INTRODUCTION

Prion-like aggregation of α-synuclein in Parkinson’s disease (PD): from gut to brain

PD is a progressive neurodegenerative disease affecting millions of people worldwide. PD symptoms include disruptions of motor behavior such as postural bradykinesia, rigidity, and resting tremors, as well as non-motor symptoms, including constipation, sleep disturbances, depression, anxiety, and impaired olfaction. The exact molecular causes of dopamine neuron loss in the substantia nigra (SN) remain unclear, but the aberrant aggregation of the protein α-synuclein is thought to be involved. The hallmark of PD is α-synuclein-containing intracellular inclusions found both in neuronal cell bodies and neuronal processes of the brain, termed “Lewy pathology,” collectively.

α-Synuclein is a small aggregation prone protein of unknown biological function. In vivo, α-synuclein forms several small homo-oligomers (i.e., dimer, tetramer, octamer) that are likely important for its endogenous functions, such as intracellular vesicular trafficking. α-Synuclein can also form progressively massive insoluble amyloid fibrils in vitro that consist of hundreds of α-synuclein proteins in a stable β-sheet conformation. Several species of α-synuclein including the monomer, dimer, tetramer, protofibrils, and full length fibrils have been implicated in PD.

The shift from endogenous α-synuclein monomer to oligomeric and fibril states have been shown to be particularly neurotoxic, though further research is required to determine what multimeric species or conformer of α-synuclein, if any, are genuinely disease causing.

There is evidence that fibrils are a disease causing species of α-synuclein. Synthetic fibrils, referred to as preformed fibrils, can be transmitted from cell-to-cell, and when injected into the brain can recapitulate symptoms and pathology of PD. The spread of α-synuclein fibrils likely involves a templating mechanism; where either intact, or fragmented (i.e., protofibrils), fibrils leave a donor cell, are subsequently taken up by a host cell, and seed further aggregation via interaction with the endogenously expressed α-synuclein of the host cell. The accumulation of intracellular fibrils may be inherently toxic, or alternatively, fibrils may exert toxicity by indirect mechanisms, such as inhibiting the normative function of endogenous α-synuclein. Disease subtypes and progression may depend on the specific “strain” or conformation of the fibril species, which affects α-synuclein structure, level of toxicity, in vivo propagation, and neuropathology. The cell-to-cell spread of fibrillar α-synuclein offers an explanation for the observations that α-synuclein pathology is observed in healthy neurons graftied into the striatum of PD patients. Indeed, α-synuclein has been shown to be released from neurons followed by subsequent spread to neurons within close proximity. The spreading behavior of α-synuclein pathology has led many researchers to hypothesize that PD results from a “prion-like” mechanism. However, a “prion-like” mechanism of PD is somewhat contradicted by the observation in some PD patients whose pathology does not adhere to the staging scheme proposed by Braak. Together, the prion-hypothesis of PD seems mechanistically viable and deserving of systematic study.
Prions are transmittable infectious particles comprised solely of protein (i.e., devoid of nucleic acids), namely prion protein (PrP), that cause fatal diseases. In general, the term “prion” refers to infectious proteins which propagate a disease by causing protein conformational changes. For PrP to become a disease causing protein it must first convert from a- helical structure to a stable b-sheet conformation. This disease causing b-sheet isoform of PrP is referred to in the literature as PrPSc. Once an animal is inoculated with PrPSc it can spread from cell-to-cell both within and between species. Environmental prions are infectious and can cause prion disease, however, prion disease can also be inherited. For example, prion diseases like Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD) occur both through a familial pattern of inheritance and sporadically. Although considerable heterogeneity exists between the pathology of prion diseases, generally PrPSc causes extensive damage to the nervous system, including vacuolation (resulting in the spongiform appearance of brain tissue), astrocytic gliosis, and PrP deposition, which together ultimately lead to death.

In the context of a-synuclein, the term “prion-like” refers to the capacity of misfolded alpha-synuclein to transfer from cell-to-cell within an organism and act as template for further aggregation. Currently, a-synuclein and aggregates of a-synuclein are not considered as infectious, communicable agents, and as a result it is debated whether these are true prions. Indeed, exposure to human growth hormone potentially contaminated with a-synuclein aggregates was not associated with PD. However, there are many similarities between the molecular behavior of a-synuclein and that of the classic prion, PrPSc. First, inoculation of rodents and monkeys with the aggregated a-synuclein and that of the classic prion, PrPSc causes extensive damage to the nervous system, including vacuolation (resulting in the spongiform appearance of brain tissue), astrocytic gliosis, and PrP deposition, which together ultimately lead to death.

