenic mice that develop more mature tangles. It is also consistent with observations that neuronal dysfunction sometimes precedes tau aggregation or occurs independently of tangle formation in animal models[101–103].

Strains: another shared property of amyloid aggregates?

Polymorphism of amyloid protein aggregates

Besides cell-to-cell transmissibility, another property common among amyloidogenic proteins is the ability to form structurally diverse fibrils, the best- studied example being PrP^{Sc} (Fig. 3a). Conformationally distinct PrP^{Sc} aggregates have been found in different ‘strains’ of prion diseases, which are characterized by specific histopathological lesion profiles (brain region–specific distribution of prion plaques and extent of spongiform changes) and clinical manifestations (incubation time before disease onset and aggressiveness of the disease)[104]. These phenotypic variations are believed to be caused by distinct PrP^{Sc} conformers, which can be propagated *in vivo* by serial passage and preserve the same phenotypes in subsequently infected animals.

An analog to prion strains has been described for other pathogenic protein aggregates. Polymorphic fibrils were shown to assemble from the same proteins with single amino acid substitutions or from proteins with identical primary structures under different fibrillization conditions, and the distinct structures can often be propagated *in vitro* or even *in vivo* via seeded fibrillization[105–111]. Importantly, conformational variants of a single protein can

exhibit different biological activities. For example, Aβ fibrils formed with or without agitation display differential toxicity in neurons[109], as do polyQ aggregates formed at different temperatures[110]. Conformational variants of synthetic α-syn fibrils not only cause differential neurotoxicities owing to disparate potencies in seeding α-syn aggregation, but also show striking differences in their ability to act as heterotypic templates to 'cross-seed' tau fibrillization[108]. Along the same line of thought, the lack of obvious neuronal loss in mice inoculated with synthetic tau fibrils[19] could well be explained by the possibility that these artificial fibrils represent a variant of fibrillar tau that is different from tau fibrils that naturally develop in brains with tauopathies. In fact, a recent study showed that heparin-induced tau fibrils are indeed structurally distinct from tau fibrils derived from Alzheimer's disease brain[112]. Given the many similarities between prion strains and conformational variants of non-prion amyloidogenic proteins, it would be reasonable to extend the scope of strains to include the latter. However, because other neurodegenerative disease–associated proteins seem to be much less transmissible than PrP^{Sc}, the operational definition of strains would need to be modified to remove the requirement of propagatability *in vivo* through serial passage.

Pathophysiological relevance of strains for non-prion proteinopathies

Similarly to prion diseases, each class of neurodegenerative protein-opathies shows tremendous heterogeneity. For example, among the various tauopathies characterized by the accumulation of tau aggregates (for example, Alzheimer's disease, argyrophilic grain disease, progressive supranuclear palsy, corticobasal degeneration and Pick's disease), there are substantial variations in clinical symptoms, age of disease onset, rate of progression, types of cells affected (neurons and/or glial cells), brain region–specific distribution of tau inclusions and morphology of tau tangles[113,114]. At the same time, considerable overlap occurs among different categories of diseases, in both clinical symptoms[115,116] and neuropathologies, whereby different protein aggregates such as NFTs and Lewy bodies frequently co-deposit in diseased brains[117]. Proteostatic stress arising from the aggregation of one protein can elicit a global effect on the structural stability of other unrelated proteins, possibly resulting in misfolding and aggregation of other proteins[118]. Filamentous aggregates consisting of one protein may also directly seed the fibrillization of another aggregation-prone protein, as supported by histological studies showing that tau and α-syn inclusions are sometimes deposited in close proximity[119,120] and also suggested by our recent study in primary neurons and transgenic mice[108].

We propose that diverse strains of protein aggregates may offer a potential explanation for both the divergence and the convergence of neurodegenerative proteinopathies (Fig. 3b). Transmissible fibrillar aggregates of different conformations may display highly variable kinetics in templated fibrillization, resulting in different progression rates of various diseases; exhibit distinct cell tropism, leading to pathology development in different cell types and progression to distinct brain areas; and differ in their ability to cross-seed aggregation of other amyloidogenic proteins, causing differential levels of comorbid pathologies. Given these variations in biological activities, conformationally diverse strains can not only potentially account for the tremendous heterogeneity among disorders with the same major protein lesions, but also explain the frequent (though variable) co-deposition of

