

Following the fate of endocytosed fibrils

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Cell-to-cell transmission of intracellular protein aggregates is considered a central event in many neurodegenerative diseases, but little is known about the underlying molecular mechanisms. A new study employs fluorescence quenching to examine the fate of α -synuclein, a key molecule in the pathology of Parkinson's disease and related disorders, in primary cultured neurons, finding that endocytosis and lysosomal processing of exogenous fibrils may explain the transmission of α -synuclein pathology.

In many neurodegenerative diseases, the buildup of abnormal protein aggregates in neurons and glial cells is closely correlated with disease symptoms and progression. Tau in Alzheimer's disease and frontotemporal lobar degeneration (FTLD)² (collectively referred to as tauopathies), α -synuclein (α -syn) in Parkinson's disease, dementia with Lewy bodies and multiple system atrophy (referred to as α -synucleinopathies), and TDP-43 in amyotrophic lateral sclerosis (ALS) and FTLD (referred to as TDP-43 proteinopathies) are the major pathological proteins. Ultrastructural and biochemical studies of aggregates in brains of patients have revealed that these proteins are accumulated as fibrous or filamentous structures that are positively stained by β -sheet binding ligands such as thioflavins. Prion-like propagation has been proposed to account for the spreading of these protein pathologies, and growing evidence, particularly with α -syn, supports this idea (1). Recombinant α -syn is a heat-stable small protein that is easy to purify in bulk and forms amyloid-like fibrils on shaking at 37 °C for ~1–2 days without any additional reagents (2). Intracerebral injection of the synthetic fibrils or brain samples from patients into transgenic or wild-type mouse brain leads to prion-like conversion of endogenous normal α -syn to a fibrous form (3, 4). Overall, the phenomenon of prion-like conversion of α -syn has

been demonstrated *in vitro*, in cultured cells and in a wild-type mouse model (5). However, the molecular mechanisms by which these proteins are transmitted from cell to cell, the trafficking pathway(s) of the incorporated proteins, their lifetime *in vivo*, and the way in which they act as seeds for prion-like propagation all remain unclear.

Initial evidence from genetic studies, neuropathological analyses, and disease models (6–8) have pointed to lysosomal defects as an important contributor to the aggregation of α -syn and other proteins. Mazzulli *et al.* (6) reported a connection between loss-of-function of glucocerebrosidase, a key molecule of glycolipid lysosomal storage disorder, and accumulation of α -synuclein in lysosomes. Tanaka *et al.* (7) demonstrated that PGRN, a causative gene of FTLD, is a secretory lysosomal protein that regulates lysosomal function and biogenesis by controlling the acidification of lysosomes; decrease of PGRN led to accumulation of TDP-43. Flavin *et al.* (8) reported that disease-associated protein fibrils of α -syn, tau, and huntingtin exon1-Q45 can rupture intracellular vesicles following endocytosis in cultured cells and that lysosomes ruptured by α -syn fibrils are targeted for autophagic degradation. In addition, they suggested that the vesicles from which Lewy bodies are derived had previously been ruptured by α -syn aggregates, based on detection of the presence of galectin 3 at the periphery of Lewy bodies (8). However, the lack of efficient methods to directly visualize protein fibrils has limited conclusive insights into their biological journey.

The work of Karpowicz, Jr. and colleagues (9) takes new strides toward mechanistic understanding by following the fate of α -syn as it interacts with an uninfected cell. First, they report an elegant method to image internalized α -syn fibrils selectively: Treating cells with GFP-labeled α -syn fibrils followed by introduction of trypan blue, a membrane-impermeable fluorescence quencher, results in the quenching of extracellular fibrils and thus selective visualization of internalized fibrils (Fig. 1). Using this method, they provide evidence that internalized α -syn fibrils are rapidly acidified along the endolysosomal pathway. Second, using mutant constructs of α -syn labeled with a fluorescent dye that is not sensitive to the low pH of the lysosomes, the authors were able to demonstrate that most of the fibrils remain for days in lysosomes after uptake; additional experiments in combination with a construct labeled with an environmentally sensitive fluorophore allowed the authors to quantitate trafficking along the endocytic pathway, determining that the fibrils pass quickly to the late endosomes and lysosomes. These data provide important confirmation of the previous studies that endocytosis is the principal uptake mech-

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² The abbreviations used are: FTLD, frontotemporal lobar degeneration; α -syn, α -synuclein.

