Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2016 | 138 (Suppl. 1) | 163–183 doi: 10.1111/jnc.13668

REVIEW

Prion-like propagation as a pathogenic principle in frontotemporal dementia

Eva-Maria Hock and Magdalini Polymenidou

Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland

Abstract

Frontotemporal dementia is a devastating neurodegenerative disease causing stark alterations in personality and language. Characterized by severe atrophy of the frontal and temporal brain lobes, frontotemporal dementia (FTD) shows extreme heterogeneity in clinical presentation, genetic causes, and pathological findings. Like most neurodegenerative diseases, the initial symptoms of FTD are subtle, but increase in severity over time, as the disease progresses. Clinical progression is paralleled by exacerbation of pathological findings and the involvement of broader brain regions, which currently lack mechanistic explanation. Yet, a flurry of studies indicate that protein aggregates accumulating in neurodegenerative diseases can act as propagating entities, amplifying their pathogenic conformation, in a way similar to infectious prions. In this prion-centric view, FTD can be divided into three subtypes, TDP-43 or FUS proteinopathy and tauopathy. Here, we review the current evidence that FTD-linked pathology propagates in a prion-like manner and discuss the implications of these findings for disease progression and heterogeneity.

Keywords: FTD, FUS, prion-like spread, tau, TDP-43.

J. Neurochem. (2016) **138** (Suppl. 1), 163–183.

***This article is part of the** Frontotemporal Dementia special issue.*

Protein aggregation in the central nervous system is a common feature of many neurodegenerative diseases. Although the main protein component and primary affected region can vary for each neurodegenerative disease, the accumulation of misfolded proteins into insoluble aggregates is a unifying hallmark and leads to progressive dysfunction and death of neurons and glial cells. Despite the diversity in clinical presentation, as well as pathological findings, increasing evidence supports a common mechanism driving neurodegeneration. Disease-related proteins are transformed from their normal conformation into fibrillar or multimeric species that act as seeds of aggregation and further sequester their native isoforms and convert them into pathological molecules. This self-perpetuating process leads to the formation of large aberrant protein aggregates, which subsequently fragment into new seeds that propagate their abnormal conformation in a template-dependent manner within one cell, as well as through the CNS by the release of seeds into the extracellular space that can be taken up by neighboring cells leading to the repetition of the propagation cycle (Aguzzi 2009; Aguzzi and Rajendran 2009; Brundin *et al.* 2010; Goedert *et al.* 2010; Lee *et al.* 2010; Polymenidou and Cleveland 2011, 2012; Jucker and Walker 2013; Walker *et al.* 2013; Guo and Lee 2014; Walker and Jucker 2015; Sanders *et al.* 2016).

Such propagation mechanisms were long thought to be exclusively associated with transmissible prion diseases, in which template-directed protein misfolding leading to the corruption of native molecules, is the basis for replication of infectious prions. In *bona fide* prion diseases (also called transmissible spongiform encephalopathies), ingestion or

Received December 22, 2015; revised manuscript received April 22, 2016; accepted May 11, 2016.

Address correspondence and reprint requests to Magdalini Polymenidou, PhD, Institute of Molecular Life Sciences, Y32-J06, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. E-mail: magdalini.polymenidou@imls.uzh.ch

Abbreviations used: 3/4R, 3/4-repeat-tau; AD, Alzheimer's disease; AGD, argyrophilic grain disease; ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant FTD; C9ORF72, chromosome 9 open reading frame 72; CBD, corticobasal degeneration; CHMP2B, charged multivesicular body protein 2B; CSF, cerebrospinal fluid; CTE, chronic traumatic encephalopathy; EWS, Ewing sarcoma protein; FET, FUS, EWS, TAF-15; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; FUS, fused in sarcoma; GRN, progranulin; hnRNP, heterogeneous ribonucleoprotein; HSPGs, heparan sulfate proteoglycans; ISF, brain interstitial fluid; MAPT, microtubule-associated protein tau; NES, nuclear export signal; NLS, nuclear localization signal; PD, Parkinson's disease; PiD, Pick's disease; prion, proteinaceous infectious particle; PrP, prion protein; PSP, progressive supranuclear palsy; RRM, RNA recognition motifs; TAF15, TATA-binding protein-associated factor 15; TDP-43, TARDBP-binding protein, 43 kDa; TD, tangle-only dementia; VCP, valosin-containing protein.

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internalization of the proteinaceous infectious agent (prion) is sufficient to transmit the disease between individuals (Prusiner 1982). Prions replicate by recruiting the normal cellular isoform of the prion protein, PrP^{C}, into ordered PrP^{Sc}-containing aggregates thus inducing a pathological conformation. This continuous conversion into a highly aggregative form triggers a widespread misfolding and fibril formation across the nervous system in a self-perpetuating manner (Aguzzi and Polymenidou 2004; Aguzzi 2009; Aguzzi and Rajendran 2009). Therefore, in prion diseases, seeded aggregation causes neurodegeneration and mice lacking the prion protein are completely resistant to the disease (Bueler *et al.* 1993). Prion pathology generally includes protein aggregation, neuronal loss, gliosis, and spongiform degeneration (Aguzzi and Polymenidou 2004). However, variations in the disease phenotypes, distinct histopathological signatures, varying incubation periods and disease progression, were found to be caused by different structural conformations of the misfolded prion protein, which are referred to as strains (Aguzzi *et al.* 2007).

Recently, the recognition of a similar propagation principle in other, non-transmissible proteinopathies has led to the term 'prion-like' (Goedert *et al.* 2010; Polymenidou and Cleveland 2011; Jucker and Walker 2013; Guo and Lee 2014). The emerging realization that pathological aggregates of amyloid-β, tau, α -synuclein, and others can act in a prion-like manner has changed the view on disease initiation and progression manner (Aguzzi 2009; Aguzzi and Rajendran 2009; Goedert *et al.* 2010; Polymenidou and Cleveland 2011, 2012; Jucker and Walker 2013; Guo and Lee 2014). While the mechanisms triggering the initial conversion of normally soluble proteins into pathogenic polymers remain unresolved, substantial evidence showed that aggregation seeds of amyloid-β (Meyer-Luehmann *et al.* 2006), tau (Clavaguera *et al.* 2009), and α-synuclein (Nonaka *et al.* 2010; Volpicelli-Daley *et al.* 2011; Luk *et al.* 2012) can induce template-dependent misfolding of native proteins *in vitro* and *in vivo*. Moreover, cell-to-cell transmission of protein aggregates has also been proven for amyloid-β, tau, α-synuclein, SOD-1, and TARDBP-binding protein, 43 kD (TDP-43) (Clavaguera *et al.* 2009; Grad *et al.* 2011; Nath *et al.* 2012; Nonaka *et al.* 2013), although both the exact spreading mechanism and nature of the transmissible entity have not been fully resolved.

These findings support an attractive hypothesis for the progressive nature of these diseases. Indeed, most neurodegenerative disorders are initially subtle but progress relentlessly, often exhibiting a clear spatiotemporal involvement of distinct, but interconnected regions of the nervous system. The molecular entity responsible for this disease spread remains controversial, but seeded aggregation of pathogenic proteins, which lead to neurotoxicity and eventually cell death, may well explain these phenomena.

Frontotemporal dementia is characterized by complex genetic causes and heterogeneous clinical presentation

Frontotemporal dementia (FTD) is the second most frequent cause of presenile dementia with age of onset typically between 45 and 65 years. It is associated with frontal and temporal lobe atrophy leading to a progressive dysfunction in behavior, personality, and language, mostly with preservation of memory (Neary *et al.* 1998; McKhann *et al.* 2001). The clinical phenotypes can be classified into behavioral variant FTD (bvFTD) and two versions of primary progressive aphasia: semantic dementia and progressive non-fluent aphasia (Gorno-Tempini *et al.* 2011; Rascovsky *et al.* 2011). Importantly, FTD clinically overlaps with amyotrophic lateral sclerosis (ALS), which is the most common motor neuron degeneration. Indeed, an estimated ~ 20% of FTD patients develop progressive paralysis and ~ 20% of ALS patients present personality changes compatible with FTD (Caselli *et al.* 1993; Neary *et al.* 2000; Lomen-Hoerth *et al.* 2002; Ng *et al.* 2015).

FTD is a heritable disease with approximately 25–50% of cases reporting a family history and thus indicating a strong genetic component (Rohrer *et al.* 2009). The first FTD-associated mutation was found in the microtubule-associated protein tau (*MAPT*) gene on chromosome 17 in 1998 (Hutton *et al.* 1998). Up till now over 50 different causal *MAPT* mutations were found accounting for 5–20% of familial FTD cases (Rohrer *et al.* 2009; Spillantini and Goedert 2013). In 2006 mutations in the progranulin (*GRN*) gene – coincidentally near *MAPT* on chromosome 17 – were identified to cause an even larger proportion of familial FTD cases (Baker *et al.* 2006; Cruts *et al.* 2006). Several much less common mutations in the valosin-containing protein (*VCP*) gene (Watts *et al.* 2004), the gene encoding charged multivesicular body protein 2B (Skibinski *et al.* 2005; Parkinson *et al.* 2006) and others have since been discovered. A major breakthrough in FTD genetics was the recognition of hexanucleotide repeat expansions in a non-coding region of the chromosome 9 open reading frame 72 (*C9ORF72*) gene, which cause ~ 25% of all familial and ~ 6% of sporadic FTD cases, making it the most common genetic cause (DeJesus-Hernandez *et al.* 2011; Renton *et al.* 2011). In people of European ancestry, *C9ORF72* hexanucleotide repeat expansions also cause ~ 40% of familial ALS and ~ 7% of sporadic ALS, whereas up to 90% of families with concurrent ALS and FTD carry *C9ORF72* mutations (Majounie *et al.* 2012) (Table 1).