different protein aggregates. Furthermore, during cell-to-cell transmission involving repetitive seeded fibrillization, one strain of pathological aggregates may evolve into another strain, as demonstrated for synthetic α-syn fibrils[108]. This could lead to brain region–specific pathologies within a single patient, such as the morphologically distinct Lewy bodies found in the substantia nigra and neocortex of patients with Parkinson's disease[3]. The variants of synthetic fibrils generated *in vitro* are not necessarily identical to fibrils formed in human brains, but considering the complexity of human brains relative to test-tube reactions, the chance of developing diverse strains of pathological aggregates in real diseased brains is exceedingly high. In fact, three recent studies have provided preliminary evidence for the presence of structural variants of pathological Aβ, tau and α-syn in human brains[42,108,121], but more studies are required to verify the existence of strains in heterogeneous disease groups and their correlation with disease phenotypes.

Therapeutic implications

Studies of prion-like transmissibility of pathogenic protein aggregates have important therapeutic implications. Cell-replacement therapy, which is designed to restore the functions of degenerating brains by replacing dying cells with healthy ones[122], may not, if used alone, provide a long-term treatment for neurodegenerative proteinopathies, because the implanted cells may eventually develop pathology owing to transmission from host cells. Indeed, several studies on patients with Parkinson's disease receiving embryonic nigral transplants showed the emergence of Lewy bodies in the young graft tissues, suggesting a possible host-to-graft transmission of α-syn pathology[123,124]. One recent study in cultured cells showed that the induction of Lewy body–like α-syn aggregates leads to the futile activation of protein-degradation systems, further stimulation of which is actually more detrimental to cells[100]. If the same is true for all pathological protein aggregates, we would need to rethink the fervently sought therapeutic approach of boosting autophagy in pathology-laden brains.

On the bright side, recent studies have opened new avenues for therapeutic interventions that could be used in combination with cell-replacement therapy. Small molecules that hinder templated recruitment by existing aggregates would inhibit further corruption of native proteins and thus block self-amplification of misfolded proteins. Because cell-to-cell transmission probably involves release and uptake of misfolded proteins, agents that could block either process would conceivably stop the spreading of pathology, thereby halting disease progression. Compounds that enhance cell membrane integrity might reduce the transcellular movement of amyloid fibrils via direct membrane penetration. As fluid-phase endocytosis seems to be a predominant uptake mechanism of several protein aggregates, agents that suppress this endocytic pathway could potentially be helpful for multiple diseases. If exosomes are truly involved in the release of aggregated proteins, inhibitors of exosome secretion may also hold therapeutic potential. However, because any cellular machinery exploited by pathological protein aggregates to accomplish intercellular transmission is likely to play important parts in many normal cellular functions, drugs affecting these pathways may have substantial side effects.

An alternative approach to inhibit cell-to-cell transmission of misfolded proteins is to sequester them in the extracellular space during transit from one cell to another. Immunotherapy, which was previously thought to be intractable for abrogating intracytoplasmic protein aggregates, would be an attractive strategy to stop the intercellular movement of target protein species. Lessons learned from Aβ immunotherapy would become useful for other pathogenic proteins[125]. In particular, careful consideration is required to decide whether active or passive immunization is the preferred method, and whether a linear epitope (a continuous stretch of amino acids in a primary structure) or a conformational epitope (discontinuous amino acids that are brought together in tertiary structure) would be more desirable. Moreover, if diverse pathological strains truly exist, they may create additional challenges for effective immunotherapy, because antibodies that exhibit high binding affinity for one strain of protein aggregates may not be equally efficient at forming complexes with another strain. However, antibodies that recognize shared conformations of multiple strains, particularly those that inhibit cross-seeding, may have a broader application than those recognizing highly specific epitopes, but the feasibility of this hypothetical approach awaits empirical testing. A major obstacle for all brain-directed therapies is the blood-brain barrier (BBB), which tightly restricts the entry of both pathogens and therapeutics into the brain. Studies on Aβ passive immunotherapy showed that only about 0.1% of circulating antibodies managed to reach the brain[126]. Therefore, to maximize therapeutic effects, it would be necessary to employ strategies that promote the uptake of antibodies across the BBB, such as engineering hybrid antibodies—which, besides binding target molecules in the brain, also interact with specific transporters or receptors on the BBB to facilitate shuttling into the brain[127].