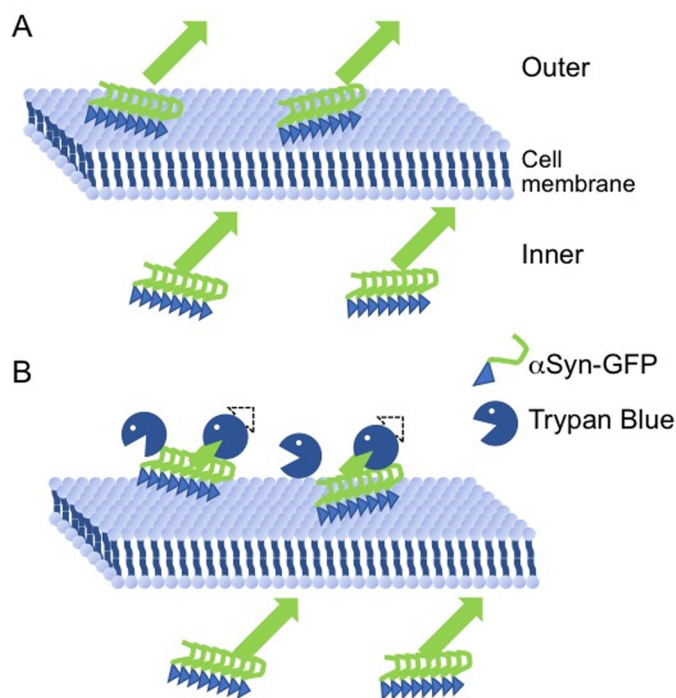


Figure 1. Selective quenching of the fluorescence from extracellular GFP-labeled α -syn fibrils by trypan blue. *A*, in the absence of trypan blue, both extra- and intracellular fibrils emit fluorescence, and they cannot be distinguished. *B*, trypan blue selectively binds to extracellular α -syn fibrils and quenches their fluorescence, so the fluorescence of intracellular α -syn fibrils is selectively observed and α -syn fibril uptake can be quantitatively characterized.

anism of extracellular α -syn fibrils in primary neurons and that lysosomal processing is the predominant fate of internalized α -syn fibrils.

Finally, the authors tackled the question of how fibrils seemingly trapped in lysosomes could be responsible for seeding the formation of new aggregates. They demonstrated that perturbation of lysosomal function with chloroquine (CHQ) causes aberrations in intracellular processing of α -syn fibrils, concomitantly with an increased rate of inclusion formation via recruitment of endogenous α -syn. These results further support the idea that defects in lysosomal activity and integrity may accelerate pathological α -syn aggregation and transmission (6–8).

The study by Lee and colleagues (9) provides exciting new evidence toward understanding aggregate transmission, yet many questions remain. For example, as the authors discuss, it is unclear whether α -syn fibril internalization requires specific receptors to mediate endocytosis, whether orthogonal routes of cell-to-cell transfer such as the use of tunneling nanotubes may be involved (10), and whether multiple mechanisms of seed uptake are operating in parallel. Second, it is not known what the possible diverse fates are for the fibrils: Could their travels end with proteolytic degradation, endocytic escape, trafficking to recycling endosomes, or other outcomes? If there are multiple trafficking routes, could this lead to bias toward distinct fates for the cargo? Finally, how are the abnormal proteins that are seeded in the cytosol processed, via recruitment to phagophores by autophagy and transfer back to lysosomes for proteolysis or secretion into the extracellular space, either in naked form or coated like exosomes? Although postmortem changes

may have erased the evidence, double-membraned phagophores have not been neuropathologically detected in intracellular inclusions in brains of patients. It is also difficult to clarify whether the secreted seeds are actually responsible for the propagation in human and model mouse brains, although it can be tested in cultured cells. On the other hand, if the proteins are degraded through proteolysis, what proportion of the proteins could be handled by the ubiquitin proteasome system *versus* what proportion would need to be processed by other systems, and how would the physical properties of the aggregates such as size influence their fate?

The new methodological approach described by Lee and colleagues (9) provides an important complement to the strains of mice and mouse embryonic fibroblast cells with knock-out of various molecules involved in ubiquitin proteasome, lysosome, or autophagy systems available to tackle these questions further. A number of animal models of seed-induced aggregation, which are extremely useful for investigation of relevant molecules and mechanisms in the brain, have also been developed (3, 4) that can recapitulate the spreading of pathologies and enable analysis without the influence of postmortem changes. It will be exciting to pursue future combination studies with these models to explore the molecular mechanisms of prion-like propagation in the neurodegenerative proteinopathies toward the development of novel therapies.

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