Frontotemporal dementia is subclassified into three distinct and non-overlapping pathologies: tau, TDP-43 and FUS

The neuropathology of clinical FTD also reflects the heterogeneity of the disorder. While frontotemporal lobar

Table 1 Summary of prion-like evidence for FTD-associated proteins in vitro, *in vivo,* and in patients

Protein	Human disease	Subcellular localization of aggregates	Seeded aggregation			Cell-to-cell spread		Experimentally transmitted disease	Strains	Pathology Staging in humans
			In vitro	In cell culture	*In vivo*	In cell culture	*In vivo*			
Tau	AD, FTLD-Tau (FTLD-17 *MAPT,* PSP, AGD, PiD, CBD), CTE	Cytoplasmic	Friedhoff *et al.* 1998;	Frost *et al.* 2009; Guo and Lee 2011; Wu *et al.* 2013; Nonaka *et al.* 2010;	Bolmont 2007, Clavaguera *et al.* 2009, 2013; Iba *et al.* 2013; Peeraer *et al.* 2015; Lasagna-Reeves *et al.* 2012;	Kfoury *et al.* 2012; Iba *et al.* 2013;	Lasagna-Reeves *et al.* 2012; de Calignon *et al.* 2012; Liu *et al.* 2012; Iba *et al.* 20l3, Ahmed *et al.* 2014; Dujardin *et al.* 2014;	Peeraer *et al.* 2015;	Sanders *et al.* 2014; Boluda *et al.* 2015	AD (Braak and Braak 1991; Braak and Braak 1995; Braak *et al.* 2006), CTE (Geddes *et al.* 1999; McKee *et al.* 2013)
TDP-43	FTLD-TDP, ALS	Mostly cytoplasmic	Furukawa *et al.* 2011;	Furukawa *et al.* 2011; Feiler *et al.* 2015;	n.d.	Nonaka *et al.* 2013; Feiler *et al.* 2015;	n.d.	n.d.		FTLD (Brettschneider *et al.* 2014), ALS (Brettschneider *et al.* 2013)
FUS	FTLD-FUS, ALS	Mostly cytoplasmic, and rare intranuclear	Nomura *et al.* 2014	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

AD, Alzheimer disease; AGD, argyrophilic grain disease; ALS, amyotrophic lateral sclerosis; CBD, corticobasal degeneration; CTE, chronic traumatic encephalopathy; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; FUS, Fused in sarcoma; PiD, Pick's disease; PSP, progressive supranuclear palsy; TDP-43, TAR DNA-binding protein of 43 kDa; n.d., not determined.

degeneration (FTLD) is the common underlying pathology of clinical FTD, several distinct disease types exist, subclassified based on the deposited protein found in abnormal intracellular inclusions (Mackenzie and Rademakers 2007; Mackenzie *et al.* 2010). The first subgroup includes ~ 40% of all FTLD cases and is defined by hyperphosphorylated tau inclusions in neurons and glia and is classified as FTLD-tau. However, most cases of FTD are not associated with tau pathology and are instead defined by neuronal inclusions originally identified by their immunoreactivity for ubiquitin and consequently termed FTLD-U (Josephs *et al.* 2004; Mackenzie *et al.* 2006). A major breakthrough followed when TDP-43 was found to be the main component in ubiquitinated pathological inclusions in most FTLD-U cases, as well as in the majority of ALS cases (Arai *et al.* 2006; Neumann *et al.* 2006). This finding not only resulted in a new subtype of FTD termed FTLD-TDP (Neumann *et al.* 2006), but it also provided a strong connection between the two disorders that had clinically been connected before (Caselli *et al.* 1993; Neary *et al.* 1998; Lomen-Hoerth *et al.* 2002). An overlapping molecular pathogenesis was further verified when the majority of tau- or TDP-43-negative inclusions were shown to be immunoreactive for a protein called fused in sarcoma (FUS) (Neumann *et al.* 2009a) shortly after FUS mutations had been detected as a cause of autosomal dominant ALS (Kwiatkowski *et al.* 2009; Vance *et al.* 2009). Interestingly, two other related DNA/RNA-binding proteins Ewing sarcoma protein (EWS) and TATA-binding protein-associated factor 15 were recently found to co-aggregate in FUS-positive inclusions in FTLD-FUS (Neumann *et al.* 2011).

While TDP-43 and FUS pathology connect FTD and ALS, FTLD-tau also shares its misfolded tau tangles with the most prominent neurodegenerative disorder, namely Alzheimer's disease (AD) (Querfurth and LaFerla 2010), whose primary pathology are insoluble, extracellular amyloid-β plaques.

Pathophysiological roles of proteins involved in FTD

The microtubule-associated protein tau forms toxic fibrils in FTD

In its natively unfolded form, tau protein is involved in microtubule formation and stabilization, as well as the regulation of axonal transport (Clavaguera *et al.* 2013). In humans, tau is abundantly found in neurons, where it concentrates in axons (Fig. 1a), but may also have a physiological function in dendrites (Binder *et al.* 1985). Six tau isoforms are expressed from a single gene (*MAPT*) by alternative mRNA splicing in the human brain (Goedert *et al.* 1989; Andreadis *et al.* 1992), each differing from the other by inclusion or exclusion of a 29- or 58-amino acid N-terminal insert and a 31-amino acid repeat in the microtubule-binding domain of tau, encoded by exon 10. Two major groups of tau isoforms can be distinguished: three isoforms with four exon 10 repeat sequences (4-repeat-tau, 4R) and three others with three repeat sequences (3-repeat tau, 3R) (Goedert *et al.* 1989).

In several disorders called tauopathies, the protein becomes hyperphosphorylated and forms insoluble filamentous inclusions making tau the most commonly misfolded protein in neurodegenerative diseases. Tauopathies include Alzheimer's disease, tangle-only dementia and FTLD-tau including frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17 *MAPT*), Pick's disease, argyrophilic grain disease, as well as progressive supranuclear palsy and corticobasal degeneration (Clavaguera *et al.* 2013; Lashley *et al.* 2015a). Hyperphosphorylation promotes the dissociation of tau from microtubules and is an early event in the process of tau aggregation. Hyperphosphorylated tau was shown to mislocalize to dendritic spines leading to synaptic abnormalities early in disease (Braak *et al.* 1994; Cochran *et al.* 2014). Many phosphorylation sites as well as responsible protein kinases and phosphatases have been identified and it has been suggested that tau may first misfold in human tauopathies, thus making it more accessible to protein kinases and less to protein phosphatases, leading to its hyperphosphorylation and increased aggregation propensity (Hanger *et al.* 2009; Clavaguera *et al.* 2013). In addition, a number of other disease-associated post-translational modifications of tau have been described, which include ubiquitination, acetylation, N-glycosylation, O-GlcNAcylation, nitration, prolyl isomerization, sumoylation, and truncation (Fig. 1b) (Lashley *et al.* 2015b; Wang and Mandelkow 2016).

Tau filaments possess a cross-β structure characteristic of amyloid fibrils and it is widely accepted that their formation in specific brain regions contributes to disease pathogenesis and can induce neurotoxicity and cellular dysfunction. Insoluble tau aggregates can be found in numerous disorders, where they exhibit a range of morphologies (Crowther and Goedert 2000), with specific conformers of distinct isoform types giving rise to distinguishable cellular tau pathologies. For instance, a mixture of 3R and 4R tau form neurofibrillary tangles can be observed in the somatodendritic compartment, as well as neuropil threads in the distal axon and dendrites in AD. Conversely, in other tauopathies only one isoform is the predominant species, as reflected by characteristic ultrastructural morphologies that can be found in either exclusively neuronal or both neuronal and glial tau filaments (Goedert *et al.* 1992).

FTDP-17 *MAPT* presents a special case of tauopathy in which mutations in the *MAPT* gene trigger abundant tau pathology (Hutton *et al.* 1998; Poorkaj *et al.* 1998; Spillantini *et al.* 1998; Goedert and Jakes 2005). Depending on the mutation, filamentous inclusions consist primarily of 3R, 4R or a mixture of all six tau isoforms and are deposited in neurons and glial cells (Ghetti *et al.* 2015). Further FTLD subtypes are also characterized by specific tau pathology

