Prion-like spread of protein aggregates in neurodegeneration

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Protein misfolding is common to most neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Recent work using animal models with intracellular α-synuclein and tau inclusions adds decisively to a growing body of evidence that misfolded protein aggregates can induce a self-perpetuating process that leads to amplification and spreading of pathological protein assemblies. When coupled with the progressive nature of neurodegeneration, recognition of such cell-to-cell aggregate spread suggests a unifying mechanism underlying the pathogenesis of these disorders.

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Neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's, and prion diseases, as well as frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are a major—and growing—public health issue for aging populations, as aging is the greatest risk factor for neurodegeneration. These conditions are characterized by the progressive dysfunction and death of neurons. The neuronal cell type most vulnerable to disease-related alterations defines the clinical picture of each disease. For example, dopaminergic neurons are lost in Parkinson's disease, which mainly presents with tremor, whereas motor neurons are lost in ALS leading to paralysis. Most neurodegenerative diseases can occur either in familial forms or sporadically, in the absence of an obvious hereditary cause. At present, there is no truly effective therapeutic intervention that slows neurodegeneration for any of the major diseases. For most, the molecular events triggering these diseases remain unknown.

Protein misfolding and accumulation in neurodegeneration

A common feature of neurodegenerative diseases is the presence of misfolded protein aggregates in affected regions of the nervous system. Although the major protein component of the pathological aggregations can be unique for each neurodegenerative disease (e.g., α-synuclein in Parkinson's or Aβ in Alzheimer's diseases), several proteins misfold and accumulate in multiple diseases. The most glaring example of the latter is TDP-43, which aggregates in ALS, FTLD, and many other conditions (Lagier-Tourenne et al., 2010). In other instances more than one accumulated protein (e.g., Aβ and tau in Alzheimer's disease) is observed in the same condition. Familial forms of neurodegenerative diseases are often linked to mutations that augment the aggregation propensity of disease-related proteins, suggesting that protein misfolding and aggregation is likely to play a decisive role in the pathogenesis of neurodegenerative diseases.

In early stages of neurodegeneration, pathological alterations, including protein aggregation and neuronal dysfunction, are localized in a confined area of the nervous system. In later stages, such alterations become more generalized and diffuse, suggesting that the pathogenic triggers spread throughout the nervous system. Indeed, apparent spreading of pathological changes has been described for all the major neurodegenerative diseases including Alzheimer's (Braak and Braak, 1991), Parkinson's (Braak et al., 2003), FTLD (Kril and Halliday, 2011), Huntington's (Deng et al., 2004), ALS (Ravits et al., 2007a,b), and of course prion diseases. In prion diseases acquired by infection, the initial site of propagation may occur outside the central nervous system (Aguzzi et al., 2008).

In this issue of the *Journal of Experimental Medicine*, Luk et al. present compelling evidence that in an animal model of Parkinson's disease, spread of the pathogenic trigger can be mediated by misfolded α-synuclein, which induces the misfolding of native α-synuclein (Luk et al., 2012). Moreover, very recent papers by de Calignon et al. (2012) and Liu et al. (2012) draw similar conclusions regarding the spreading of misfolded tau. Both of these latter papers use an elegant model in which tau aggregates form specifically in the entorhinal cortex, resembling early Alzheimer's disease. These three papers add to a growing body of evidence supporting the view that misfolded protein propagation underlies the progression of several, if not all, neurodegenerative diseases (Aguzzi 2009; Aguzzi and Rajendran, 2009; Polymenidou and Cleveland, 2011).

Self-perpetuating seeded aggregation and spreading

In the best known example of protein misfolding within the nervous system, the prion diseases, seeded aggregation is not only a critical feature of neurodegeneration. It is also the cause of neurodegeneration. Indeed, the infectious prion replicates by recruiting the normal prion protein PrP^{C} into the pathological PrP^{Sc}-containing aggregates, and inducing a pathological conformation of the native endogenous protein (Prusiner, 1982; Aguzzi and Polymenidou, 2004). This type

