Neurobiology of Disease 77 (2015) 257–265

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

From molecule to molecule and cell to cell: Prion-like mechanisms in amyotrophic lateral sclerosis

Leslie I. Grad, Sarah M. Fernando, Neil R. Cashman *

Department of Medicine (Neurology), Brain Research Centre, University of British Columbia, 2211 Wesbrook Mall, Vancouver BC, Canada, V6T 2B5

ARTICLE INFO

Article history:
Received 6 January 2014
Revised 13 August 2014
Accepted 9 February 2015
Available online 17 February 2015

Keywords:
Amyotrophic lateral sclerosis
Propagated protein misfolding
Protein aggregation
Prion-like
Intercellular transmission
Exosomes
Superoxide dismutase 1 (SOD1)
TAR DNA-binding protein 43 (TDP-43)

ABSTRACT

Prions, self-proliferating infectious agents consisting of misfolded protein, are most often associated with aggressive neurodegenerative diseases in animals and humans. Akin to the contiguous spread of a living pathogen, the prion paradigm provides a mechanism by which a mutant or wild-type misfolded protein can dominate pathogenesis through self-propagating protein misfolding, and subsequently spread from region to region through the central nervous system. The prion diseases, along with more common neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and the tauopathies belong to a larger group of protein misfolding disorders termed proteinopathies that feature aberrant misfolding and aggregation of specific proteins. Amyotrophic lateral sclerosis (ALS), a lethal disease characterized by progressive degeneration of motor neurons is currently understood as a classical proteinopathy; the disease is typified by the formation of inclusions consisting of aggregated protein within motor neurons that contribute to neurotoxicity. It is well established that misfolded/aggregated proteins such as SOD1 and TDP-43 contribute to the toxicity of motor neurons and play a prominent role in the pathology of ALS. Recent work has identified propagated protein misfolding properties in both mutant and wild-type SOD1, and to a lesser extent TDP-43, which may provide the molecular basis for the clinically observed contiguous spread of the disease through the neuroaxis. In this review we examine the current state of knowledge regarding the prion-like properties of proteins associated with ALS pathology as well as their possible mechanisms of transmission.

Contents

Introduction

The identification of prions, or infectious proteins, over thirty years ago (Diener et al., 1982; Prusiner, 1982) as the transmissible agent responsible for a myriad of brain wasting diseases such as scrapie in

* Corresponding author.
E-mail address: neil.cashman@vch.ca (N.R. Cashman).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.02.009
0969-9961/

sheep, bovine spongiform encephalopathy (BSE), also known as mad cow disease in cattle, and the devastating human diseases kuru and Creutzfeldt–Jakob disease (CJD), among others was a landmark development in the fields of infectious disease and protein biochemistry. The transmission of disease between organisms without the requirement of nucleic acid was deemed heretical for its time, and even now the concept faces occasional bouts of controversy. Since then, the singularity of pathological mammalian prion protein (PrP) as the sole example of a true infectious protein has come under constant challenge; from earlier studies in yeast (Patino et al., 1996) and other fungi (Uptain and Lindquist, 2002) to more recent work in human neurodegenerative diseases (Aguzzi, 2009; Guest et al., 2011). As such, aberrant protein misfolding and aggregation are a part of the pathobiology of numerous relatively common neurodegenerative disorders including Parkinson's disease (PD), the tauopathies and Alzheimer's disease (AD), known collectively as proteinopathies. Specific proteins in these diseases have been identified that appear to emulate the propagating protein mechanism of prions: amyloid-β (Aβ) in AD (Eisele et al., 2010; Meyer-Luehmann et al., 2006), α-synuclein in PD (Luk et al., 2012, 2009), and tau protein in AD, frontotemporal dementia (FTD) and other dementias (Clavaguera et al., 2009; Frost et al., 2009; Guo and Lee, 2011).

The level of experimental evidence describing the prion-like activities implicated in these diseases varies considerably. While evidence of a prion-like mechanism in AD and PD has populated the literature for over a decade, a relative newcomer to the prion paradigm is amyotrophic lateral sclerosis (ALS). When one examines the clinical pathology and epidemiology of ALS, its inclusion into the family of prion-like proteinopathies is quite reasonable. For example, the most significant clinical feature of ALS is its relentless, but contiguous spread through the neuroaxis; pathology starts at one or more focal points (Sekiguchi et al., 2014) of onset, possibly beginning in cortical neurons (Braak et al., 2013), and spreads in a spatiotemporal fashion through adjacent neuroanatomical regions (Ravits et al., 2007; Ravits and La Spada, 2009). Given the similarities in progression and anatomical spread among neurodegenerative proteinopathies, including the classical prion diseases, a likely mechanism to account for this observation that has gained traction in recent years is the region to region prion-like spread of propagated protein misfolding. In addition, this mechanism also accounts for how a mutant or even wild-type protein can dominate pathogenesis of a phenotypically diverse disease such as ALS, akin to when the normal cellular isoform of prion protein (PrP^{C}) converts to its pathologically dominant form PrP^{Sc} in prion disease. To date at least two proteins associated with the pathobiology of ALS have demonstrated multiple aspects of prion-like activity: SOD1 and TDP-43. This review attempts to summarize the known experimental evidence for prion-like activity of these proteins, and comment on their possible mechanisms of intercellular spread.

An introduction to ALS

ALS, also known as Lou Gehrig's disease in the United States, is a rapidly progressive fatal neuromuscular condition characterized by degeneration of the upper and lower motor neurons causing progressive paralysis and spasticity that affects the muscles of mobility, speech, swallowing and respiration (Bradley, 2009; Cleveland and Rothstein, 2001), although cognitive function is usually spared with only 5% of patients developing frank frontotemporal dementia (FTD) (Phukan et al., 2007). It is the most common form of motor neuron disease worldwide and has a global incidence of about two in 100,000. Half of affected individuals die within 3 years, and less than 20% survive for more than 5 years (Strong and Rosenfeld, 2003). The aetiology of ALS is unknown; however, similar to other neurodegenerative diseases such as AD and CJD, the disease can be divided into two categories, sporadic and familial. 90–95% of ALS cases are sporadic (SALS) where only some predisposing gene mutations have been identified, such as the ataxin-2 repeat expansions (Elden et al., 2010). The remainder of cases are familial (FALS) (Haverkamp et al., 1995), which are predominantly associated with Mendelian-inherited mutations in genes encoding Cu/Zn superoxide dismutase (SOD1), TAR-DNA binding protein 43 (TDP-43), fused in sarcoma/translocated in liposarcoma (FUS/TLS), and C9ORF72, but have been associated with mutations in other genes as well (ALS Mutation Database, 2007; Deng et al., 2011; Stewart et al., 2012; Wu et al., 2012). Clinically, both categories are very similar suggesting that a common downstream pathogenic mechanism, regardless of disease origin, may lie at the heart of the disease (Kabashi et al., 2007).

ALS is considered a protein misfolding disorder, based on its neuropathology, and as such is classified as a proteinopathy, similar to other neurodegenerative diseases. The post-mortem pathology of ALS patients typically features loss of motor neurons in the brain stem and ventral horn of the spinal cord accompanied by astrocyte activation and proliferation of microglia (Philips and Robberecht, 2011). Spinal cord histology often reveals abnormal accumulations of ubiquitinated proteinaceous inclusions in motor neurons and neural accessory cells, which are thought to be the result of the aggregation of misfolded protein. Protein misfolding can be triggered by a multitude of factors, including genetic mutation, oxidation, aberrant post-translational modification, dysfunctional proteostasis and seeded polymerization. In FALS cases where a SOD1 mutation is identified, the primary component of these protein inclusions is SOD1 itself (Kato et al., 2000). In sporadic cases where SOD1 mutations have been excluded there is recent evidence supporting the presence of misfolded SOD1-containing inclusions as well (Bosco et al., 2010; Forsberg et al., 2010; Grad et al., 2014; Pokrishevsky et al., 2012), although this observation is not one of consensus (Kerman et al., 2010). A more universally agreed observation is that TDP-43 is a primary component of cytosolic inclusions in the vast majority of SALS, in combination with its depletion from the nucleus, where the majority of native TDP-43 normally resides (Neumann et al., 2006).