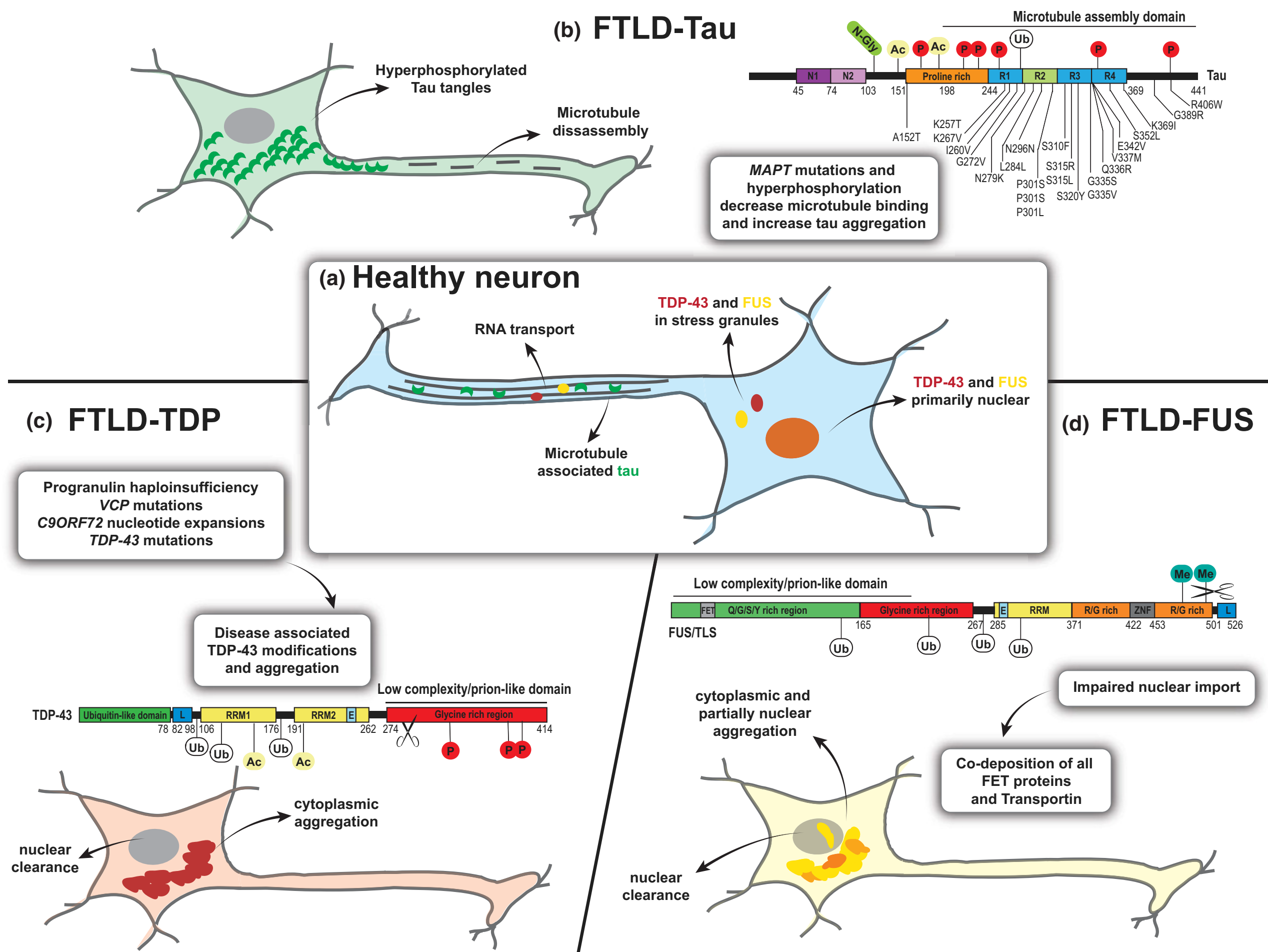

Fig. 1 The three main types of FTD pathology are characterized by the accumulation of distinct protein aggregates. (a) In healthy neurons, tau (green crescent) is mainly distributed in axons and regulates microtubule stability. TDP-43 (red) and FUS (yellow) are primarily found in the nucleus, where they are involved in several steps of RNA metabolism. In the cytoplasm, both proteins participate in dynamic RNA granules, facilitating axonal transport or forming as part of the cell stress response. (b) In pathological conditions of FTLD-tau, the protein is dissociated from microtubules and accumulates into pathogenic tangles in the cytoplasm. Mutations in the microtubule-binding region of the microtubule-associated protein tau (*MAPT*) gene are found FTLD-17 *MAPT* and can lead to splicing changes and decreased microtubule-binding affinity. Hyperphosphorylation (P) and other post-translational modifications (N-Glyc (N-glycosylation), Ac (Acetylation), Ub (Ubiquitination) can further enhance the detachment, resulting in microtubule disassembly and the formation of tau aggregates in the cytoplasm (example modification sites are shown). (c) In FTLD-TDP-43 several underlying genetic causes (*GRN, VCP, TDP-43, C9ORF72* mutations) and sporadic cases are linked by a common TDP-43 pathology. TDP-43 undergoes several disease-associated modifications including phosphorylation, ubiquitination and C-terminal cleavage and forms pathogenic inclusions in the cytoplasm leading to a redistribution of the protein, associated with nuclear clearance. (d) Although called FTLD-FUS, all FET proteins have now been found to co-aggregate in ubiquitinated inclusions in severe cases of sporadic FTD. Factors impairing the nuclear import of these proteins might lead to cytoplasmic accumulation and following aggregation because of strongly aggregation-prone low complexity domains. C9ORF72: chromosome 9 open reading frame 72, E: Nuclear export signal, FET: FUS, TAF-15, EWS protein interacting domain, FTD: frontotemporal dementia, FTLD: frontotemporal lobar degeneration, FUS: fused in sarcoma, L: nuclear localization signal, N1, N2: near-amino-terminal inserts, Q/G/S/Y-rich: glutamine-glycine-serine-tyrosine rich region, R1, R2, R3, R4: carboxy-terminal repeat domain, R/G-rich: arginine/glycine rich region, RRM: RNA recognition motif, TDP-43: TAR DNA-binding protein 43, VCP: valosin-containing protein, ZNF: zinc finger domain.

with typically one isoform subtype: pick bodies composed primarily of 3R tau in Pick's disease (Delacourte *et al.* 1996), tufted astrocytes and numerous neurofibrillary tangles in subcortical nuclei in progressive supranuclear palsy (Flament *et al.* 1991), and 4R tau astrocytic plaques with abundant thread pathology in corticobasal degeneration (Ksiezak-Reding *et al.* 1994). As a primary tauopathy that leads to severe neuronal loss, astrocytic gliosis, and spongiosis (Morris *et al.* 2001), FTLD-tau demonstrates that dysfunction and subsequent accumulation of the tau protein

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per se suffices to cause neurodegeneration and dementia (Clavaguera *et al.* 2015).

The ribonuclear protein TDP-43 accumulates in cytoplasmic aggregates in FTD and ALS

The discovery of TDP-43 as the main component of ubiquitin-positive, cytoplasmic inclusions in approximately 50% of patients with FTD in 2006, rendered FTLD-TDP the most frequent pathological subtype and enormous efforts have been made ever since to uncover the underlying mechanisms of TDP-43 pathogenesis. This finding also strongly highlighted the pathological link between FTD and ALS, which are now recognized as the two ends of the same disease spectrum (Ling *et al.* 2013).

TDP-43 is a 414 amino acid protein encoded by the *TARDBP* gene on chromosome 1. It is a ubiquitously expressed and highly conserved heterogeneous nuclear ribonucleoprotein, involved in multiple steps of RNA maintenance and processing (Lagier-Tourenne *et al.* 2010; Polymenidou *et al.* 2012). Two RNA recognition motifs (RRM1 and RRM2) in the center of the protein enable nucleic acid binding to UG-repeat sequences (Polymenidou *et al.* 2011; Lukavsky *et al.* 2013), which might be supported by an atypical ubiquitin-like domain, recently found in the N-terminal part of the protein (Qin *et al.* 2014). In the nucleus, TDP-43 plays a critical role in regulating alternative RNA splicing, as well as modulating the levels of long-intron containing RNAs important for neuronal activity (Polymenidou *et al.* 2011; Lagier-Tourenne *et al.* 2012). By regulating the stability of its own mRNA, TDP-43 can autoregulate its protein levels (Ayala *et al.* 2011; Polymenidou *et al.* 2011; Bembich *et al.* 2014). In addition, TDP-43 has recently been reported as a repressor of cryptic exons, which, when included, trigger the degradation of respective mRNAs (Ling *et al.* 2015). Although predominantly localized in the nucleus, TDP-43 contains a nuclear localization signal (NLS) and a nuclear export signal, which allow for classical Importin α/β-mediated shuttling (Winton *et al.* 2008).

Indeed, several roles have been proposed for TDP-43 outside the nucleus (Fig. 1a) and nuclear efflux can be influenced by both neuronal activity (Wang *et al.* 2008) and stress (Bentmann *et al.* 2012). TDP-43 is a key component in RNA transport granules and plays an important role in regulating local translation at distal locations, a function that is particularly important for neurons (Alami *et al.* 2014). Furthermore, TDP-43 was shown to associate with stress granules (Liu-Yesucevitz *et al.* 2010; Dewey *et al.* 2011; Bentmann *et al.* 2012), which are non-membranous cytoplasmic foci composed of non-translating messenger ribonucleoprotein complexes that transiently form in cells upon stress, thus selectively stalling protein synthesis (Anderson and Kedersha 2009). The C-terminus of TDP-43 harbors an aggregation-prone glycine-rich region important for protein–protein interactions (Buratti *et al.* 2005) that is necessary for its recruitment into stress granules, as deletion of this domain excluded TDP-43 from these compartments (Dewey *et al.* 2011).