Conclusions and future directions

A rapidly growing body of literature has provided compelling evidence supporting the intercellular transmission of pathological protein aggregates through templated recruitment as a potential unifying mechanism that underlies the progression of various neurodegenerative proteinopathies. Furthermore, the exact conformation of the template may dictate its recruitment properties, possibly giving rise to heterogeneity among diseases characterized by the same protein lesions. Although these studies have revolutionized our conception of these diseases, more questions remain. For example, it is still unclear what triggers the generation of the misfolded protein seeds that initiate the disease cascade, why only certain proteins aggregate in a disease-specific manner if the main trigger is a global deterioration of protein homeostasis, what determines the selective vulnerability of different brain regions and what roles glial cells have in the transmission of protein aggregates. Numerous studies suggest that prefibrillar species may be more detrimental than fibrillar aggregates[26,128], but the exact nature of these early species in the aggregation pathway and their relevance for human diseases require further studies. The biochemical identities of transmissible species also need to be better defined, as most existing studies simply use sonicated synthetic fibrils or crude preparations of brain homogenates, which may contain mixed forms of misfolded proteins. Moreover, although synthetic fibrils have been shown competent as seeds, they may not be identical to the pathological aggregates derived *in vivo*, which seem to be more potent seeds in transmission. Thus, future research should clarify the

differences between these sources of seeds and investigate the potential parts other cellular components play in influencing transmissibility.

From a translational point of view, a detailed molecular dissection of pathways mediating the release and uptake of protein aggregates, which may or may not be the same for different pathogenic proteins, would provide new targets for drug development. Specifically, future studies should determine the exact endocytic pathways involved in the internalization of protein aggregates and verify whether aggregates are released from cells in a free-floating form or enclosed by vesicles, as this will be crucial for the design of effective therapies. Finally, more effort should be directed toward generating animal models of neurodegenerative diseases without overexpression, which would provide better recapitulations of sporadic diseases; so far, this goal has only been partially attained for α-syn[16]. For other proteins, researchers may need to create strains of aggregates that are more potent than existing ones in propagating pathology, possibly by using specific combinations of cofactors or by amplifying pathological aggregates derived *in vivo*. With a collaborative effort on the issues mentioned above, we would gain better understanding of the pathogenesis of neurodegenerative proteinopathies and, hopefully, find cures for these devastating diseases.

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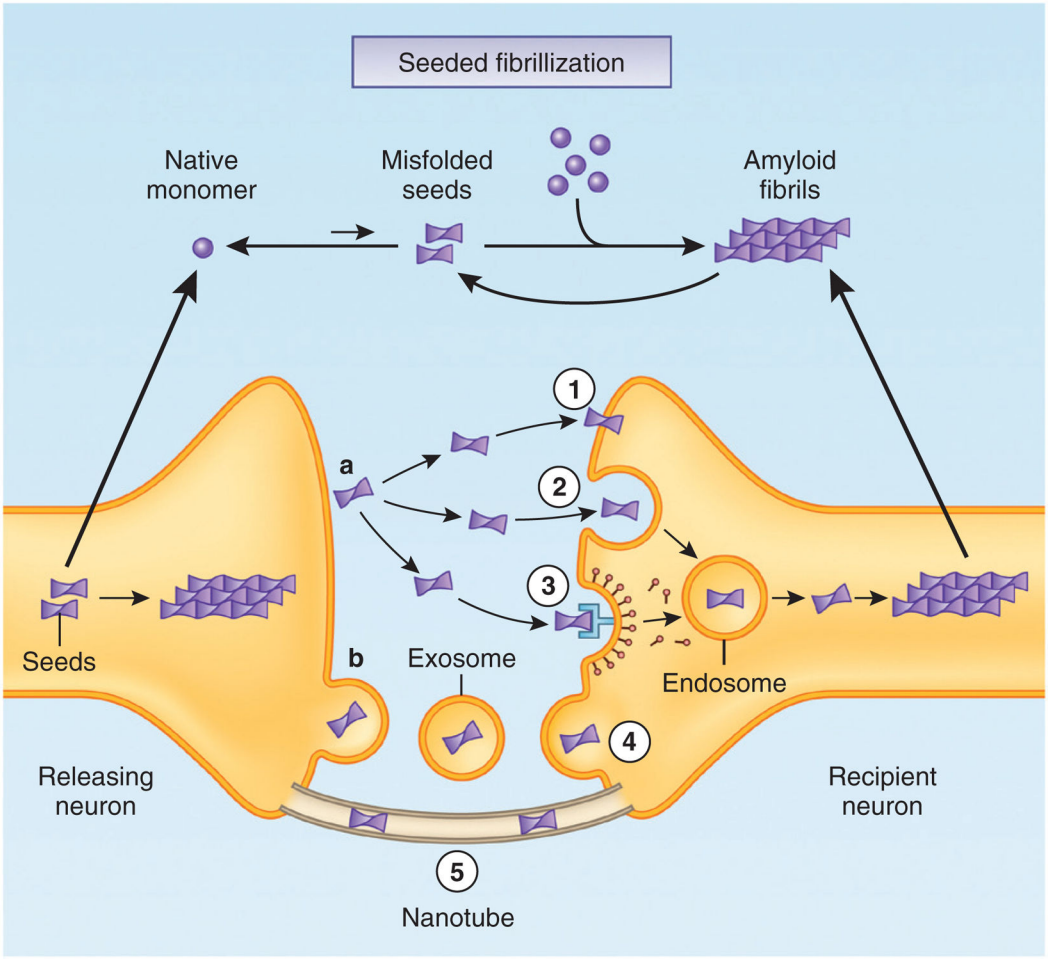