Pathological SOD1 in ALS

Mutations in the gene encoding SOD1, a ubiquitously-expressed free-radical scavenging enzyme, were the first genetic cause of ALS to be identified (Rosen, 1993) and are implicated in ~20% of all FALS cases. The primary function of SOD1 is the conversion of the highly toxic free-radical superoxide to water and hydrogen peroxide (Beckman and Koppenol, 1996). To date, over 150 different disease-causing SOD1 mutations have been identified (ALS Mutation Database, 2007; Andersen et al., 2003). Despite the intrinsic stability of the native SOD1 enzyme, the majority of these mutations induce misfolding and subsequent aggregation. SOD1 aggregation occurs through a mechanism by which the highly-stable native SOD1 homodimer is disrupted producing misfolded monomer intermediates that can be incorporated into higher-order oligomeric structures (Rakhit et al., 2004, 2007). However, genetic mutation is not the only way to destabilize, misfold and aggregate SOD1. Aberrant oxidation or post-translational modification of wild-type (WT) SOD1 has been observed to mimic the aggregation-prone effects of mutant SOD1 *in vitro* (Casoni et al., 2005; Rakhit et al., 2004, 2002) in a concentration-dependent manner (Rakhit et al., 2004). There is increasing evidence that all types of ALS, including non-SOD1-linked familial and sporadic cases are associated with SOD1 misfolding, oxidation and aggregation (Matias-Guiu et al., 2008; Synofzik et al., 2012). Inclusions containing aggregated SOD1 have been detected in spinal cord tissues from both FALS and SALS patients (Chou et al., 1996a, 1996b; Shibata et al., 1994) in addition to biochemical, genetic and immunological evidence of misfolded SOD1 in cases of SOD1-excluded SALS (Bosco et al., 2010; Broom et al., 2008; Forsberg et al., 2010; Grad et al., 2014; Gruzman et al., 2007; Pokrishevsky et al., 2012). Misfolded SOD1 is therefore a prime

candidate as a common molecular determinant for all forms of ALS and may play a key role in disease pathogenesis.

Mutations in SOD1 can impair enzymatic function; however loss of superoxide dismutase function is not the cause of disease as SOD1 knockout mice do not develop an ALS-like motor neuron disease. Conversely, a mutant *SOD1* transgenic mouse developed a progressive motor neuron disease despite increased SOD1 enzymatic activity over and above the mouse endogenous protein (Gurney et al., 1994). Instead, misfolding of SOD1 conferred by mutation, oxidation or other cell stresses is generally thought to acquire a toxic gain of function (Andersen et al., 1995; Gurney et al., 1994; Reaume et al., 1996), although the precise nature of this toxicity and its specificity for motor neurons remain to be elucidated. The idea that misfolded/aggregated SOD1 directly contributes to neurotoxicity is lent credence by the observation that all transgenic mouse strains expressing ALS-causing SOD1 mutants develop large intracellular cytosolic inclusions in motor neurons and accessory neural cells that coincide with the onset of motor neuron disease and increase throughout the course of disease progression (Bruijn et al., 1997; Cleveland, 1999). Moreover, in its misfolded pathological form SOD1 can react non-specifically with a variety of substrates, causing it to actually become a net producer of reactive oxygen and nitrogen species (Said Ahmed et al., 2000), as opposed to a free-radical scavenger, which can subsequently lead to intracellular damage of cellular protein, lipid, and nucleic acid (Beckman et al., 1993, 2001). Toxicity of misfolded SOD1 has also been attributed to other deleterious cellular effects including: cytoskeletal and mitochondrial disruption, caspase activation, microglial activation, and proteasomal and autophagy pathway disruption (Bard et al., 2012; Bendotti et al., 2012; Cleveland and Liu, 2000).

Intermolecular conversion of SOD1

Central to the prion schema is the 'protein-only' hypothesis that stipulates that misfolded protein alone is necessary and sufficient to induce further misfolding in native protein, without the requirement of nucleic acids or other co-factors (Prusiner, 1982). That is to say, one molecule can literally impart its misfold on a neighbouring molecule. Although the depth of experimental evidence is not as established compared to the prion protein, an ever-growing body of evidence in the literature suggests that misfolded SOD1 has the ability to transfer conformational information from one molecule to another, meeting at least the intermolecular requirements of prion-like activity (Chia et al., 2010; Grad et al., 2011; Munch et al., 2011). SOD1, a small soluble ubiquitously-expressed 153-residue protein, exists as a homodimer in its normal enzymatically active form. The native holo-enzyme contains an intramolecular disulphide bond within each monomer, which contributes to its high conformational stability and resistance to proteolytic digestion (Ratovitski et al., 1999). Despite this, SOD1 is highly prone to destabilization when mutated or aberrantly oxidized. In its mutated or post-translationally modified state SOD1 has a high propensity to misfold and form multimeric species and aggregates. Under denaturing conditions wild-type and mutant forms of SOD1 can spontaneously form aggregates and fibrils *in vitro* (Chia et al., 2010), where the relative propensity for aggregation is dependent upon the SOD1 mutant variant (Prudencio et al., 2009b). Indications of *in vivo* SOD1 fibril formation have been observed in transgenic mice expressing mutant SOD1 (Furukawa et al., 2008; Wang et al., 2002). However, the formation of amyloid fibrils does not appear to be a consistent feature of SOD1 in human disease as SOD1-positive inclusions from FALS patients do not stain with amyloid-specific dyes (Kerman et al., 2010).

Intramolecular conversion of native WTSOD1 to a pathological form has been indirectly observed in a series of *in vivo* studies. Acceleration of motor neuron degeneration in mutant SOD1 mouse models is observed upon co-expression of human WTSOD1 (Jaarsma et al., 2000; Wang et al., 2009), possibly through intermolecular stabilization of mutant species via WTSOD1 interactions (Fukada et al., 2001) or via formation of non-native disulphide interactions that induce insoluble aggregate formation (Deng et al., 2006). Co-aggregation of mutant and WTSOD1 has also been detected in tissue derived from familial ALS patients (Bruijn et al., 1998), suggestive of intermolecular conversion in the pathology of the disease in patients. Direct evidence of intermolecular WTSOD1 conversion to a misfolded isoform has been observed in mesenchymal and neuron-like human cell lines (Grad et al., 2011; Pokrishevsky et al., 2012). Expression of misfolded human SOD1 mutants can convert endogenous WTSOD1 to a misfolded form, revealed by conformation-specific antibodies that recognize epitopes only accessible when the protein is in a misfolded state. The induced misfolding by mutant SOD1 results in enhanced protease sensitivity, which is interpreted as an indicator of global loosening of the polypeptide backbone. Conversion of WTSOD1 by a mutant conformer has also been shown to occur in a cell-free system, thus eliminating any extraneous protein, lipid or nucleic acid co-factors from the process (Grad et al., 2011) thus further supporting the 'protein-only' paradigm. Interestingly, intermolecular conversion of WTSOD1 by a misfolded template is a sequence- and/or structure-dependent process akin to the species barrier observed for classical prion diseases such as chronic wasting disease, a transmissible spongiform encephalopathy that is thus far restricted to certain cervid species (Raymond et al., 2000). Conversion of human WTSOD1 to a misfolded form is restricted to the exposure of a single amino acid residue, a tryptophan (Trp) at position 32 (Grad et al., 2011), indicating a potential point of contact between the converting and converted species that is separate from the dimer interface region, once thought as a more intuitive point of contact between SOD1 molecules. Trp32 is the only tryptophan residue present in the human WTSOD1 amino acid sequence and has previously been identified as a site prone to oxidative modification and a potentiator of aggregation (Taylor et al., 2007). SOD1 constructs featuring ALS-causing mutations, but with a serine substitution at position 32 failed to induce WTSOD1 misfolding in human cell lines (Grad et al., 2011). Furthermore, mouse endogenous SOD1, which inherently possesses a serine at position 32, is also inert to conformational conversion of human SOD1 *in vitro* (Grad et al., 2011). *In vivo*, human WTSOD1 does not accelerate motor neuron disease in mice expressing mutant murine SOD1 with a G86R substitution (Audet et al., 2010), analogous to the human G85R mutation linked to FALS. Furthermore, murine SOD1 is not incorporated into aggregates of human mutant SOD1 (Deng et al., 2006; Prudencio et al., 2009a; Wang et al., 2009). Given the collective evidence, SOD1 satisfies the intermolecular characteristics of prion-like protein conversion that adheres to sequence/structure specificity reminiscent of the species barrier observed for certain prion diseases.