In disease, the physiological, predominantly nuclear localization of TDP-43 is disturbed. Affected neurons and glial cells show a mislocalization to the cytoplasm with the formation of pathogenic inclusions (Fig. 1c) (Arai *et al.* 2006; Neumann *et al.* 2006; Igaz *et al.* 2008; Van Deerlin *et al.* 2008). This cytoplasmic aggregation prevents its return to the nucleus, leading to nuclear clearance. Moreover, pathological TDP-43 inclusions present a specific biochemical signature including polyubiquitination, hyperphosphorylation, and proteolytic cleavage of the highly aggregation-prone C-terminus, which is co-deposited in the cytoplasm along with the full-length protein (Arai *et al.* 2006; Neumann *et al.* 2006). Interestingly, the majority of missense mutations linked to FTD or ALS in TDP-43 are localized in the aggregation-prone C-terminal domain (Lagier-Tourenne *et al.* 2010; Ling *et al.* 2013). The latter also contains a glutamine/asparagine-rich low complexity or prion-like domain that shares similarities with yeast prions (Cushman *et al.* 2010; Fuentealba *et al.* 2010). Yeast prion proteins contain regions that can switch their conformation between an intrinsically unfolded and an aggregated state that can impose its conformation to an unfolded counterpart, in a mechanism similar to mammalian prions (Cushman *et al.* 2010). Disease-linked point mutations in this already aggregative prion-like domain of TDP-43 significantly increase its aggregation propensity, which hints to a causative link between TDP-43 aggregation and disease (Johnson *et al.* 2009; Guo *et al.* 2011; Molliex *et al.* 2015). Importantly, in the cortex and hippocampus of FTLD-TDP patients, phosphorylated C-terminal fragments of TDP-43 predominate over the full-length protein in the cytoplasmic inclusions (Igaz *et al.* 2008), suggesting a key role in neurodegeneration. The precise pathogenic mechanisms triggered by TDP-43 remain unknown and it remains unclear whether TDP-43 toxicity results from the loss of its normal function, from direct toxicity of the cytoplasmic misfolded protein deposits or a combination of the two possibilities (Lagier-Tourenne *et al.* 2010; Polymenidou and Cleveland 2012; Ling *et al.* 2013).

Nucleocytoplasmic transport defects cause cytoplasmic aggregation of FUS in FTD

Soon after the identification of TDP-43 as a genetic and pathological link between FTD and ALS, another DNA/RNA-binding protein, named FUS – also referred to as TLS for translocated in liposarcoma – was found in the pathological inclusions of about 5–10% of FTLD (Neumann *et al.* 2009a) and approximately 4% of familial ALS cases (Kwiatkowski *et al.* 2009; Vance *et al.* 2009). This discovery provided further evidence that ALS and FTD are closely

related disorders and emphasized the pathogenic involvement of RNA-binding proteins in these conditions. However, it quickly became clear that while in ALS-FUS missense mutations in the *FUS* gene account for most familial ALS and rare sporadic cases, no genetic alterations have been associated with the vast majority of FTLD-FUS cases. Yet, in the absence of FUS mutations, wild-type FUS misaccumulates in distinct, atypical clinical subgroups of FTD patients (Neumann *et al.* 2009a), namely neuronal intermediate filament inclusion disease (Neumann *et al.* 2009b) and basophilic inclusion body disease (Munoz *et al.* 2009) without TDP-43 or tau pathology (Urwin *et al.* 2010).

FUS is a 526 amino acid, ubiquitously expressed ribonucleoprotein and belongs to the FET (FUS, EWS, TAF-15) family of proteins together with EWS and TAF-15 (Bertolotti *et al.* 1996). It can bind nucleic acids through its C-terminal part, which contains an RRM, two glycine/arginine-rich motifs and a zinc finger domain. The N-terminal part of FUS comprises a low complexity domain harboring a glutamine-glycine-serine-tyrosine (Q/G/S/Y)-rich region and the R/G-rich motifs, which render the protein highly aggregation-prone (Sun *et al.* 2011) and which is predicted to attain prion-like properties (Cushman *et al.* 2010; Polymenidou and Cleveland 2011). An atypical nuclear localization signal (PY-NLS) and a nuclear export signal allow Transportin-mediated shuttling of the predominantly nuclear protein to the cytoplasm (Lee *et al.* 2006), suggesting that FUS is involved in RNA metabolism pathways that take place in both cellular compartments (Lagier-Tourenne *et al.* 2010). Being not only structurally, but also functionally similar to TDP-43, FUS has been implicated in transcriptional repression, splicing regulation, and micro-RNA processing (Lagier-Tourenne *et al.* 2010). Although TDP-43 and FUS bind a distinct spectrum of RNAs, they share a preference for mRNAs derived from genes with exceptionally long introns and 45 common RNA targets, mostly important for normal neuronal function, are significantly down-regulated upon depletion of either TDP-43 or FUS in the adult mouse brain and in cultured human neurons (Polymenidou *et al.* 2011; Lagier-Tourenne *et al.* 2012). Interestingly, several studies show altered splicing of the *MAPT* mRNA upon FUS depletion (Lagier-Tourenne *et al.* 2012). In primary neurons, a loss of FUS leads to the expression of a longer tau isoform (Orozco *et al.* 2012) that has also been found to be increased in patients with FTD and Parkinsonism (Hutton *et al.* 1998).

In the cytoplasm, similar to TDP-43, FUS can partition in stress granules (Bentmann *et al.* 2012) and plays a role in RNA transport granules, which carry mRNAs to dendritic spines for local translation (Kanai *et al.* 2004; Fujii and Takumi 2005; Elvira *et al.* 2006). Moreover, FUS interacts with the motor proteins kinesin and actin (Kanai *et al.* 2004; Yoshimura *et al.* 2006) and is essential for spine morphology (Fujii and Takumi 2005). Nevertheless, FUS predominantly localizes to the nucleus of healthy cells, whereas it is redistributed to the cytoplasm of affected cells, where it accumulates and aggregates (Fig. 1d). This mislocalization induces a partial loss of nuclear protein (Vance *et al.* 2009; Mackenzie *et al.* 2010), albeit to a lesser extent compared to TDP-43 proteinopathies. Strikingly, while both pathogenic FUS (Neumann *et al.* 2009a; Urwin *et al.* 2010) and TDP-43 (Neumann *et al.* 2006) inclusions are immunoreactive for p62 and ubiquitin, they seem mutually exclusive.

Evidence for prion-like behavior of FTD-associated proteins

In vitro seeding and *in vivo* propagation and spreading of misfolded tau was shown in multiple models

In its functional state tau is a hydrophilic protein that is natively unfolded with very little secondary structure, which makes it dynamic and highly soluble. Thus, its assembly into highly ordered β-sheet structures that form insoluble amyloid fibers in disease is counterintuitive and several fibrillization enhancing tricks have been necessary to reproduce their formation *in vitro* or in cell culture. Adding polyanionic substances, RNA or fatty acids has been shown to promote fibril assembly of recombinant, full-length tau protein *in vitro* by overcoming the nucleation barrier (Goedert 1996; Kampers *et al.* 1996; Chirita *et al.* 2003). Indeed, tau fibrillization follows a nucleation-dependent mechanism, and initiating tau aggregation with preformed tau fibrils can bypass this nucleation step and accelerate fibrillization of monomeric tau (Friedhoff *et al.* 1998). Similarly, highly soluble tau resists aggregation despite high over-expression and spontaneous hyperphosphorylation in cultured cells. Addition of minute quantities of preformed fibrils, however, is sufficient to recruit large amounts of soluble tau into filamentous inclusions with high efficiency (Friedhoff *et al.* 1998; Frost *et al.* 2009). Thus, a mechanism of template-directed misfolding of soluble tau through recruitment to an initial aggregated seed has been suggested to underlie the propagation of tauopathies. Exogenously supplied recombinant tau filaments can be internalized by cells and seed aggregation by recruitment of endogenous tau into misfolded conformations (Frost *et al.* 2009; Guo and Lee 2011; Wu *et al.* 2013). Newly misfolded tau was competent to initiate further aggregation and could transfer between co-cultured cells. The seed-dependent polymerization and transcellular propagation of tau was also demonstrated by over-expression of an aggregation-prone mutant in cells leading to amyloid filaments that were released directly into the extracellular space and subsequently taken up by co-cultured cells, inducing tau fibrillization.

The seeding and spreading of tau pathology via cell-to-cell transfer of aggregates was also demonstrated in mouse models *in vivo*. In an initial proof-of-principle study, brain homogenates of transgenic mice expressing mutant human tau with filamentous tauopathy were injected into mice

transgenic for human wild-type tau (Clavaguera *et al.* 2009). Upon inoculation, the originally inclusion-free mice developed filaments consisting of wild-type human tau in both neurons and oligodendrocytes. This induced tau pathology progressing over time from the injection site to neighboring and more distant, anatomically connected brain regions. Similar results were obtained using synthetic preformed tau fibrils to initiate extensive filamentous tau pathology in young asymptomatic mutant human tau over-expressing mice (Iba *et al.* 2013; Peeraer *et al.* 2015). Furthermore, isolated tau oligomers from AD patient brains were able to seed and propagate widespread amyloidogenic tau plaques composed of endogenous murine tau, upon injection into the hippocampus of normal C57BL/6 mice (Lasagna-Reeves *et al.* 2012).

The spreading ability of tau pathology was further confirmed by cell-specific expression of the human mutant tau in the entorhinal cortex. Several months after the appearance of the first tau inclusions in the transgene-expressing neurons, distant axonally connected cells in the hippocampus also developed filamentous tau pathology (de Calignon *et al.* 2012; Liu *et al.* 2012). Indeed, several studies have shown that induced tau pathology was able to propagate to anatomically connected brain regions indicating neuronal transport mechanisms and transsynaptic spread involved in the proliferation of neurofibrillary pathology (Iba *et al.* 2013; Ahmed *et al.* 2014; Dujardin *et al.* 2014). Neuronal activity can not only modulate tau protein secretion into the extracellular space in culture (Yamada *et al.* 2014), but it was further shown that synaptic contacts and neuronal activation can significantly potentiate neuron-to-neuron propagation of tau pathology (Calafate *et al.* 2015).

While the above *in vitro* and *in vivo* evidence strongly supports a template-dependent tau seeding and spreading mechanism, a recent study pointed to a significant deviation from the classical prion-like mechanism, by demonstrating that spreading of tau tangles does not depend on endogenous tau expression *in vivo* (Wegmann *et al.* 2015). Importantly, however, removing endogenous tau reduced neurotoxicity in this paradigm, potentially highlighting the fact that tau tangles do not equate the neurotoxic species. In agreement with this view, tau was shown to misfold into smaller aggregates prior to assembly into fibrils, and these smaller species, which are more efficiently taken up by neurons (Wu *et al.* 2013), may be the primary neurotoxic moiety.