Figure 1.

Potential mechanisms mediating cell-to-cell transmission of cytosolic protein aggregates. (**a**, **b**) Misfolded protein seeds (for example, oligomers and protofibrils) first form in the cytoplasm of the releasing neuron (left), where soluble native monomers are recruited into large intracellular aggregates and a positive feedback loop can be initiated by generation of more seeds through fragmentation or secondary nucleation. A small amount of protein aggregates can be released into the extracellular space in the 'naked' form (**a**) or via membrane-bound vesicles such as exosomes (**b**). Free-floating seeds may directly penetrate the plasma membrane of the recipient neuron (1) or enter by fluid-phase endocytosis (2) or receptor-mediated endocytosis (3), whereas exosomes containing seeds may fuse with the membrane of the recipient neuron (4). Intercellular transfer of seeds may also occur by nanotubes that directly connect the cytoplasm of two cells (5). Internalized seeds then nucleate the fibrillization of native monomers in the cytoplasm of the recipient neuron.

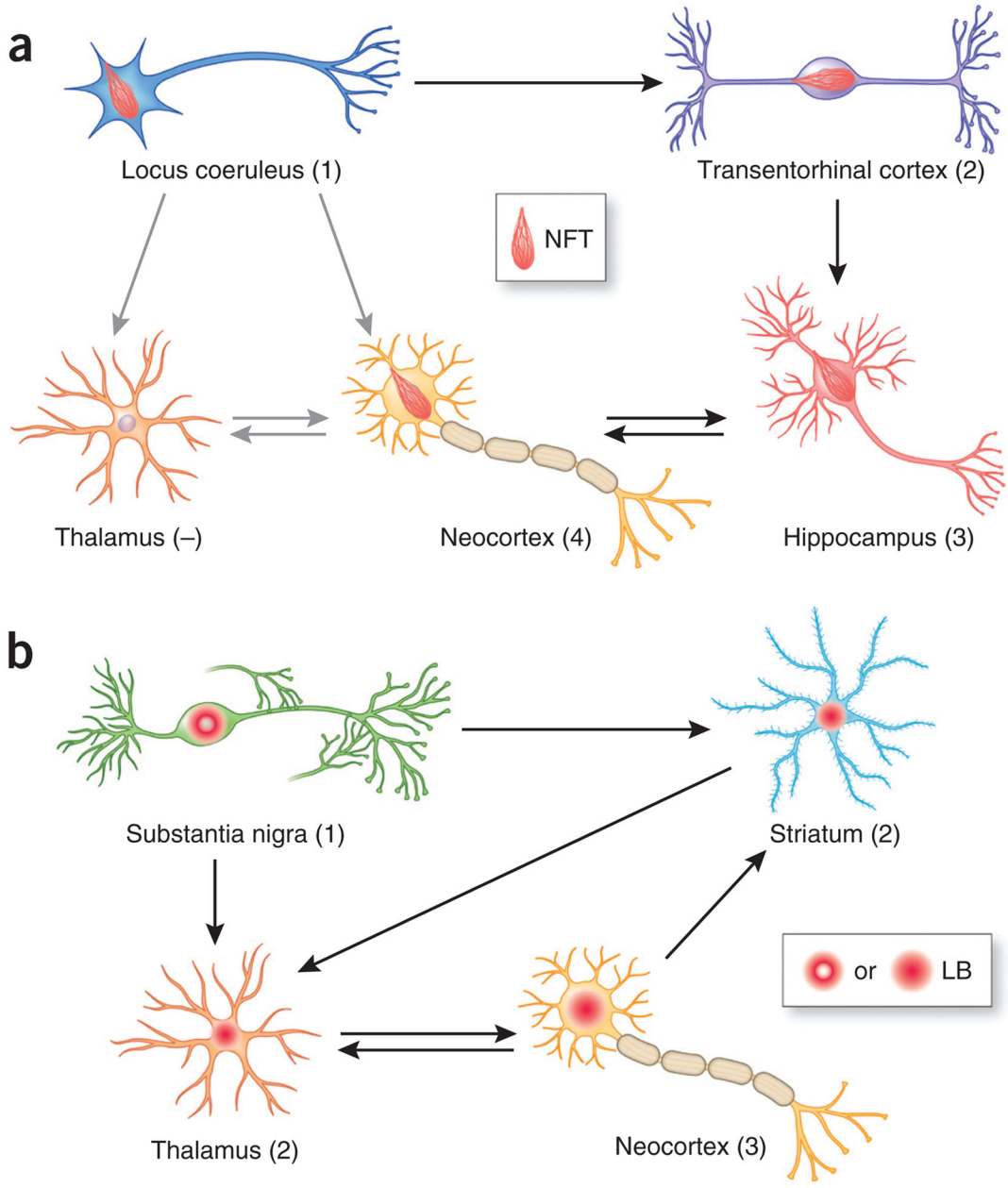

Figure 2.

Hypothetical model accounting for the stereotypical progression of pathologies in Alzheimer's and Parkinson's diseases. (**a**, **b**) Key brain regions developing NFTs (**a**) or Lewy bodies (LB) (**b**) are shown for each disease; numbers in parentheses indicate the relative temporal sequence of pathology progression (−, lack of pathology). Major neuronal projections are indicated by arrows, with black arrows indicating projections that hypothetically contribute to the spreading of pathology and grey arrows showing lack of transmission. Brain areas affected by tau and α-syn inclusions at the early stages of the diseases are different, probably owing to brain region–specific vulnerability to the aggregation of the disease proteins. Subsequently affected brain regions may acquire transmissible protein aggregates along both anterograde and retrograde connections, although not all regions connected with affected areas develop pathology. For example, the thalamus is relatively resistant to the accumulation of

NFTs despite direct connections with the locus coeruleus. Therefore, brain region–specific vulnerability combined with network connections gives rise to the characteristic onset and progression patterns of neuropathology for different disease proteins.