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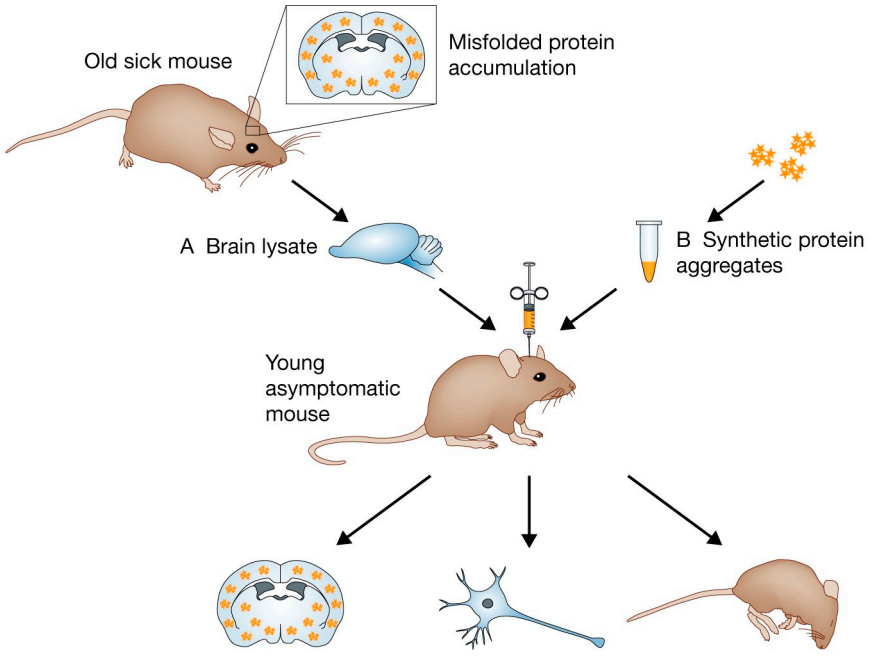
of cyclic amplification can be replicated in vitro, using minute amounts or "seeds" of aggregated PrP^{Sc} and an excess of natively folded cellular prion protein (Castilla et al., 2005). Although such propagation mechanisms were long thought to exclusively underlie transmissible prion diseases, in the past decade accumulating evidence suggests that several other proteins follow similar general molecular mechanisms of self-perpetuating seeded aggregation and cell-to-cell spreading in vitro and in cell culture models, as well as when introduced focally into animals (Fig. 1).

Misfolded α-synuclein spreading in Parkinson's disease

The pathological hallmark of Parkinson's disease is the presence of compact round inclusions of aggregated proteins called Lewy bodies in the cytoplasm of affected cells (Braak et al., 2003). The primary protein component of Lewy bodies is α-synuclein, a largely synaptic protein that plays a key causative role in the pathogenesis of Parkinson's disease. Indeed, point mutations (Polymeropoulos et al., 1997; Krüger et al., 1998; Zarranz et al., 2004) and duplications or triplications (Singleton et al., 2003) in the gene encoding α-synuclein have been associated with familial forms of Parkinson's disease.

Spreading and in vivo seeding of α-synuclein aggregation was first demonstrated by the induction of Lewy bodies within normal neuronal stem cells transplanted into Parkinson's disease patients (Kordower et al., 2008; Li et al., 2008). This paradigm was subsequently replicated in mice (Desplats et al., 2009; Hansen et al., 2011). Moreover, α-synuclein exhibits a seeding behavior when introduced into cultured cells (Desplats et al., 2009; Nonaka et al., 2010; Hansen et al., 2011). Most recently, fibrils formed in vitro from



	Induction of aggregation		Neuronal loss		Fatal disease	
	Lysate	Synthetic	Lysate	Synthetic	Lysate	Synthetic
PrP^{Sc}	Yes[1]	Yes[2]	Yes[1]	Yes[2]	Yes[1]	Yes[2]
Aβ	Yes[3]	No[3]	Yes[3]	No[3]	No[3]	No[3]
α-synuclein	Yes[4,5]	Yes[5]	Yes[4,5]	Yes[5]	Yes[4,5]	Yes[5]
Tau	Yes[6]	n.d.	Yes[6]	n.d.	No[6]	n.d.