The low complexity domains of TDP-43 and FUS mediate the formation of self-propagating, complex aggregates

Initial evidence suggests that a mechanism, similar to the spreading and seeding of tau, could underlie TDP-43 and FUS proteinopathies and several studies have demonstrated that both proteins are intrinsically aggregation-prone and can behave in a prion-like manner, both *in vitro* and in cell culture. *In vitro* studies using recombinant, aggregated TDP-43 (Furukawa *et al.* 2011) or FUS (Nomura *et al.* 2014) showed that minute amounts were sufficient to induce misfolding and aggregation of their corresponding natively folded protein, implicating template-dependent propagation. The strong tendency of TDP-43 and FUS to aggregate is mediated by the low complexity (Kato *et al.* 2012), or prion-like (Cushman *et al.* 2010) domain found in both proteins; the N-terminal part of FUS (amino acids 1-239) and the C-terminal part of TDP-43 (amino acids 274-414), which rank highly based on algorithms that identify aggregative and prion-forming propensities (Alberti *et al.* 2009; Toombs *et al.* 2010). FUS is characterized by an even higher aggregation propensity than TDP-43 and it was shown to spontaneously aggregate into pore-like oligomeric species that rapidly assemble into filamentous structures in a cell-free system within minutes (Couthouis *et al.* 2011; Sun *et al.* 2011). However, this capacity to aggregate is not enhanced by disease-linked mutations affecting the NLS (Ju *et al.* 2011; Sun *et al.* 2011).

Over-expression of TDP-43 prion-like domain, induced protein accumulation and cell toxicity (Furukawa *et al.* 2004; Johnson *et al.* 2009; Zhang *et al.* 2009; Liu-Yesucevitz *et al.* 2010; Guo *et al.* 2011; Pesiridis *et al.* 2011), whereas deletion of the same domain from TDP-43 (Johnson *et al.* 2009; Furukawa *et al.* 2011) or FUS (Sun *et al.* 2011) interfered with aggregate formation, indicating their requirement for efficient seeding. While short recombinant or synthetic peptides derived from the prion-like domain can acquire toxic amyloid forms, purified full-length TDP-43 does not appear to have a classic amyloid structure, but rather forms small pore-like oligomers and short fibrils (Johnson *et al.* 2009; Couthouis *et al.* 2011; Shimonaka *et al.* 2016), which can cluster together in large complexes with resemblance to pathological inclusions (Fang *et al.* 2014). Going a step further, it was demonstrated that aggregates reconstituted *in vitro* from recombinant wild-type or mutant TDP-43 or short synthetic peptides could seed aggregation of wild-type or endogenously expressed protein in cell culture (Furukawa *et al.* 2011; Shimonaka *et al.* 2016).

While this has not yet been shown for FUS, it was recently reported that co-expression of mutant and wild-type FUS induces aggregation of the otherwise soluble wild-type FUS (Nomura *et al.* 2014). In this study, a rather rare mutation located in the prion-like domain changed the aggregation propensity of FUS and led to intranuclear inclusions, similar to what can be found in cases of atypical FTLD-FUS (Neumann *et al.* 2009a; Nomura *et al.* 2014).

Supporting the prion paradigm and self-templating properties of TDP-43 further, aggregated TDP-43 isolated from ALS and FTD patient brains was able to act as a propagative seed and induce protein misfolding and accumulation in TDP-43 transfected cultured cells (Nonaka *et al.* 2013; Feiler *et al.* 2015). Moreover, the induced TDP-43 aggregates persisted within cells when the original pathogenic seed was

no longer present and could then act as new misfolding templates that exhibit properties consistent with prion-like propagation (Nonaka *et al.* 2013). Recent evidence furthermore suggests that oligomeric TDP-43 can be transmitted horizontally between cell somata likely via microvesicles, trigger oligomerization, and exert toxicity in recipient cells (Feiler *et al.* 2015). Neuron-to-neuron transmission experiments further indicated both anterograde and retrograde transsynaptic spreading of TDP-43 oligomers and provide additional evidence of the propagative properties of TDP-43 (Feiler *et al.* 2015).

To date, no report of prion-like propagation of TDP-43 or FUS in animal models could be found. However, a recent mouse model expressing a regulatable human TDP-43 construct lacking the NLS indicated that existing intracellular TDP-43 inclusions can be cleared *in vivo,* which can halt further neurodegeneration (Walker *et al.* 2015). Suppression of transgene expression after disease onset dramatically decreased the accumulation of insoluble, phosphorylated, cytoplasmic TDP-43, reversed the loss of endogenous nuclear mouse TDP-43 and prevented further motor neuron loss in this model (Walker *et al.* 2015). Similar results were obtained in a study using an inducible TDP-43 transgene with a point mutation where suppression of transgene expression in mice with overt neurodegeneration for only 1 week was sufficient to clear pathological TDP-43 and to significantly improve motor and behavioral deficits (Ke *et al.* 2015). This suggests that the presence of intracellular pathological TDP-43 at a certain point might not be sufficient for cell-to-cell spread and propagation of disease pathology without a continuous pool of cytoplasmic TDP-43. However, small quantities of transmitted pathological TDP-43 in patients could lead to disease amplification over extended time periods (Walker *et al.* 2015).

While the prion-like domains of TDP-43 and FUS have been incriminated for their aggregation propensity, other protein regions seem to be contributing to this behavior. The FUS prion-like domain alone is insufficient to cause aggregation, but requires the RNA-binding motif and a glycine-rich RGG domain (Sun *et al.* 2011). Intriguingly, this RGG domain contains a short low complexity region (amino acids 391–407), which might allow the protein to self-aggregate using its two prion-like domains (Gitler and Shorter 2011). Moreover, in contrast to most observations focusing on the C-terminal domain of TDP-43, two recent studies highlight the significance of the N-terminal region, which was reported to mediate RNA binding thereby ensuring proper protein function, such as splicing regulation, as well as promoting aggregation (Zhang *et al.* 2013; Qin *et al.* 2014). This unexpected function is facilitated by an unusual structural switch from a folded ubiquitin-like domain upon DNA binding in low protein concentrations to an unfolded and aggregation-prone structure with increasing protein concentrations (Qin *et al.* 2014).

Increase in local concentration and RNA association in stress granules may initiate TDP-43 and FUS aggregation

The mechanisms driving the onset of FTD and many other neurodegenerative disorders still remain unknown. While mutations in TDP-43 seem to facilitate protein aggregation in subcellular compartments, alternative etiologies may contribute to pathogenesis in the majority of tau-negative cases including sporadic forms of FTLD-TDP and familial forms with *GRN*, *C9ORF72*, or *VCP* mutations all leading to a similar TDP-43 pathology. Likewise, no genetic cause has been identified in aggressive forms of FTLD-FUS. Thus, attractive candidates to trigger disease are environmental stimuli, such as cellular stress and the implication of both TDP-43 (Colombrita *et al.* 2009; Moisse *et al.* 2009; Liu-Yesucevitz *et al.* 2010; McDonald *et al.* 2011) and FUS (Andersson *et al.* 2008; Dormann *et al.* 2010; Bentmann *et al.* 2012) in stress granule assembly offers a plausible mechanism for aggregate nucleation and thereby, disease initiation and possible seeding (Ito and Suzuki 2011).

Indeed, the formation of stress granules *per se* is mediated by the ordered aggregation of TIA-1, an essential stress granule component that possesses a prion-like domain, which is not only required for TIA-1′s nucleation, but can even be replaced by another prion-like domain from a yeast prion protein without affecting the size or number of stress granules (Gilks *et al.* 2004). Aggregated TIA-1 can then recruit mRNAs and other proteins, including TDP-43 (Colombrita *et al.* 2009; Liu-Yesucevitz *et al.* 2010; McDonald *et al.* 2011) and FUS (Andersson *et al.* 2008) into these cytoplasmic foci, which are transiently formed and resolved in a strictly regulated manner under physiological conditions (Buchan and Parker 2009; Wolozin 2012). However, since high protein concentration is a main determinant for protein aggregation, the increase in local TDP-43 and FUS concentration within stress granules could facilitate aggregation initiation. With the persistence of cellular stress during aging, these granules may act as precursors of pathologic inclusions, which may transform into irreversible aggregates and form seeds of aggregation (Polymenidou and Cleveland 2011; Maniecka and Polymenidou 2015). This mechanism may be further assisted by the association with RNA molecules that can act as scaffolds mediating the ordered aggregation of TDP-43 and FUS within these cytoplasmic granules. Indeed, the main RNA-binding region of FUS is the arginine-glycine-glycine zinc finger domain, which is most important for stress granule recruitment, while both RRM1 and the C-terminal glycine-rich domain of TDP-43 are required for SG localization (Bentmann *et al.* 2012). Furthermore, the scaffolding capacity of RNA has been shown in the *in vitro* aggregation of the mammalian prion protein (Deleault *et al.* 2003), as RNA and phospholipids were necessary to generate infectious prions with purified PrP (Wang *et al.* 2010). In addition, RNA was

shown to mediate the formation of fibril-like FUS assemblies *in vitro* (Schwartz *et al.* 2013).