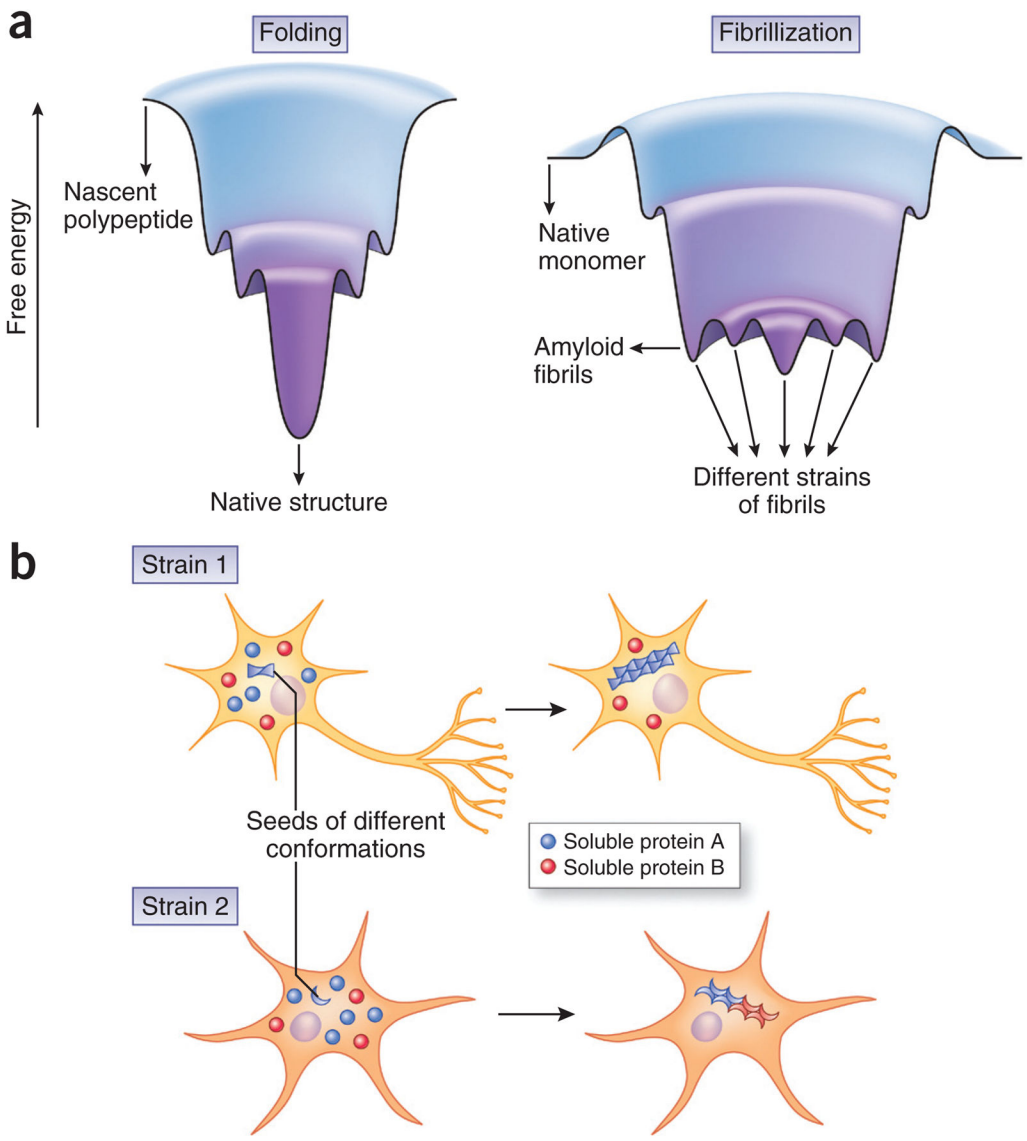

Figure 3.

Pathological strains underlying the divergence and convergence of neurodegenerative proteinopathies. (**a**) A possible explanation for the existence of multiple fibrillar conformers for a single polypeptide is that the energy landscape of fibrillization is different from that of normal protein folding. Whereas the energy landscape of protein folding resembles a funnel with a global minimum that corresponds to the native structure[129,130], the landscape for abnormal protein aggregation may resemble rugged valleys with numerous local minima corresponding to different strains of fibrils. According to this speculative protein-aggregation landscape, multiple strains of fibrillar aggregates could theoretically emerge in one fibrillization event, but the exact environmental conditions may favor the generation and propagation of one strain over the others; one conformational variant could also directly morph into another if the energy barrier between them is overcome. Indeed, this is what was observed for the Darwinian evolution of prion strains in cultured cells and living animals[131,132]. A similar evolution of strains was recently reported for α-syn fibrils generated from recombinant protein, whereby serial passage *in vitro* resulted in the conversion of one strain of α-syn fibrils into the other[108]. (**b**) Different strains of amyloid fibrils may exhibit distinct cell tropism (indicated by different shapes of affected cells) and differential efficiency in seeding homotypic monomers (blue