[1]Initially by Chandler 1961 and replicated by many; [2]Wang *et al*. 2010; [3]Meyer-Luehmann *et al*. 2006; [4]Mougenot *et al*. 2011; [5]Luk *et al*. 2012; [6]Clavaguera *et al*. 2009

Figure 1. Scheme summarizing evidence for seeded aggregation and cell-to-cell spreading in animal models of neurodegeneration. The figure depicts the experimental paradigm originally used to replicate infectious prions in mice, which is now used to replicate spreading of misfolded Aβ, α-synuclein, and tau. Protein aggregate containing brain lysates from old sick mice (A) or pure recombinant fibrils aggregated in vitro (B) are introduced in the brains of young asymptomatic mice by injection. It is important to note that some prion-containing lysates (Chandler, 1961) or synthetic prion aggregates (Wang et al., 2010) can transmit disease to wild-type nontransgenic mice, whereas all other aggregates have thus far only been shown to induce aggregation and neuronal dysfunction in transgenic mice expressing the human versions of the respective proteins.

pure recombinant wild-type α-synuclein induced pathological aggregates of endogenous α-synuclein in primary neurons, which caused synaptic dysfunction and neuronal death (Volpicelli-Daley et al., 2011).

Luk et al. (2012) used transgenic mice expressing human α-synuclein carrying a Parkinson's disease–linked mutation (A53T). Like most transgenic mice expressing mutant proteins associated with human neurodegenerative diseases, the α-synuclein^{A53T} mice are indistinguishable from control littermates at birth and grow normally in the absence of any signs of disease until they reach 1 yr of age. These mice begin accumulating α-synuclein inclusions in their brains beginning at ∼8 mo of age, and accumulation intensifies by 12 mo of age, when they develop a severe movement disorder which is fatal over a 3-mo disease course (Giasson et al., 2002).

Luk et al. (2012) find that injection of brain lysate from α-synuclein^{A53T} mice at the terminal stage of disease (which is enriched in α-synuclein aggregates) into the cortex and striatum of young asymptomatic α-synuclein^{A53T} mice accelerates disease initiation (Fig. 1). Remarkably, this injection also quickens death (Luk et al., 2012), consistent with another recent paper (Mougenot et al., 2011). α-Synuclein aggregate-containing lysates induced α-synuclein pathology in recipient mice as early as 30 d after injection and then progressively spread. This pathological effect was entirely reliant on the combination of endogenous and mutant α-synuclein expression in recipient mice, as injection of the same material in α-synuclein knockout mice induced neither pathology nor clinical signs, and the inoculum was rapidly degraded. This reliance on endogenous native protein expression resembles the effect seen upon prion infection of mice lacking the cellular prion protein (Büeler et al., 1993).

These findings strongly suggest that exogenous α-synuclein aggregates induce aggregation of endogenously expressed α-synuclein through a seeding reaction and that this mechanism underlies the observed amplification. Indeed, the presence of α-synuclein aggregates in the inoculum was the trigger of the acceleration of disease and pathology in injected mice, as injection of brain lysates from young A53T mice lacking α-synuclein aggregates had no effect. Most importantly, the consequences of injecting old A53T brain lysates could be reproduced using pure bacterially produced recombinant α-synuclein fibrils aggregated in vitro. In fact, using different amounts of recombinant α-synuclein aggregates resulted in a dose-dependent effect on induction of pathology.

So, how do the intracellular α-synuclein aggregates spread from cell to cell? This key question remains unanswered. Nevertheless, a thorough immunohistochemical analysis (Luk et al., 2012) was consistent with spreading by passage between synaptically connected neurons. In fact, inoculation into either striatum or cortex produced different patterns of α-synuclein pathology in recipient mice, each consistent with spreading to neurons connected to the site of deposition. Simultaneous injection in both cortex and striatum produced a composite distribution.

Aβ and tau aggregation and spreading in Alzheimer's disease

Alzheimer's disease is the most common form of dementia and affects one in eight people above the age of 65. Pathologically, the disease is characterized by the accumulation of extracellular Aβ plaques that form from a highly insoluble proteolytic fragment of the normal amyloid precursor protein (APP) and cytoplasmic neurofibrillary tangles consisting of the microtubule-associated protein tau (Braak and Braak, 1991). In the past 10 yr it has been established that Aβ aggregation in transgenic mice expressing human APP is hastened by the presence of preformed Aβ aggregates (Kane et al., 2000; Meyer-Luehmann et al., 2006; Eisele et al., 2010). In particular, accelerated aggregation of Aβ occurs in human APP transgenic mice after intracerebral injection of brain extracts from autopsy material of human Alzheimer's disease patients (Kane et al., 2000) or aged Alzheimer's disease model mice (Meyer-Luehmann et al., 2006), both of which contain Aβ aggregates. Intraperitoneal administration of Aβ-containing extracts also induced Aβ aggregation in the vicinity of brain blood vessels (Eisele et al., 2010), reminiscent of cerebral β-amyloid angiopathy associated with Alzheimer's disease in humans (Thal et al., 2008). Notably, Alzheimer's disease-related aggregation spread is not limited to Aβ aggregates. Intracerebral injection of mutant tau aggregate-containing brain extract seeds widespread aggregation of normal human tau in transgenic mice that do not otherwise develop aggregates (Clavaguera et al., 2009).