After the initiation phase, another characteristic shared by TDP-43 and FUS could potentially contribute to their self-perpetuating aggregation. The levels of both proteins are strongly autoregulated through binding to their own mRNA transcripts and regulating the RNA processing pathways (Ayala *et al.* 2011; Polymenidou *et al.* 2011; Lagier-Tourenne *et al.* 2012; Zhou *et al.* 2013). The formation of pre-inclusions with sequestration of TDP-43 or FUS in the cytoplasm would cause a reduction in nuclear protein, which in turn would lead to increased levels of stable *TARDBP* or *FUS* transcripts. Consequently and enhanced by the additional cell stress because of the formation of pre-inclusions, TDP-43 or FUS protein levels rise providing abundant substrate for continuous seeded aggregation, and thus contributing to the growth of pathogenic deposits (Maniecka and Polymenidou 2015). Increased levels of TDP-43 mRNA measured in the brains of patients affected by various forms of FTLD (Mishra *et al.* 2007; Chen-Plotkin *et al.* 2008) suggests that such a feed-forward mechanism could enable and enhance aggregate propagation.

Interestingly, both TDP-43 and FUS were found to colocalize with stress granule proteins, including TIA-1 (Fujita *et al.* 2008; Liu-Yesucevitz *et al.* 2010), PABP-1 (Bentmann *et al.* 2012), and eIF3 (Liu-Yesucevitz *et al.* 2010) in ALS and FTD patients. Several stressors, such as oxidative, osmotic, heat shock, and ER stress were able to recapitulate the cytoplasmic redistribution and stress granule association of TDP-43 (Colombrita *et al.* 2009; Liu-Yesucevitz *et al.* 2010; Dewey *et al.* 2011; McDonald *et al.* 2011; Leggett *et al.* 2012). Importantly, some of these stress-induced granules turned into insoluble aggregates and were shown to persist after removal of the stressor (Dewey *et al.* 2011; Cohen *et al.* 2015; Feiler *et al.* 2015; Kabuta *et al.* 2015). Importantly, recent elegant biophysical studies (Burke *et al.* 2015; Molliex *et al.* 2015; Patel *et al.* 2015) support the hypothesis that soluble and dynamic stress granules can transform into insoluble and irreversible aggregates *in vitro*. Indeed, FUS and other RNA-binding proteins containing low complexity domains can form dynamic liquid-like assemblies *in vitro* (Han *et al.* 2012; Kato *et al.* 2012; Burke *et al.* 2015; Molliex *et al.* 2015; Patel *et al.* 2015) that resemble flexible cellular stress granules. Over time these dynamic assemblies can mature into rigid, large protein structures, reminiscent to pathological protein aggregates seen in patients (Burke *et al.* 2015; Molliex *et al.* 2015; Patel *et al.* 2015). Taken together, evidence suggests that stress granules may initiate or facilitate TDP-43 and FUS seeding within the cytoplasm of diseased cells.

Notably, despite its unrelated structure and function, tau has also been shown to associate with stress granule proteins (Vanderweyde *et al.* 2012). Patients with FTLD-tau and AD show large amounts of stress granules that colocalize with tau tangles and induction of stress granules in cells overexpressing tau and TIA-1 appears to be able to induce tau pathology (Vanderweyde *et al.* 2012). Furthermore, tau binding on RNA was shown to promote its aggregation into paired helical filaments (Kampers *et al.* 1996). Thus, stress granule formation is implicated in all subtypes of FTD (Vanderweyde *et al.* 2012).

Molecular mechanisms of cell-to-cell spread: what is transmitted and how?

Pathways of transcellular transport include active and passive mechanisms of release and uptake

Cell-to-cell propagation requires specific transmission events and the mechanisms by which misfolded protein complexes spread from one neuron to the next have not been fully determined. The protein seed needs to be released from one cell and get internalized by another where it has to be able to convert endogenous monomers to a pathogenic conformation and thus amplify. The properties of the transmitted protein aggregates, as well as the exact mechanisms, which underlie transcellular spread are still largely unknown and highly debated. The latter may include exocytosis, vesicle mediated (exosomes or pre-synaptic vesicles) or passive release by degeneration of a cell. The receiving neuron might internalize the protein by macropinocytosis, receptor-mediated endocytosis, vesicle membrane fusion or direct penetration. Direct transfer could also happen via tunneling nanotubes (Fig. 2).

Although tau is predominantly an intracellular protein, multiple studies have shown that it is physiologically released into the extracellular space in cell culture and the mouse brain (Pooler *et al.* 2013b). It has been detected in the cerebrospinal fluid (CSF) and the brain interstitial fluid of wild-type and transgenic mice (Yamada *et al.* 2011) and both healthy and AD individuals (Arai *et al.* 1995). It is, however, still controversial, whether tau is released in a free soluble form or inside membrane vesicles. Several studies have detected tau in various kinds of vesicles including exosomes (Saman *et al.* 2012), ectosomes (Dujardin *et al.* 2014), microvesicles (Simon *et al.* 2012), and synaptic vesicles (Pooler *et al.* 2013a) in cultured cells and animal models. Consistent with these models, tau was also found in exosomes in the CSF of healthy controls and AD patients (Saman *et al.* 2012), however, no data are available on FTLD-tau patients. In addition, free tau seems to be released via unconventional secretory and vesicle independent mechanisms (Karch *et al.* 2012; Chai *et al.* 2012).

Increased levels of TDP-43 have been detected in the CSF of ALS patients (Kasai *et al.* 2009), with some full-length TDP-43 and TDP-43 CTFs being found in the exosomal fraction (Feneberg *et al.* 2014; Ding *et al.* 2015). Cell culture models also suggest the possible transfer of TDP-43 via exosomes (Nonaka *et al.* 2013) or microvesicles (Feiler

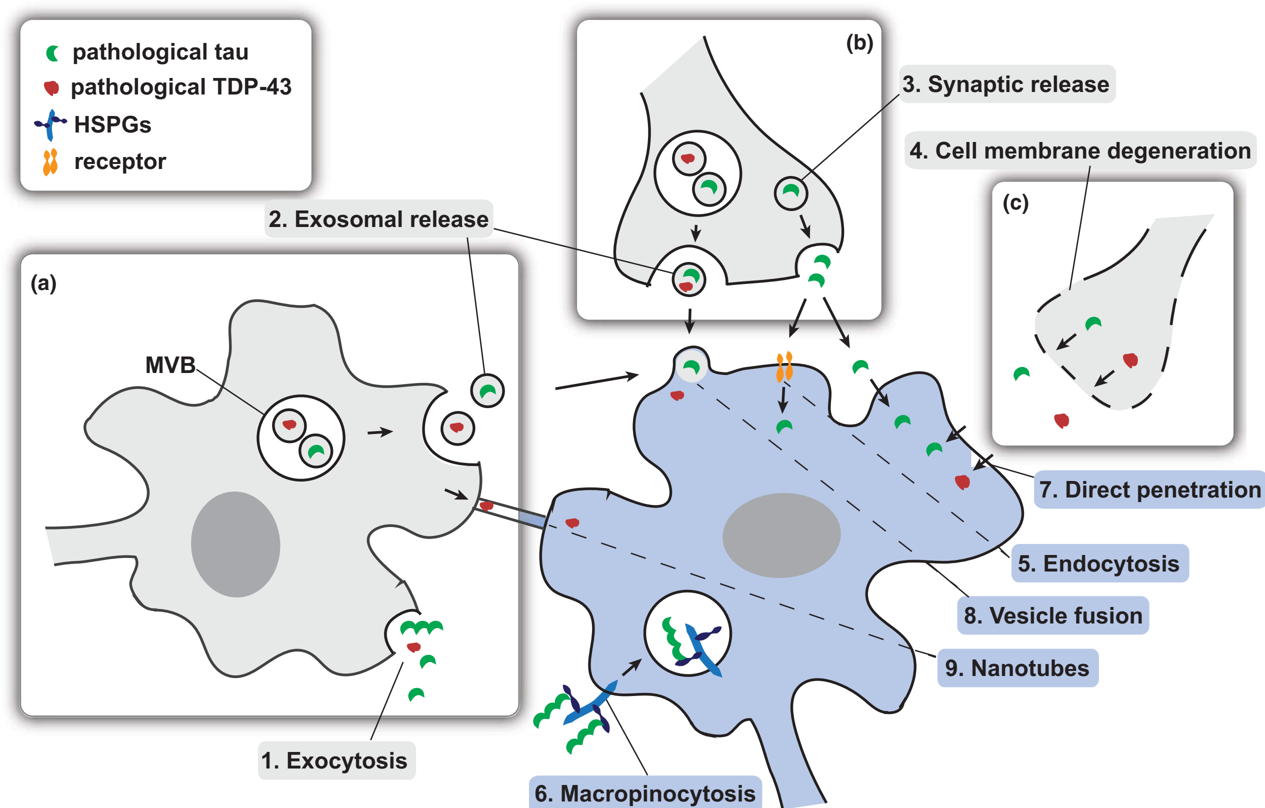

Fig. 2 Proximity-dependent and transsynaptic mechanisms of cell-to-cell spread. The transmission of pathological seeds (tau: green; TARDBP-binding protein, 43 kD (TDP-43): red) may happen between neighboring cells within the same brain region in a proximity-dependent pattern through active release (a) or passive leakage (c) whereas spreading between distant anatomically connected brain regions may rely on synaptic connectivity (b). The protein seeds may be released from the donor cell (gray) via exocytosis (1), packaged into vesicles such as exosomes (released from multivesicular bodies (MVBs)) (2) or dispensed by synaptic vesicles (3), with all these mechanisms having been suggested for tau and TDP-43. Furthermore, pre-synaptic membrane leakage because of the degeneration of neurons can lead to extracellular protein seeds (4). Once released, free protein seeds may be internalized by receptor-mediated endocytosis (5) or heparan sulfate proteoglycan (HSPG)-dependent macropinocytosis (6) or directly penetrate the recipient cell (blue) (7). Membrane-coated seeds can fuse with the receiving neuronal membrane (8). Direct transfer between two cells could also happen via tunneling nanotubes (9).

et al. 2015) and tunneling nanotubes (Ding *et al.* 2015). While many of the above mechanisms may also apply to FUS, there is currently no experimental evidence on the extent and mode or release and uptake of FUS aggregates by cells.