Mechanisms of propagated SOD1 misfolding

Another key characteristic of the prion paradigm is the ability for newly misfolded protein substrate to provide new template for additional rounds of misfolding. Two mechanisms have been proposed to account for this conversion process: nucleated polymerization, in which the misfolded species is intrinsically less stable as a monomer but becomes more stable than its native counterpart when recruited to a multimeric aggregate; and template-directed misfolding, in which the misfolded pathological conformer is more stable than the native form but is kinetically inaccessible without catalysis by interaction with the misfolded form (Horwich and Weissman, 1997). Evidence for both template-directed misfolding and nucleated polymerization have been observed for SOD1 and suggests that propagated misfolding of both mutant and WTSOD1 spans a continuum between the two models. *In vitro* surface plasmon resonance experiments with natively folded WTSOD1 suggest that the intracellular conversion of WTSOD1 likely occurs in a template-directed fashion (Grad et al., 2011). Given the intrinsic stability of homodimeric native SOD1, and that co-expression of

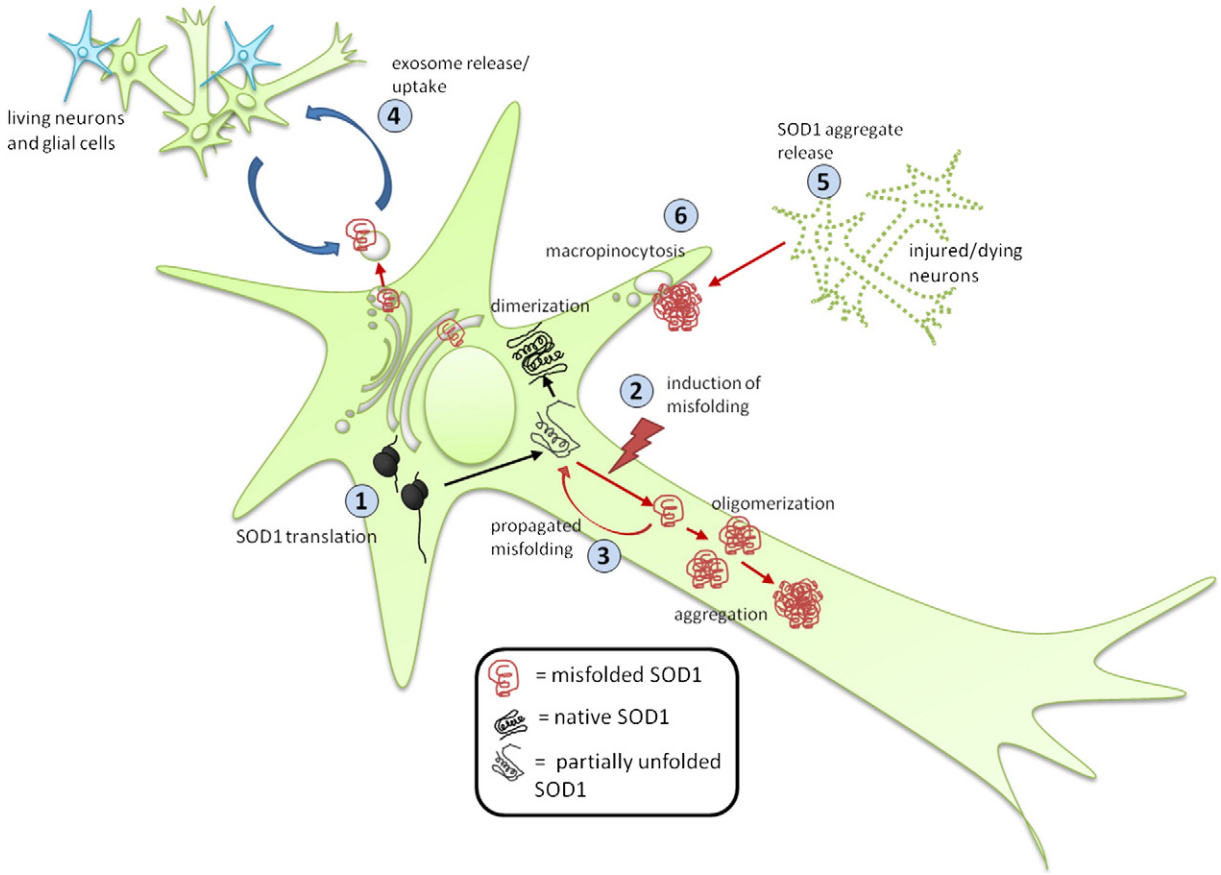

Fig. 1. Intermolecular conversion and intercellular transmission of SOD1. Native WTSOD1 or near-native SOD1 mutants are often highly protease resistant making their ability to misfold thermodynamically unfavourable. One explanation is that misfolded SOD1 seed utilizes post-translational intermediates of WT or mutant SOD1 that remain partially unfolded and susceptible to induced pathological misfolding (1). In addition, SOD1 misfolding can be induced by variety of intracellular and extracellular stresses (2). Once a misfolded template is present, it can induce subsequent cycles of propagated protein misfolding (3), converting neighbouring native SOD1 molecules into pathological isoforms, which can subsequently form oligomers and aggregates over time. Misfolded SOD1 can also accumulate in the ER–Golgi system where it can enter the vesicle-mediated secretory pathway, becoming selectively incorporated onto the outer surface of exosomes, exit the cell via secretion (4) and be taken up by neighbouring cells. Alternatively, during later stages of disease when neural cells are injured and dying, large proteinaceous aggregates containing misfolded SOD1 are released (5) and subsequently taken up by neighbouring cells via macropinocytosis (6).

WTSOD1 has been observed to stabilize mutant misfolded SOD1 (Prudencio et al., 2010; Witan et al., 2009), it is possible that nascent WTSOD1 polypeptide newly-emerged from the cell's translation machinery may provide a thermodynamically favourable substrate for seeded polymerization to occur (Fig. 1), although this remains to be experimentally verified.

Propagated misfolding between molecules of mutant SOD1, which are already prone to aggregate, likely use a combination of template-directed misfolding and seeded polymerization, depending on the intrinsic aggregation properties and structural stabilities of different mutants, which plays an essential role in disease progression and patient survival (Wang et al., 2008). Crystal structures from recombinant metal-deficient mutant SOD1 proteins reveal assemblies of aligned beta-sheets forming amyloid-like filaments and water-filled nanotubes that result in enzymatic dysfunction (Elam et al., 2003) suggesting that this conformational rearrangement in the metal-deficient enzyme could contribute to SOD1-linked FALS pathology. For nucleated polymerization to occur, a pre-formed high-order structure acts as a nucleus for soluble protein to come into contact with. The soluble protein subsequently becomes incorporated into the aggregate via conformational change. Recombinant apo-SOD1 without disulphide bonds, ALS mutant SOD1 protein or insoluble SOD1-containing aggregates isolated from transgenic mouse models of ALS expressing mutant SOD1 have all been shown to possess spontaneous fibrillization and seeding activity *in vitro* under various non-physiological conditions (Chia et al., 2010; Furukawa et al., 2008). Recent observations suggest that protein to protein conversion induced by both mutant (Munch et al., 2011) and over-expressed WTSOD1 (Grad et al., 2011) can be self-sustaining. Induced misfolding of SOD1 can persist even in the absence of the misfolded seed in cell culture, suggesting that newly misfolded SOD1 can act as template for subsequent cycles of misfolding (Grad et al., 2014). Exogenously-applied aggregated mutant human SOD1 can induce subsequent aggregation of soluble transgenically-expressed mutant SOD1 in mouse neuroblastoma cells (Munch et al., 2011). Similarly, exogenous fibrils of recombinant mutant human SOD1 can be taken up by mouse neuroblastoma cells expressing the same human SOD1 mutant and trigger intracellular aggregation (Furukawa et al., 2013).