spheres) as well as in cross-seeding heterotypic monomers (red spheres). These properties could result in distinct neuropathological profiles, including differences in the distribution of pathology and extent of comorbid pathologies, and ultimately lead to heterogeneous clinical manifestations.

Table 1

Summary of studies demonstrating the transmissibility of non-prion protein aggregates

		Seeded aggregation in different model systems		
Protein	**Type of seed**	**Non-neuronal cells**	**Neuronal cells**	**Mice**
Aβ	Synthetic fibrils	Not tested	Not tested	Yes[22]
	Mouse brain lysates	Not tested	Not tested	Yes[22,41,72]
	Human brain lysates	Not tested	Not tested	Yes[41]
Tau	Synthetic fibrils	Yes[17,58]	Yes[62,133]	Yes[19]
	Mouse brain lysates	Not tested	Not tested	Yes[18]
	Human brain lysates	Not tested	Not tested	Yes[29,42]
α-Syn	Synthetic fibrils	Yes[134]	Yes[15,133]	Yes[16,20,21]
	Mouse brain lysates	Not tested	Not tested	Yes[20]
	Human brain lysates	Not tested	Not tested	Yes[21]
TDP-43	Synthetic fibrils	Yes[135]	Not tested	Not tested
	Human brain lysates	Not tested	Yes[136]	Not tested
SOD1	Synthetic fibrils	Not tested	Yes[57]	Not tested
PolyQ	Synthetic fibrils	Yes[56]	Not tested	Not tested