Extracellularly released protein needs to be taken up by surrounding cells. Macropinocytosis, as a form of fluid-phase uptake, has been proposed as a mechanism for several extracellular aggregates including tau (Frost *et al.* 2009; Holmes *et al.* 2013). This process relies on heparan sulfate proteoglycans (HSPGs) as membrane receptors for binding, internalization, seeding, and transcellular propagation of tau (Holmes *et al.* 2013). While this process involves direct binding of free tau seeds, a new study suggests that HSPGs can also facilitate the internalization of exosomes (Christianson *et al.* 2013), and might thus offer penetration and propagation mechanisms for many other aggregated protein species. Intriguingly, TDP-43 seems to be much more efficiently taken up when packaged into microvesicles/exosomes in cultured cells and primary neurons (Feiler *et al.* 2015). FTD-associated proteins might take advantage of several of these mechanisms simultaneously to spread between neighboring cell bodies or synaptically connected neurons.

The importance of transcellular spreading via release and reuptake in disease propagation and pathology has been demonstrated using extracellular anti-tau antibodies. Several antibodies have been shown to prevent tau spreading in cell culture (Kfoury *et al.* 2012), as well as in mouse models (Chai *et al.* 2011; Yanamandra *et al.* 2013, 2015) by blocking uptake of certain types of tau fibrils and promoting clearance by microglia (Funk *et al.* 2015). These studies strongly support the beneficial role of tau immunotherapy as it leads to reduced tau pathology, decreased seeding activity, as well as improved cognitive deficits in animal models (Boutajangout *et al.* 2011; Chai *et al.* 2011; Yanamandra *et al.* 2013, 2015).

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While promoting microglial aggregate uptake and clearance decreased tau pathology in some studies, microglia have also been found to promote propagation and disease progression by phagocytosing and secreting tau protein in exosomes (Asai *et al.* 2015). Depleting microglia or inhibiting exosome synthesis was shown to significantly reduce tau propagation *in vitro* and *in vivo* (Asai *et al.* 2015).

Properties of transporting pathologic species are elusive

However, it is still unclear which protein species – monomer, oligomer, or aggregate – can efficiently spread and is responsible for transmitting pathology. Most extracellular tau seems to be monomeric and while a recent study suggests that trimers are the minimal unit of propagation leaving monomers unlikely to spread pathology as they are not spontaneously internalized (Mirbaha *et al.* 2015), another group showed that monomeric tau is sufficient to initiate the nucleation of aggregate seeds in an HSPG-independent manner (Michel *et al.* 2014). Further studies, however, found no seeding activity for monomeric tau and small oligomers, but showed the requirement for short fibrils (Jackson *et al.* 2016) or even very large, high molecular weight species to transfer pathology efficiently (Takeda *et al.* 2015).

The properties of the propagation efficient TDP-43 particles are even less defined. Recent evidence suggests that TDP-43 can be transmitted horizontally in a dimeric/oligomeric form using the microsome/exosome pathway (Feiler *et al.* 2015). However, in this setting, TDP-43 dimers/oligomers might represent a physiological state, different from the small pore-like oligomeric structures found in transgenic mice and FTD patients (Fang *et al.* 2014). Furthermore, it has not been explored if TDP-43 particles are released in a complex bound to nucleic acids or interacting proteins. This would pose an interesting question with regard to uptake mechanisms and further oligomerization and seeding properties in the recipient cell.

Neurodegenerative changes gradually intensify from most to less affected regions of the human nervous system

Disease staging studies and implications for disease progression

Intriguingly, many neurodegenerative diseases not only share the general pathology of misfolded protein aggregates, but also clinically present a characteristic progressive nature. Progressing disease symptoms have therefore been hypothesized to coincide with the propagation of pathology across anatomically connected regions of the nervous system. Indeed, neuropathological studies have recently recognized that characteristic anatomical patterns can be found in various neurodegenerative diseases and that the progression of these stereotypical patterns is linked to an increasing severity of the disease phenotype (Brettschneider *et al.* 2015). Staging studies could show that pathological events initiate very early in a certain CNS area and progress sequentially in a topographically predicted manner through anatomical connections.

Early staging attempts of disease progression were based on clinical signs and brain atrophy (Broe *et al.* 2003; Mioshi *et al.* 2010; Kril and Halliday 2011). One of the best described cases of progressive neurodegenerative changes is ALS, where motor neuron loss was shown to initiate focally and progress gradually away from this initial focus, which generally coincides with the spinal cord level that defines the original symptoms (Ravits *et al.* 2007a,b; Ravits and La Spada 2009; Ravits 2014). Later efforts concentrated on analyzing aggregate size and number of affected cells with pathological inclusions as molecular correlates of the systematic symptom spread. Indeed, a gradual distribution of pathological TDP-43 aggregates was found in ALS and FTD patients throughout the neuraxis (Geser *et al.* 2009). Furthermore, a four stage model describing the sequential spread of TDP-43 pathology in ALS was proposed based on the progressive dissemination of intraneuronal phospho-TDP-43 positive aggregates. According to this model, ALS starts in the cerebral neocortex and the somatomotor neurons of the spinal cord and the lower brainstem and spreads in an anterograde manner along corticofugal axonal pathways to connected subcortical areas and distant CNS sites (Braak *et al.* 2013; Brettschneider *et al.* 2013). Similarly, a recent study focused on phospho-TDP-43 positive inclusions in bvFTD suggests a sequential spreading pattern along a fronto-occipital gradient with the first lesions starting in orbitofrontal areas and the amygdala, followed by lesions appearing later in premotor, primary motor, parietal and, finally, occipital areas of the cortex (Brettschneider *et al.* 2014).

While, the expansion and stereotypical spread of tau pathology across the nervous system have been clearly characterized in AD (Braak and Braak 1995; Braak *et al.* 2006) and chronic traumatic encephalopathy (Braak and Braak 1995; Geddes *et al.* 1999; Braak *et al.* 2006; McKee *et al.* 2013), one recent study also relates the neuropathological progression of tauopathy with clinical symptoms of bvFTD. Spreading sequentially from the frontotemporal and neocortical regions to the subcortical structures, the primary motor cortex and finally the visual cortex tau pathology reflected the evolution of clinical symptoms and directly correlated with disease duration (Irwin *et al.* 2016).

Collectively, the above data support the spatiotemporal spread of pathologic aggregates throughout the nervous system, although the alternative hypothesis of a temporally selective regional vulnerability of neurons to lesion formation (Walsh and Selkoe 2016) has not been formally excluded.

Protein aggregates preferentially spread across neuronal networks in FTD

More and more evidence supports the idea that neuronal connectivity defines disease-spreading pathways and that pathology is transmitted along neural networks through propagation of self-templating misfolded protein aggregates. After disease origination at an initial focal point, anatomically neighboring regions may lie at risk of involvement through proximity-dependent transfer of released pathological seeds. However, recent imaging studies relating degeneration patterns to neural connectivity suggest a 'network-based neurodegeneration' hypothesis supporting the model of transsynaptic propagation of toxicity along functionally and anatomically connected networks (Raj *et al.* 2012; Zhou *et al.* 2012; Agosta *et al.* 2015). For each disease type specific neural networks emerged with their critical center areas and functional and anatomical connectivity profiles resembling disease-associated atrophy patterns. Modeling analysis thus supports a pathology model of transneuronal spread based on connectional strength that defines disease vulnerability (Zhou *et al.* 2012).

In bvFTD progressive atrophy patterns were found to resemble a network called the salience network. The latter, which includes the anterior cingulate cortex and frontoinsula, as well as the amygdala and striatum (Rosen *et al.* 2002; Broe *et al.* 2003; Seeley 2008), is important for socioemotional tasks relying on attentional selection and self-regulation of behavior (Seeley *et al.* 2007). bvFTD patients exhibit reduced connectivity in the salience network, which has been correlated with clinical severity, apathy, and disinhibition scores (Zhou *et al.* 2010; Filippi *et al.* 2013). Furthermore, changes in the white matter tracts connecting key regions of the salience network reflect the propagation of pathology and have been proposed as a marker to assess *in vivo* spreading of pathological proteins (Agosta *et al.* 2012; Lam *et al.* 2014).

In the context of cell-to-cell propagation of toxic seeds, the network hypothesis offers a plausible route for the pathological spread that defines clinical presentation. The original site of pathology may determine the network involved and eventually the clinical phenotype (Sanders *et al.* 2016). Inoculation studies (Iba *et al.* 2013), as well as region-selective expression of mutant tau (de Calignon *et al.* 2012; Liu *et al.* 2012) in mice strongly support the selective spreading of pathology along known anatomical networks, Indeed, pathology in the entorhinal cortex was induced by tau pathology initiated in the hippocampus, while injection into the striatum led to pathology in the substantia nigra and the thalamus (de Calignon *et al.* 2012; Liu *et al.* 2012; Iba *et al.* 2013).

Multiple conformations of pathogenic protein assemblies may contribute to disease heterogeneity

Aside from the network-specific involvement, the molecular principles of the prion paradigm may also offer an explanation for the high variability in pathology and clinical presentation in neurodegenerative diseases. Indeed, the concept of strains is well established for prion diseases, where several distinct self-propagating conformers or strains underlie different pathological manifestations and disease phenotypes in humans and mice (Aguzzi *et al.* 2007). Recently, other neurodegenerative disease-associated proteins are also revealing strain-like properties including α-synuclein (Guo *et al.* 2013) and Aβ (Petkova *et al.* 2005; Heilbronner *et al.* 2013). Strain manifestation seems particularly plausible for tau, given the variety of clinical and neuropathological characteristics in tauopathies with differences in regional involvement, disease duration, age of onset, isoform expression, and fibril morphology.