Intercellular transmission of SOD1 misfolding

It is clear that regional spread of protein misfolding and aggregate formation is a prevalent feature of neurodegenerative disease, with the spread of misfolded protein seed playing the critical role of expanding the pathology beyond the site of original protein conversion. A misfolded protein that cannot escape the local cellular environment in which it was formed has no way to effectively transmit its misfold to other anatomical regions. Indeed, intercellular transmission of propagated misfolding and aggregation of pathogenic PrP^{Sc} is a hallmark of the prion diseases. As mentioned before, release and uptake of protein aggregates or proto-fibrils is one possible mechanism for intercellular spread in ALS, and likely occurs when dying neurons release their contents to the extracellular environment. For example, exogenous mutant (Munch et al., 2011) and WTSOD1 (Grad et al., 2014) aggregates have been shown to efficiently penetrate the cell membrane of neuron-like

cells in a macropinocytosis-dependent mechanism, and become self-perpetuating in recruiting soluble SOD1 into insoluble aggregates. Aggregates of misfolded SOD1 likely represent a conformationally and thermodynamically stable form (Guest et al., 2011) that can better survive the relative hostilities of the extracellular environment than lower-order soluble forms.

Although formation of large protein aggregates and fibrils is an observable pathological characteristic of ALS, there is a question as to whether these protein structures or other forms are responsible for neurotoxicity. In other neurodegenerative diseases, there is growing evidence pointing to *soluble* misfolded protein as the primary toxic species as opposed to large protein aggregates, inclusions or fibrils, which appear for the most part to be pathologically inert (Haass and Selkoe, 2007). In Alzheimer's disease, it is smaller oligomeric forms of amyloid-β that impair synaptic function (Kayed et al., 2003; Lesne et al., 2006), plasticity and memory (Shankar et al., 2008). Likewise, there is growing evidence that pre-fibrillar oligomers of α-synuclein are responsible for disease progression in Parkinson's disease (El-Agnaf et al., 2006; Lashuel et al., 2002) as opposed to the hallmark Lewy bodies, which may simply serve as benign "dumps" of aggregated protein. It is reasonable that a similar paradigm exists for SOD1 misfolding in ALS, which could explain why misfolded SOD1 is detectable in sporadic cases, but not the large protein inclusions usually observed in mutant SOD1-associated familial ALS (Bosco et al., 2010; Forsberg et al., 2010; Grad et al., 2014) insinuating that misfolded SOD1 in SALS is present as smaller soluble species not readily detectable by gross histochemical staining of tissue sections.

Before neuronal cell death occurs in ALS, pathology spreads from region to region in a spatiotemporal manner (Ravits and La Spada, 2009) suggesting an active cellular process that may be more relevant to the early stages of the disease. One mechanism by which this can occur is through small 30–80 nm-wide membrane-bounded vesicles, called exosomes, which normally transport various cargo such as proteins and nucleic acids between cells. Exosomes have been increasingly implicated in the spread of misfolded or aggregated proteins involved in neurodegenerative diseases, such as Alzheimer's disease (Perez-Gonzalez et al., 2012), Parkinson's disease (Danzer et al., 2012; Emmanouilidou et al., 2010) and the prion diseases (Coleman et al., 2012; Fevrier et al., 2004). The evidence supporting an analogous mechanism involved in ALS is ever-increasing. Exosomes are formed within intracellular endosomes by membrane invagination, resulting in the formation of multivesicular bodies (MVBs). MVBs are normally involved in the lysosome pathway, trafficking proteins to the lysosome for proteolysis. Alternatively, MVBs can fuse with the plasma membrane, leading to the release of its intraluminal vesicles into the extracellular milieu as exosomes (Keller et al., 2006). SOD1 itself is a known resident protein of exosomes from multiple cell types and species (Kim et al., 2013). Subsequent studies have identified exosomes as the secretion mechanism for both wild-type and mutant SOD1 in a motor neuron-like cell model (Gomes et al., 2007), potentially mediated by chromogranins (Urushitani et al., 2006), acidic components of neurotransmitter-enriched secretory vesicles (Huttner et al., 1991) that could serve as a secretory chaperone. Further evidence suggests that mutant SOD1 oligomers accumulate in the endoplasmic reticulum–Golgi compartments of the endocytic pathway prior to its subsequent secretion (Urushitani et al., 2008). More recent work has established that both mutant and wild-type misfolded SOD1 can be secreted from neuron-like cells via exosomes (Basso et al., 2013); these exosomes can then be subsequently taken up by fresh cells where the misfolded SOD1 cargo provides a template for subsequent induction of protein misfolding (Grad et al., 2014) (Fig. 1). Misfolded SOD1 is immunologically detectable on the outer leaflet of extracellular vesicle membranes (Grad et al., 2014) and is therefore accessible for targeting by therapeutic molecules in the extracellular milieu. More recently, mouse astrocyte-derived exosomes were observed to efficiently transfer mutant SOD1 to spinal neurons and subsequently cause selective motor neuron death (Basso et al.,

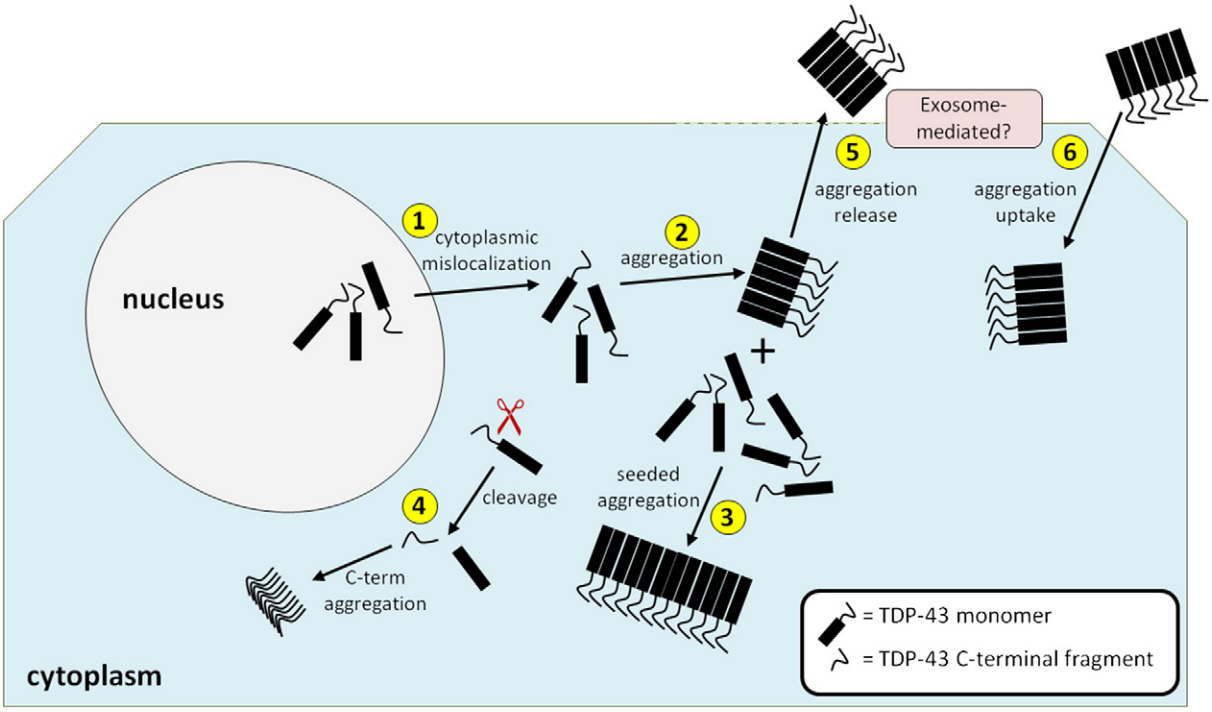

Fig. 2. Pathways of pathological TDP-43. TDP-43 translocation from the nucleus to the cytoplasm occurs either via mutation or cell stressors (1). Cytoplasmic TDP-43 has a propensity to aggregate (2) via its C-terminal fragment (CTF), forming nucleating seeds that recruit other soluble TDP-43 molecules into larger aggregates (3). Similarly, TDP-43 can be proteolytically cleaved, releasing its CTF, which can form aggregates with other CTFs via its prion-like domain, forming inclusions (4). Pathological TDP-43 is known to be released from cells, and has also been detected as a component of exosomes, implicating these vesicles as a mode of transport out of the cell (5). Conversely, TDP-43 aggregate uptake (6) has also been observed, but the mechanism by which it occurs has yet to be identified.

2013), providing further evidence that exosome-mediated transmission of misfolded SOD1 is pathogenic.