The presence of neurofibrillary tangles in Alzheimer's disease correlates well with cognitive dysfunction and neuronal loss. In fact, the presence of tau inclusions in a particular set of neurons found in layer II of the entorhinal cortex is associated with mild Alzheimer's disease, suggesting that these may be among the initial alterations in this disease (Gómez-Isla et al., 1996). Two very recent independent studies (de Calignon et al., 2012; Liu et al., 2012) demonstrate that aggregated tau can initiate neurofibrillary tangle formation in vivo. Both teams used bi-transgenic mice carrying an activator transgene driving expression of the tet transactivator under the entorhinal-specific neuropsin gene promoter and a responder transgene encoding the mutant tau^{P301L} (the responder transgene is expressed only in presence of the tet transactivator). Mice described in both studies exhibited progressive alterations of tau resembling those typically found in autopsies of human patients. These alterations include misfolding, hyperphosphorylation (revealed by immunohistochemical methods), and the appearance of ordered fibrils (revealed by Gallyas silver staining and thioflavin S staining). In fact, a careful time-course analysis (de Calignon et al., 2012) revealed progressive alteration of tau from misfolding and hyperphosphorylation to formation of ordered cytoplasmic aggregates.

Most importantly, both groups demonstrated that although all of these alterations were originally restricted to the entorhinal cortex, where the mutant tau transgene is active, they spread to

synaptically connected regions over time. This spread of tau pathology beyond the entorhinal cortex could not be explained by mutant tau^{P301L} synthesis within those additional regions, as the use of laser capture microdissection confirmed the complete absence (de Calignon et al., 2012) or highly reduced (Liu et al., 2012) expression of the mutant tau^{P301L} transgene in brain areas outside of the entorhinal cortex. This latter finding is highly reminiscent of the α-synuclein results reported in this issue (Luk et al., 2012).

The progression of pathological tau alterations that occurs outside the site of transgene expression very likely involves the induction of an altered conformation of endogenous mouse tau through a seeding reaction, because mouse tau is recruited into the cytoplasmic inclusions (de Calignon et al., 2012). Moreover, expression of mutant tau^{P301L} in the entorhinal cortex caused not only selective loss of the neurons expressing the transgene, but also synaptic degeneration within the neuronal circuits of these neurons, strongly suggesting that the spread of pathological alterations of tau is accompanied by and/or causes dysfunction within the respective neurons. Consistent with this are the concomitant astrogliosis and microgliosis observed in areas with axonal degeneration (de Calignon et al., 2012). Lastly, similar to what is now reported for α-synuclein (Luk et al., 2012), de Calignon et al. (2012) provided evidence that astrocytes, but not microglia, can take up and potentially amplify tau aggregates.

A common molecular pathway in neurodegeneration

More aggregated proteins characterizing additional neurodegenerative diseases may behave similarly to α-synuclein and tau. For example, RNA-binding proteins associated with neurodegeneration (e.g., TDP-43 and FUS/TLS) may behave in such a manner, as several RNA-binding proteins contain domains with high aggregation propensity (King et al., 2012). In fact, a new study provides compelling evidence that such domains may facilitate the formation of subcellular structures—such as stress granules—via the reversible polymerization into dynamic amyloid-like fibers (Kato et al., 2012), suggesting a functional role for the aggregation-prone domains of RNA-binding proteins involved in disease.

It is important to emphasize that there is currently no evidence that any other neurodegenerative disease besides prion diseases can be transmitted between individuals by natural routes of transmission/infection or as a result of a medical intervention. Rather, all evidence described here relates to propagation of misfolded protein aggregates within an organism, and the term prionoid was introduced to distinguish these events from bona fide, infectious prions (Aguzzi, 2009). Nevertheless, the prion-like replication that occurs within affected cells, followed by transfer from cell to cell provides a molecular pathway for disease spread within the nervous system after focal generation of an initiating misfolding event. This unifying mechanism of neurodegeneration offers opportunities for therapeutic interventions based on agents that may disrupt the cascade of events leading to the propagation of protein misfolding.

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