Indeed, seeded aggregation of filaments consisting of either 3R or 4R isoforms was only successful in cells expressing the same isoform suggesting specificity in the assembly of amyloid fibers (Nonaka *et al.* 2010). Furthermore, two studies have recently shown that tau can adopt different stable conformational states that can propagate through recruitment of native tau. Brain extracts from patients with different tauopathies were able to induce differential patterns of spread and tau deposition in mice (Sanders *et al.* 2014; Boluda *et al.* 2015). Similarly, inoculation with distinct tau strains produced *in vitro* result in unique and consistent pathologies upon serial passages in mice (Sanders *et al.* 2014), thus showing stable conformational integrity analogous to prion strains. In addition, a recent biochemical analysis of pathological tau aggregates from patient brains found distinct C-terminal band patterns indicating disease specific conformations (Taniguchi-Watanabe *et al.* 2016).

Similarly, distinct patterns of TDP-43 pathology have been described. FTLD-TDP can be pathologically divided into four subtypes (A–D), based on the predominant form of inclusions found and each type is associated mostly with a certain disease phenotype and often a mutation in a specific FTLD-TDP related gene (Mackenzie *et al.* 2011). For example, type A is defined by large amounts of short dystrophic neurites and oval-shaped neuronal cytoplasmic inclusions and associated with bvFTD and mutations in the progranulin gene. Some evidence suggests that distinct structural conformations of TDP-43 might underlie these pathological subtypes (Tsuji *et al.* 2012). Different mutations or post-translational modifications could introduce variations in the protein fold that would further lead to differences in the misfolded conformers. Distinct pathogenic templates could have unique characteristics leading to cell or region-specific preferences, varying propagation, and seeding properties, and different levels of toxicity (Smethurst *et al.* 2015). Indeed, trypsin and chymotrypsin digestion of sarkosyl-insoluble TDP-43 inclusions from patients produced specific protease-resistant TDP-43 fragments leading to a distinct banding pattern for subtypes A-C (Hasegawa *et al.* 2011; Tsuji *et al.* 2012). Moreover, seeding aggregation with *in vitro* aggregated synthetic TDP-43 peptides (Shimonaka *et al.* 2016)

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as well as homogenates from ALS and FTD patients (Nonaka *et al.* 2013) in cell culture models resulted in various types of pathologies showing the same distinct biochemical characteristics as observed in the original seeds. Interestingly, seeded aggregation required the interaction of the peptide seed with the identical peptide sequence of the host protein (Shimonaka *et al.* 2016). This indicates, that conformation-specific templating of TDP-43 can lead to differentially misfolded conformers, which furthermore can cause different patterns of pathology and disease phenotypes in a strain-like manner.

In conclusion, prion-like phenomena may well explain the heterogeneity in clinical and pathological presentation of FTD, as well as the molecular basis of disease progression and spread in the nervous system. Further studies are needed to decipher the particulars of this intriguing pathogenic pathway.

Conclusions and open questions

In conclusion, multiple lines of evidence support a template-directed misfolding and propagation of pathology of FTD-associated proteins, although the state of research varies for each protein. Indeed, more evidence has accumulated over the years for the propagation of tau, whose involvement in neurodegeneration dates back to almost 20 years now (Hutton *et al.* 1998), while much less is known for the more recently identified TDP-43 (Neumann *et al.* 2006) and FUS (Kwiatkowski *et al.* 2009; Vance *et al.* 2009).

Several intriguing questions remain unanswered. Here, we list some of those and propose potential experimental approaches and key considerations when attempting to address them.

What are the exact properties of the pathologic state? Which of those define propagation and/or neurotoxicity?

Defining structural properties of protein aggregates remains a technologically challenging task, and few techniques can reliably assess them. One of them is solid-state NMR, which was used to define the beta-sheet core of aggregated tau (Daebel *et al.* 2012), albeit no such studies have been reported for TDP-43 or FUS, so far. Other techniques focusing on the biophysical properties of protein assemblies that have been used for prion particles (Laferriere *et al*. 2013), or more recent assays based on detection of conformational protein changes via mass spectrometry (Feng *et al.* 2014) may shed light to these critical questions.

What is the role of post-translational modifications in aggregation, seeding, and toxicity?

All FTD-associated proteins can be distinguished from their native counterparts via specific post-translational modifications (Fig. 1), which serve as markers for the detection of the pathologic state in patient tissues. Yet the exact role of these modifications in aggregation initiation seeding, spreading, and toxicity remains unclear.

Can patient-derived or *in vitro* formed TDP-43 and/or FUS aggregates trigger pathology and disease in experimental animals?

Understanding the biophysical and structural features of patient-derived aggregates is key for addressing this important question. A potential challenge here is that these aggregates most likely do not form beta-sheet conformations in patients, so their mode of propagation may be entirely different from prions and other classical amyloids. When attempting *in vivo* inoculation experiments, one must confirm the preservation (in the case of human brain-derived material) or faithful reconstitution (when using *in vitro* formed aggregates) of authentic, patient-like pathologic conformation. The potential sequestration of RNA or other proteins within these pathologic complexes may further complicate the issue.

Do any of these phenomena apply to dipeptide repeat proteins accumulating in C9orf72 FTD?

While it is conceivable that dipeptide repeat proteins (Mori *et al.* 2013), which originate from repeat-associated non-ATG translation (Zu *et al.* 2011) specifically in C9orf72 hexanucleotide repeat expansion carriers (DeJesus-Hernandez *et al.* 2011; Renton *et al.* 2011) may propagate and spread throughout the brain, no experimental evidence exists currently to support this. Similar to the other FTD-associated proteins, dipeptide repeat proteins are forming insoluble aggregates, which are neurotoxic in experimental models (Mizielinska *et al.* 2014). Yet, they are conceptually distinct from tau, TDP-43 and FUS, because of the lack of known native, stable protein isoform, which would supply a constant source of substrate, necessary for seeding and amplification. This resembles the case of polyglutamine and other repeat expansion diseases, which intriguingly were shown to transport between neurons (Pecho-Vrieseling *et al.* 2014; Brahic *et al.* 2016) and from glia to neurons (Pearce *et al.* 2015) in a prion-like manner.

Can we capture the propagating entity to stall disease progression in patients?

This is probably the most important question from the viewpoint of FTD patients. Indeed, a major implication of the spatiotemporal disease spread is the potential existence of an extracellular 'propagon' that could be therapeutically targeted and neutralized. Antibodies are excellent candidates for this purpose and tau immunotherapy has been explored with promising results (Funk *et al.* 2015; Sigurdsson 2016). Similar approaches may prove effective for TDP-43 and FUS proteinopathies in the future.

Acknowledgements and conflict of interest disclosure

The authors are grateful to Polymenidou laboratory members for critical comments and suggestions. MP has been awarded a Swiss National Science Foundation Professorship and a Career

Terminology

Considering the growing amount of evidence, it has become clear that the concept of prion-like processes has extended into many other neurodegenerative diseases requiring a clarification and expansion of the terminology that describe the underlying phenomena. However, while pathological aggregates propagating in other neurodegenerative diseases fundamentally share the molecular mechanisms underlying the replication of infectious prions, there is one substantial difference, namely inter-organism transmission, which to date remains exclusive to prion diseases. This critical factor has led to some discrepancy in the terminology within the scientific community.

Some definitions are focused on the shared molecular actions, meaning the ability of a protein assembly to relay a specific stable conformation via template-directed amplification and propose to expand the term prion to non-infectious protein assemblies (Sanders *et al.* 2016). This seems counterintuitive, especially since the term prion was denoted as 'proteinaceous infectious agent' (Prusiner 1982). Focusing on the same underlying principles, but acknowledging the discrepancy of the term 'infectious', others suggest to change the definition broadly to 'proteinaceous nucleating particle' (Walker and Jucker 2015) or establish the term prionoid (Aguzzi 2009) to differentiate the pathological proteins causative for non-infectious diseases from bona fide infectious prions and additionally to avoid public health concerns (Brettschneider *et al.* 2015). Similarly, the term propagon was proposed recently for all misfolded proteins that can initiate misfolding and aggregation of native proteins and thus propagate spreading of pathological conformations, to avoid confusion and be able to specify further (e.g., molecular propagon, tissue propagon) (Eisele and Duyckaerts 2016; Uchihara *et al.* 2016). In the absence of a unifying definition, in this review the self-perpetuating propagation of misfolding and aggregation, resembling that of prion amplification, is discussed as prion-like mechanism.

The template-dependent and self-perpetuating conversion of large amounts of natively folded protein into a pathological conformation by minute quantities of misfolded aggregates is termed seeding. While this expression is mostly used in *in vitro* assays using recombinant protein, it could also describe the early molecular events happening in a cell. Nucleation defines the initial phase of this conversion process leading to the formation of a highly ordered 'nucleus' or 'seed of aggregation'. The mechanisms of how pathological aggregates propagate within one organism using cellular connections, exo/endocytosis mechanisms and possibly others is not yet completely understood and often called cell-to-cell spreading.

Development Award from the Human Frontier Science Program. This work was supported by the Clinical Research Priority Program Small RNAs of the University of Zurich and the NCCR RNA&Disease. MP and E-MH are grateful for receiving a PhD Grant from the Neuroscience Center Zurich. The authors have no conflict of interest to declare.

All experiments were conducted in compliance with the ARRIVE guidelines.

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