Misfolding of TDP-43 in neurodegenerative disease

TAR-DNA-binding protein of 43-kDa (TDP-43) is a ubiquitously-expressed 414 amino acid protein localized primarily to the nucleus, and was originally identified as a major component of the ubiquitin-positive neuronal and glial inclusions found in post-mortem FTD brains (Neumann et al., 2006; Sampathu et al., 2006). TDP-43 was first thought to be involved in early embryonic development as disruption of *TARDBP* expression is lethal at the embryonic stage (Sephton et al., 2010; Wu et al., 2010). However the protein is now known to be involved in gene regulation, and mRNA splicing and localization (Johnson et al., 2009). It is an established disease hallmark of not only FTD but of ALS where SOD1 mutations are excluded, providing a potential mechanistic link between the two disorders. In both FTD and ALS, TDP-43 is found mislocalized to the cytoplasm (Fig. 2), aggregated, hyperphosphorylated, and ubiquitinated within proteinaceous inclusions (Arai et al., 2006; Neumann et al., 2006). Further investigation into TDP-43 has determined that the protein is directly related to disease pathogenesis as opposed to simply being an end-stage marker of disease as multiple laboratories identified mutations in *TARDBP*, the gene encoding TDP-43, in both FALS and SALS patients (Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008; Yokoseki et al., 2008). However, the precise role of TDP-43 in disease pathology presently remains ambiguous; it is unclear whether pathology is caused by a toxic gain-of-function due to cytoplasmic mislocalization, loss-of-function due to its resultant nuclear depletion, or a combination thereof. The TDP-43 protein sequence consists of two RNA-recognition motifs and a C-terminal glycine-rich domain, cleaved fragments of which are found in ALS-associated aggregates (Neumann et al., 2006; Rutherford et al., 2008). The C-terminal region of TDP-43 contains a prion-like domain that is ranked highly compared to other mammalian proteins predicted to carry sequences based on algorithms that identify prion propensity (Alberti et al., 2009; Toombs et al., 2010). The C-terminal region of TDP-43, specifically amino acids 277–414, is predicted to be prone to misfolding and includes the glycine-rich domain proposed to be involved in protein–protein interactions (Mousavi and Hotta, 2005). Intriguingly, almost all of the *TARDBP* mutations identified in ALS are located in exon six encoding the C-terminal domain (Rutherford et al., 2008; Sreedharan et al., 2008) and result in autosomal dominant inheritance (Pesiridis et al., 2009). Yeast models of TDP-43 proteinopathy successfully recapitulate the cytoplasmic mislocalization and toxicity seen in disease. These models show that the prion-like domain of TDP-43 is essential for its aggregation and cellular toxicity, demonstrating a proposed role in human disease (Johnson et al., 2008). Other studies have shown that the prion-like domain is essential for normal protein function as deletion mutants fail to properly splice normal TDP-43 targets while substitution of a yeast prion domain into the C-terminus of TDP-43 successfully rescues function (Wang et al., 2012). On the basis of its C-terminal prion-like domain and the presence of C-terminal fragments within neuronal inclusions, TDP-43 clearly has strong potential for prion-like behaviour (summarized in Fig. 2).

Seeded aggregation of TDP-43

Studies into TDP-43 propagated misfolding provide evidence that the mechanism of seeded aggregation is most likely employed. Similar to some mutant SOD1 species, TDP-43 and its derived fragments have a high propensity to aggregate *in vitro* (Furukawa et al., 2011; Johnson et al., 2009) and mutations expressed within the protein can enhance this property (Guo et al., 2011; Johnson et al., 2009). As stated before, the C-terminal region of TDP-43 is necessary and sufficient for aggregation (Johnson et al., 2008, 2009). Remarkably, even truncation constructs featuring just the C-terminal portion of the protein are aggregation-competent *in vitro* and in cultured cells (Furukawa et al., 2011; Guo et al., 2011; Liu-Yesucevitz et al., 2010). Pathological TDP-43 is primarily comprised of aggregates of its C-terminal fragment (CTF) that can form detergent-insoluble (Arai et al., 2006) fibril-like structures (Hasegawa et al., 2008) localized to the cytoplasm (Fig. 2). However, recapitulation of detergent-insoluble fibrils of full-length TDP-43 or CTFs in a cell culture model system has been difficult to achieve without the presence of non-native epitope tags (Furukawa et al., 2011; Nonaka et al., 2009). Nevertheless, work by Furukawa *et al.* has demonstrated that exogenously applied detergent-insoluble aggregates of full-length TDP-43 generated *in vitro* can be taken up by human embryonic kidney cells, and act as seed for the aggregation of endogenous TDP-43 (Furukawa et al., 2011). These induced aggregates are detergent-insoluble and ubiquitinated, similar to that observed for pathological TDP-43 in ALS patients, suggesting that seeded aggregation of TDP-43 is an important aspect of disease pathology. Interestingly, seeded aggregation is a phenomenon observed for several proteinopathies, including the prion diseases (Aguzzi and Rajendran, 2009), Alzheimer's disease (Hardy and Selkoe, 2002; Meyer-Luehmann et al., 2006) and Parkinson's disease (Luk et al., 2009).

In further support of the prion paradigm, self-templating properties have also been observed for TDP-43. Aggregated TDP-43 isolated from the brains of ALS and frontotemporal lobar degeneration patients exogenously applied to cultured human neuroblastoma cells can serve as seed for the self-propagation of additional ubiquitinated and phosphorylated TDP-43 aggregates (Nonaka et al., 2013). In addition, Nonaka *et al.* found that the seeding ability of tissue-derived aggregated TDP-43 is resistant to heat, protease digestion and formic acid, similar to that found for pathological prion protein. However, it is unclear if induced aggregation persists even in the absence of the original templating seed, indicative of an authentic prion-like self-propagating mechanism. Most importantly, the induced intracellular aggregates of TDP-43 were toxic to cultured neuroblastoma cells, possibly via a mechanism involving proteasome dysfunction (Nonaka et al., 2013), establishing a solid link between propagated TDP-43 misfolding and disease pathology. It should be noted that possible cell-to-cell spread has also been observed for propagated TDP-43 aggregation as phosphorylated aggregates of TDP-43 expressed in cultured neuroblastomas have been shown to induce TDP-43 aggregation in cells that did not originally contain them (Nonaka et al., 2013). Furthermore, TDP-43 pathology in ALS is clinically observed to spread in a sequential pattern through the neuroaxis in agreement with the aforementioned cell biology data, and consistent with a requisite behaviour for the emerging prion paradigm for this protein (Brettschneider et al., 2013). It should be noted that exosome-mediated transmission may not be specific to just SOD1 in ALS. Possible evidence for exosome involvement in TDP-43 intercellular transmission has also been observed recently. Full-length TDP-43 is enriched in exosome fractions isolated from human neuroblastoma cells treated with seed from ALS patient extract (Nonaka et al., 2013).

Prion-like activity in other proteins associated with ALS

Since the initial discovery of TDP-43 involvement in neurodegenerative disease, a host of other RNA-binding proteins belonging to the heterogeneous ribonucleoprotein family and containing prion-like domains have been identified and linked to ALS pathobiology. Fused in sarcoma (FUS), Ewing sarcoma breakpoint region 1 (EWSR), and TATA-binding protein associated factor 15 (TAF15) are three such proteins which share similar domain architecture and together form the TET family of proteins (reviewed in (Tan and Manley, 2009)). FUS mutations causing ALS were first identified in 2009 and were shown to cause mislocalization of the protein from the nucleus to the cytoplasm and inclusion formation similar to TDP-43 (Kwiatkowski et al., 2009; Vance et al., 2009). Likewise, expression of mutant FUS in yeast causes

aggregation and cytotoxicity (Gitler and Shorter, 2011). Like TDP-43, FUS is an RNA-processing enzyme and contains a prion-like domain; however the FUS prion-like domain is located in the N-terminus of the protein. The minimal protein region of FUS required for aggregation is larger than in TDP-43; in FUS, the prion-like domain alone is insufficient to cause aggregation and requires the RNA-binding RRM and glycine-rich RGG domains (Sun et al., 2011). Interestingly, the RGG domain of FUS also contains a small prion-like region which opens up the possibility that FUS may self-aggregate using its two prion-like domains (Gitler and Shorter, 2011). FUS has different binding partners than TDP-43, and the two do not co-localize within pathological inclusions in the cytoplasmic (Huang et al., 2010; Sun et al., 2011) despite their structural, functional and pathological similarities suggesting that the two proteins are involved in pathology via distinct, albeit related, mechanisms. TAF15, which serves as a component of the scaffold for assembly of the RNA transcription complex, was identified in a systematic survey used to screen candidates similar to TDP-43 or FUS which could be involved in ALS (Couthouis et al., 2011). Like other TET family members, TAF15 has a QGSY-rich N-terminal region which is also predicted to be a prion-like domain (King et al., 2012). Recent work has demonstrated that TET members bind with each other via their N-terminal prion-like domains to form homo- and hetero-complexes, a process which appears to mediate stress granule formation in normal cells but can be dysregulated in disease, resulting in the cytoplasmic inclusions routinely observed (Thomsen et al., 2013). EWSR1 is an RNA-binding protein also identified in a screen for proteins with prion-like domains, and disease-causing mutations in EWSR1 were discovered in ALS patients (Couthouis et al., 2011). Further work is needed to determine whether TET family members propagate in a prion-like manner in ALS similar to SOD1 or TDP-43, and whether their prion-like domains play a role in this process. The above studies suggest that RNA-binding proteins normally self-associate through their prion-like domains, and that disease-causing mutations in these domains lead to aggregation, perhaps through unregulated polymerization with each other (Thomsen et al., 2013). However, evidence of self-propagation of protein misfolding or intercellular transmission of these proteins is yet to be presented.

Can ALS be considered a true prion disease?

The cell to cell transmission of propagated protein misfolding now appears to be a common mechanism in neurodegenerative diseases of aging; however, the question remains whether ALS or any of the other non-prion protein neurodegenerative diseases mentioned in this review can be truly considered as authentic prion diseases. One would argue that despite fulfilling certain required characteristics of pathological prion proteins, such as evidence of intermolecular protein recruitment and conversion, intercellular transmission, and neuroanatomical migration, SOD1 and other analogous prion-like proteins involved in neurodegenerative proteinopathies do not share key traits of the classical prion diseases, namely transmission between organisms (Guest et al., 2011) and the evidence of strains that vary in their infectivity and toxicity (Legname et al., 2005). Moreover, there is little evidence in the literature to date that resolutely confirms the protein-only hypothesis in any of the other non-classical prion diseases in a reductionist *in vitro* system; the lone exception may be for SOD1 as template-directed misfolding was observed in a completely cell-free environment using purified recombinant protein (Grad et al., 2011).

When it comes to organismal infectivity no other pathologically misfolded protein comes close to the virulence of PrP^{Sc} in prion disease. To date, there is no published experimental evidence demonstrating that any neurodegenerative proteinopathy, other than the classical prion diseases, can spread from individual to individual in humans or experimental model systems via natural infection pathways such as oral or intravenous inoculation. A recent study examining the use of human growth hormone derived from the cadavers of AD and PD patients reaffirms that person-to-person transmissibility for Alzheimer's and Parkinson's diseases is highly unlikely to occur (Irwin et al., 2013). However, one cannot ignore the more demonstrative observations of facilitated trans-organism disease transmission when intraperitoneal inoculation of misfolded amyloid-beta-enriched brain extract from Alzheimer's patients into transgenic mice induces amyloidosis in the brain, a region considerably non-local to the site of injection (Eisele et al., 2010). It is therefore likely that the point of entry of other prion-like agents is crucial for disease propagation, indicating a limited mode of transmission compared to PrP^{Sc}, the prototypical highly-infectious prion. Sensitivity to the extracellular environment may explain the lack of evidence thus far for organism-to-organism spread of ALS, at least via misfolded SOD1. Unlike PrP^{Sc}, which is a highly-stable and protease-resistant isoform compared to normal PrP^{C} and represents the epitome of a transmissible pathological protein-only agent, many pathological species of misfolded SOD1 become increasingly protease-sensitive and unstable (Grad et al., 2011; Ratovitski et al., 1999). This would make a misfolded SOD1 transmissible particle less likely to stand up to the environmental rigours of person-to-person transmission, although the presence of native wild-type SOD1 has been shown to increase the stability of mutant misfolded SOD1 (Weichert et al., 2013). Other factors that may confound interorganism infection of SOD1 or TDP-43 misfolding/aggregation include misfolding kinetics, i.e. it simply takes too long for suitable levels of misfolded template to replicate to levels that facilitate infection, and host attributes that may simply be incompatible for misfolded proteins of ALS to transmit between individuals. However, SOD1 does appear to meet the intermolecular and intercellular requirements associated with prions, and TDP-43 clearly has the ability to recruit soluble monomer into larger insoluble aggregates. Taken together, the literature suggests that the pathological agents of neurodegenerative proteinopathies could all be considered prions on a molecular and cellular scale. However, for prion proteins in general transmissibility and toxicity characteristics fall within a wide spectrum, with PrP^{Sc} representing the extreme end (highly transmissible, highly toxic) and proteins associated with more relatively common neurodegenerative proteinopathies, such as SOD1, TDP-43, amyloid-β or α-synuclein, grouped near the other end as less robust transmissible agents. There is no doubt that further *in vivo* characterization of misfolded protein transmissibility in ALS is required to determine the risks, if any, for interorganism transmission, and how much of a role misfolded protein propagation plays in motor neuron disease progression and pathology. Needless to say, the uniqueness of prions may have somewhat diminished in the past decade, but the importance in understanding their biology with respect to more common proteinopathies has never been greater; the significance of which is even higher for a disease like ALS, which lacks any type of intervention that significantly delays disease onset or slows progression. Targeting the prion-like mechanisms of this disease may provide much needed hope for an effective intervention.

Acknowledgments

NRC is the Canada Research Chair in Neurodegeneration and Protein Misfolding Diseases at the University of British Columbia, and is supported by donations from the Webster Foundation, the Allen T. Lambert Neural Research Fund and the Temerty Family Foundation, and also by grants from PrioNet Canada (20R65301/F1100079), the Canadian Institutes of Health Research (CIHR) (JNM-123677; 00028-007), and Biogen-Idec Corp. SF is supported by a University of British Columbia four-year graduate student fellowship.

References

Aguzzi, A., 2009. Cell biology: beyond the prion principle. Nature 459, 924–925.
Aguzzi, A., Rajendran, L., 2009. The transcellular spread of cytosolic amyloids, prions, and prionoids. Neuron 64, 783–790.

Alberti, S., et al., 2009. A systematic survey identifies prions and illuminates sequence features of prionogenic proteins. Cell 137, 146–158.

ALS Mutation Database. http://reseq.biosciencedbc.jp/resequence/SearchDisease.do?targetId=1. The University of Tokyo.

Andersen, P.M., et al., 1995. Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. Nat. Genet. 10, 61–66.

Andersen, P.M., et al., 2003. Sixteen novel mutations in the Cu/Zn superoxide dismutase gene in amyotrophic lateral sclerosis: a decade of discoveries, defects and disputes. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 4, 62–73.

Arai, T., et al., 2006. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem. Biophys. Res. Commun. 351, 602–611.

Audet, J.N., et al., 2010. Wild-type human SOD1 overexpression does not accelerate motor neuron disease in mice expressing murine Sod1(G86R). Neurobiol Dis 40 (1), 245–250.

Bard, F., et al., 2012. Sustained levels of antibodies against Abeta in amyloid-rich regions of the CNS following intravenous dosing in human APP transgenic mice. Exp. Neurol. 238, 38–43.

Basso, M., et al., 2013. Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis. J. Biol. Chem. 288, 15699–15711.

Beckman, J.S., Koppenol, W.H., 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am. J. Physiol. 271, C1424–C1437.

Beckman, J.S., et al., 1993. ALS, SOD and peroxynitrite. Nature 364, 584.

Beckman, J.S., et al., 2001. Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci. 24, S15–S20.

Bendotti, C., et al., 2012. Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. Prog. Neurobiol. 97, 101–126.

Bosco, D.A., et al., 2010. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat. Neurosci. 13, 1396–1403.

Braak, H., et al., 2013. Amyotrophic lateral sclerosis–a model of corticofugal axonal spread. Nat. Rev. Neurol. 9, 708–714.

Bradley, W.G., 2009. Updates on amyotrophic lateral sclerosis: improving patient care. Ann. Neurol. 65 (Suppl. 1), S1–S2.

Brettschneider, J., et al., 2013. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. Ann. Neurol. 74, 20–38.

Broom, W.J., et al., 2008. 50 bp deletion in the promoter for superoxide dismutase 1 (SOD1) reduces SOD1 expression in vitro and may correlate with increased age of onset of sporadic amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 9, 229–237.

Bruijn, L.I., et al., 1997. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. Neuron 18, 327–338.

Bruijn, L.I., et al., 1998. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. Science 281, 1851–1854.

Casoni, F., et al., 2005. Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: possible multifunctional role in the pathogenesis. J. Biol. Chem. 280, 16295–16304.

Chia, R., et al., 2010. Superoxide dismutase 1 and tgSOD1 mouse spinal cord seed fibrils, suggesting a propagative cell death mechanism in amyotrophic lateral sclerosis. PLoS ONE 5, e10627.

Chou, S.M., et al., 1996a. Colocalization of NOS and SOD1 in neurofilament accumulation within motor neurons of amyotrophic lateral sclerosis: an immunohistochemical study. J. Chem. Neuroanat. 10, 249–258.

Chou, S.M., et al., 1996b. Role of SOD-1 and nitric oxide/cyclic GMP cascade on neurofilament aggregation in ALS/MND. J. Neurol. Sci. 139 (Suppl.), 16–26.

Clavaguera, F., et al., 2009. Transmission and spreading of tauopathy in transgenic mouse brain. Nat. Cell Biol. 11, 909–913.

Cleveland, D.W., 1999. From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. Neuron 24, 515–520.

Cleveland, D.W., Liu, J., 2000. Oxidation versus aggregation—how do SOD1 mutants cause ALS? Nat. Med. 6, 1320–1321.

Cleveland, D.W., Rothstein, J.D., 2001. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat. Rev. Neurosci. 2, 806–819.

Coleman, B.M., et al., 2012. Prion-infected cells regulate the release of exosomes with distinct ultrastructural features. FASEB J. 26, 4160–4173.

Couthouis, J., et al., 2011. A yeast functional screen predicts new candidate ALS disease genes. Proc. Natl. Acad. Sci. U. S. A. 108, 20881–20890.

Danzer, K.M., et al., 2012. Exosomal cell-to-cell transmission of alpha synuclein oligomers. Mol. Neurodegener. 7, 42.

Deng, H.X., et al., 2006. Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. Proc. Natl. Acad. Sci. U. S. A. 103, 7142–7147.

Deng, H.X., et al., 2011. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477, 211–215.

Diener, T.O., et al., 1982. Viroids and prions. Proc. Natl. Acad. Sci. U. S. A. 79, 5220–5224.

Eisele, Y.S., et al., 2010. Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. Science 330, 980–982.

El-Agnaf, O.M., et al., 2006. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. FASEB J. 20, 419–425.

Elam, J.S., et al., 2003. Amyloid-like filaments and water-filled nanotubes formed by SOD1 mutant proteins linked to familial ALS. Nat. Struct. Biol. 10, 461–467.

Elden, A.C., et al., 2010. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1069–1075.

Emmanouilidou, E., et al., 2010. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J. Neurosci. 30, 6838–6851.

Fevrier, B., et al., 2004. Cells release prions in association with exosomes. Proc. Natl. Acad. Sci. U. S. A. 101, 9683–9688.

Forsberg, K., et al., 2010. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS ONE 5, e11552.

Frost, B., et al., 2009. Propagation of tau misfolding from the outside to the inside of a cell. J. Biol. Chem. 284, 12845–12852.

Fukada, K., et al., 2001. Stabilization of mutant Cu/Zn superoxide dismutase (SOD1) protein by coexpressed wild SOD1 protein accelerates the disease progression in familial amyotrophic lateral sclerosis mice. Eur. J. Neurosci. 14, 2032–2036.

Furukawa, Y., et al., 2008. Complete loss of post-translational modifications triggers fibrillar aggregation of SOD1 in the familial form of amyotrophic lateral sclerosis. J. Biol. Chem. 283, 24167–24176.

Furukawa, Y., et al., 2011. A seeding reaction recapitulates intracellular formation of Sarkosyl-insoluble transactivation response element (TAR) DNA-binding protein-43 inclusions. J. Biol. Chem. 286, 18664–18672.

Furukawa, Y., et al., 2013. Intracellular seeded aggregation of mutant Cu,Zn-superoxide dismutase associated with amyotrophic lateral sclerosis. FEBS Lett. 587, 2500–2505.

Gitcho, M.A., et al., 2008. TDP-43 A315T mutation in familial motor neuron disease. Ann. Neurol. 63, 535–538.

Gitler, A.D., Shorter, J., 2011. RNA-binding proteins with prion-like domains in ALS and FTLD-U. Prion 5, 179–187.

Gomes, C., et al., 2007. Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. Neurosci. Lett. 428, 43–46.

Grad, L.I., et al., 2011. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc. Natl. Acad. Sci. U. S. A. 108, 16398–16403.

Grad, L.I., et al., 2014. Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. Proc. Natl. Acad. Sci. U. S. A. 111, 3620–3625.

Gruzman, A., et al., 2007. Common molecular signature in SOD1 for both sporadic and familial amyotrophic lateral sclerosis. Proc. Natl. Acad. Sci. U. S. A. 104, 12524–12529.

Guest, W.C., et al., 2011. Toward a mechanism of prion misfolding and structural models of PrP(Sc): current knowledge and future directions. J. Toxicol. Environ. Health A 74, 154–160.

Guo, J.L., Lee, V.M., 2011. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. J. Biol. Chem. 286, 15317–15331.

Guo, W., et al., 2011. An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. Nat. Struct. Mol. Biol. 18, 822–830.

Gurney, M.E., et al., 1994. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. Science 264, 1772–1775.

Haass, C., Selkoe, D.J., 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat. Rev. Mol. Cell Biol. 8, 101–112.

Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353–356.

Hasegawa, M., et al., 2008. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann. Neurol. 64, 60–70.

Haverkamp, L.J., et al., 1995. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. Brain 118 (Pt 3), 707–719.

Horwich, A.L., Weissman, J.S., 1997. Deadly conformations—protein misfolding in prion disease. Cell 89, 499–510.

Huang, E.J., et al., 2010. Extensive FUS-immunoreactive pathology in juvenile amyotrophic lateral sclerosis with basophilic inclusions. Brain Pathol. 20, 1069–1076.

Huttner, W.B., et al., 1991. The granin (chromogranin/secretogranin) family. Trends Biochem. Sci. 16, 27–30.

Irwin, D.J., et al., 2013. Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. JAMA Neurol. 70, 462–468.

Jaarsma, D., et al., 2000. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol. Dis. 7, 623–643.

Johnson, B.S., et al., 2008. A yeast TDP-43 proteinopathy model: exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. Proc. Natl. Acad. Sci. U. S. A. 105, 6439–6444.

Johnson, B.S., et al., 2009. TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. J. Biol. Chem. 284, 20329–20339.

Kabashi, E., et al., 2007. Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis? Ann. Neurol. 62, 553–559.

Kabashi, E., et al., 2008. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat. Genet. 40, 572–574.

Kato, S., et al., 2000. New consensus research on neuropathological aspects of familial amyotrophic lateral sclerosis with superoxide dismutase 1 (SOD1) gene mutations: inclusions containing SOD1 in neurons and astrocytes. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 1, 163–184.

Kayed, R., et al., 2003. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300, 486–489.

Keller, S., et al., 2006. Exosomes: from biogenesis and secretion to biological function. Immunol. Lett. 107, 102–108.

Kerman, A., et al., 2010. Amyotrophic lateral sclerosis is a non-amyloid disease in which extensive misfolding of SOD1 is unique to the familial form. Acta Neuropathol. 119, 335–344.
Kim, D.K., et al., 2013. EVpedia: an integrated database of high-throughput data for systemic analyses of extracellular vesicles. J. Extracell. Vesicles 2.
King, O.D., et al., 2012. The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res. 1462, 61–80.
Kwiatkowski Jr., T.J., et al., 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.
Lashuel, H.A., et al., 2002. Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J. Mol. Biol. 322, 1089–1102.
Legname, G., et al., 2005. Strain-specified characteristics of mouse synthetic prions. Proc. Natl. Acad. Sci. U. S. A. 102, 2168–2173.
Lesne, S., et al., 2006. A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440, 352–357.
Liu-Yesucevitz, L., et al., 2010. Tar DNA binding protein-43 (TDP-43) associates with stress granules: analysis of cultured cells and pathological brain tissue. PLoS ONE 5, e13250.
Luk, K.C., et al., 2009. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc. Natl. Acad. Sci. U. S. A. 106, 20051–20056.
Luk, K.C., et al., 2012. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338, 949–953.
Matias-Guiu, J., et al., 2008. Superoxide dismutase: the cause of all amyotrophic lateral sclerosis? Ann. Neurol. 64, 356–357 (author reply 358).
Meyer-Luehmann, M., et al., 2006. Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. Science 313, 1781–1784.
Mousavi, A., Hotta, Y., 2005. Glycine-rich proteins: a class of novel proteins. Appl. Biochem. Biotechnol. 120, 169–174.
Munch, C., et al., 2011. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc. Natl. Acad. Sci. U. S. A. 108, 3548–3553.
Neumann, M., et al., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.
Nonaka, T., et al., 2009. Truncation and pathogenic mutations facilitate the formation of intracellular aggregates of TDP-43. Hum. Mol. Genet. 18, 3353–3364.
Nonaka, T., et al., 2013. Prion-like properties of pathological TDP-43 aggregates from diseased brains. Cell Rep. 4, 124–134.
Patino, M.M., et al., 1996. Support for the prion hypothesis for inheritance of a phenotypic trait in yeast. Science 273, 622–626.
Perez-Gonzalez, R., et al., 2012. The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space. J. Biol. Chem. 287, 43108–43115.
Pesiridis, G.S., et al., 2009. Mutations in TDP-43 link glycine-rich domain functions to amyotrophic lateral sclerosis. Hum. Mol. Genet. 18, R156–R162.
Philips, T., Robberecht, W., 2011. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 10, 253–263.
Phukan, J., et al., 2007. Cognitive impairment in amyotrophic lateral sclerosis. Lancet Neurol. 6, 994–1003.
Pokrishevsky, E., et al., 2012. Aberrant localization of FUS and TDP43 is associated with misfolding of SOD1 in amyotrophic lateral sclerosis. PLoS ONE 7, e35050.
Prudencio, M., et al., 2009a. Modulation of mutant superoxide dismutase 1 aggregation by co-expression of wild-type enzyme. J. Neurochem. 108, 1009–1018.
Prudencio, M., et al., 2009b. Variation in aggregation propensities among ALS-associated variants of SOD1: correlation to human disease. Hum. Mol. Genet. 18, 3217–3226.
Prudencio, M., et al., 2010. An examination of wild-type SOD1 in modulating the toxicity and aggregation of ALS-associated mutant SOD1. Hum. Mol. Genet. 19, 4774–4789.
Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science 216, 136–144.
Rakhit, R., et al., 2002. Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. J. Biol. Chem. 277, 47551–47556.
Rakhit, R., et al., 2004. Monomeric Cu,Zn-superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial amyotrophic lateral sclerosis. J. Biol. Chem. 279, 15499–15504.
Rakhit, R., et al., 2007. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. Nat. Med. 13, 754–759.
Ratovitski, T., et al., 1999. Variation in the biochemical/biophysical properties of mutant superoxide dismutase 1 enzymes and the rate of disease progression in familial amyotrophic lateral sclerosis kindreds. Hum. Mol. Genet. 8, 1451–1460.
Ravits, J.M., La Spada, A.R., 2009. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. Neurology 73, 805–811.
Ravits, J., et al., 2007. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology 68, 1571–1575.
Raymond, G.J., et al., 2000. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. EMBO J. 19, 4425–4430.
Reaume, A.G., et al., 1996. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat. Genet. 13, 43–47.
Rosen, D.R., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 364, 362.
Rutherford, N.J., et al., 2008. Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. PLoS Genet. 4, e1000193.
Said Ahmed, M., et al., 2000. Increased reactive oxygen species in familial amyotrophic lateral sclerosis with mutations in SOD1. J. Neurol. Sci. 176, 88–94.
Sampathu, D.M., et al., 2006. Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. Am. J. Pathol. 169, 1343–1352.
Sekiguchi, T., et al., 2014. Spreading of amyotrophic lateral sclerosis lesions—multifocal hits and local propagation? J. Neurol. Neurosurg. Psychiatry 85, 85–91.
Sephton, C.F., et al., 2010. TDP-43 is a developmentally regulated protein essential for early embryonic development. J. Biol. Chem. 285, 6826–6834.
Shankar, G.M., et al., 2008. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat. Med. 14, 837–842.
Shibata, N., et al., 1994. Cu/Zn superoxide dismutase-like immunoreactivity in Lewy body-like inclusions of sporadic amyotrophic lateral sclerosis. Neurosci. Lett. 179, 149–152.
Sreedharan, J., et al., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.
Stewart, H., et al., 2012. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. Acta Neuropathol. 123, 409–417.
Strong, M., Rosenfeld, J., 2003. Amyotrophic lateral sclerosis: a review of current concepts. Amyotroph. Lateral Scler. Other Motor Neuron Disord. 4, 136–143.
Sun, Z., et al., 2011. Molecular determinants and genetic modifiers of aggregation and toxicity for the ALS disease protein FUS/TLS. PLoS Biol. 9, e1000614.
Synofzik, M., et al., 2012. Mutant superoxide dismutase-1 indistinguishable from wild-type causes ALS. Hum. Mol. Genet. 21, 3568–3574.
Tan, A.Y., Manley, J.L., 2009. The TET family of proteins: functions and roles in disease. J. Mol. Cell Biol. 1, 82–92.
Taylor, D.M., et al., 2007. Tryptophan 32 potentiates aggregation and cytotoxicity of a copper/zinc superoxide dismutase mutant associated with familial amyotrophic lateral sclerosis. J. Biol. Chem. 282, 16329–16335.
Thomsen, C., et al., 2013. A conserved N-terminal motif is required for complex formation between FUS, EWSR1, TAF15 and their oncogenic fusion proteins. FASEB J. 27, 4965–4974.
Toombs, J.A., et al., 2010. Compositional determinants of prion formation in yeast. Mol. Cell. Biol. 30, 319–332.
Uptain, S.M., Lindquist, S., 2002. Prions as protein-based genetic elements. Annu. Rev. Microbiol. 56, 703–741.
Urushitani, M., et al., 2006. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat. Neurosci. 9, 108–118.
Urushitani, M., et al., 2008. The endoplasmic reticulum–Golgi pathway is a target for translocation and aggregation of mutant superoxide dismutase linked to ALS. FASEB J. 22, 2476–2487.
Van Deerlin, V.M., et al., 2008. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol. 7, 409–416.
Vance, C., et al., 2009. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323, 1208–1211.
Wang, J., et al., 2002. Fibrillar inclusions and motor neuron degeneration in transgenic mice expressing superoxide dismutase 1 with a disrupted copper-binding site. Neurobiol. Dis. 10, 128–138.
Wang, Q., et al., 2008. Protein aggregation and protein instability govern familial amyotrophic lateral sclerosis patient survival. PLoS Biol. 6, e170.
Wang, L., et al., 2009. Wild-type SOD1 overexpression accelerates disease onset of a G85R SOD1 mouse. Hum. Mol. Genet. 18, 1642–1651.
Wang, I.F., et al., 2012. The self-interaction of native TDP-43 C terminus inhibits its degradation and contributes to early proteinopathies. Nat. Commun. 3, 766.
Weichert, A., et al., 2013. Wild-type Cu/Zn superoxide dismutase stabilizes mutant variants by heterodimerization. Neurobiol. Dis. 62, 479–488.
Witan, H., et al., 2009. Wild-type Cu/Zn superoxide dismutase (SOD1) does not facilitate, but impedes the formation of protein aggregates of amyotrophic lateral sclerosis causing mutant SOD1. Neurobiol. Dis. 36, 331–342.
Wu, C.H., et al., 2012. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 488, 499–503.
Wu, L.S., et al., 2010. TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. Genesis 48, 56–62.
Yokoseki, A., et al., 2008. TDP-43 mutation in familial amyotrophic lateral sclerosis. Ann. Neurol. 63, 538–542.