Routes of prion infectivity in the gut
How might a-synuclein prion particles from food infect a human host? One possibility is transcellular antigen sampling via microfold cells (M cells), dendritic cells, and to some extent columnar enterocytes. Antigen sampling is the process of controlled absorption of luminal contents and the subsequent transport of these particles to the gut-associated lymphoid tissue (GALT) to stimulate B-cell IgA production. This hypothesis was based on the observation that in many, but not all, PD patients Lewy body pathology has been documented in colonic biopsies can accurately distinguish between PD from Alzheimer disease (AD) and control subjects. However, this hypothesis is challenged by the observation that peripheral pathology/insult may lead to CNS pathology, further work is required to clarify the site for the introduction of an unknown pathogen and the subsequent initiation of PD pathology.

Several recent studies have, in part, supported this “gut to brain” hypothesis of PD. a-Synuclein is endogenously expressed in enteric neurons throughout the gastrointestinal (GI) system and PD-like a-synuclein pathology has been documented in intestinal tissues of PD patients and prodromal-PD patients. Local injections of a-synuclein into the vagal nerve of rats can induced PD-like pathology throughout CNS structures. Similar results have been seen with local injection of a-synuclein into the rodent olfactory bulb, skeletal muscle, and intestinal wall. Bidirectional transport of a-synuclein pathology is likely, because rodent models overexpressing a-synuclein in the mid-brain show a-synuclein pathology in the GI system. PD pathology in the CNS can be induced in rats via GI administration of the neurotoxin rotenone. The spread of administered or rotenone-induced alpha-synuclein fibrils from the gut to the brain of rodents can be abolished by severing the vagal nerve. The transport of pathological a-synuclein from the gut to the brain via the vagal nerve has also been directly observed in rats by utilizing live cell imaging. In concordance with the apparent spread of a-synuclein pathology via the vagal nerve, some epidemiological studies found that a full truncal, but not super-selective, vagotomy was associated with a decreased incidence of PD in humans. Furthermore, pathology has been detected in the GI tract 8 years before the clinical onset of PD (though this finding has not yet been replicated). Despite these findings, the hypothesis that pathology begins in the gut of human patients and then spreads to the CNS has not been documented unequivocally. Several recent studies have, in part, supported this hypothesis of PD. Several recent studies have, in part, supported this hypothesis of PD.
Dendritic cells, which are primarily antigen presenting cells, can also actively sample GI luminal contents/pathogens.97 Dendritic cells migrate between tight junctions and sample the luminal contents directly, including PrPSc.98 Sampled luminal antigens are then presented to T cells of the GALT. α-Synuclein binds to lymphocyte-activated activation gene 3 (LAG3) with nanomolar affinity, and binding to LAG3 appears to drive neuropathology.99 LAG3 is expressed in T cells,100 B cells,101 some dendritic cells,102 and neurons.32 Therefore, misfolded α-synuclein sampled from the intestinal lumen would be sampled and transported to the lymphoid tissue containing LAG3 positive T cells. Subsequent neuronal interaction with T cells could initiate and drive aggregation in the GI tissue.99

Leaky gut could be another gateway for the entry of exogenous prion-like particles, including α-synuclein.103 Normally the semi-permeable barrier through which the GI tract prevents unwanted exposure to luminal contents. However, under conditions of infection, inflammation, and GI disease the gut barrier can become permeable allowing potentially toxic luminal contents to pass the protective epithelial barrier.89 Dietary fiber deprivation has also been shown to degrade the intestinal barrier and enhance pathogen entry.104 Hence, a leaky gut could potentially expose submucosal neurites and/or the underlying immune tissues to luminal contents. Recently, it was found that many PD patients contain activated T cells against the n-terminal of α-synuclein, suggesting PD may be a result of autoimmunity against this antigen.105 The entry of protein particles through leaky gut has been hypothesized as a factor leading to autoimmunity.106 Hence, it is an intriguing possibility that under conditions of a “leaky gut” dietary α-synuclein could enter host and result in an adaptive immune response.

Once in the gut α-synuclein may infect host neurons or immune cells through several mechanisms.107 Passive and/or receptor mediated endocytosis could be involved with the uptake of α-synuclein into the host neurons in the gut. Once α-synuclein binds to either LAG3 receptors on neurons102 or to the membrane via electrostatic interactions it can be endocytosed into the host enteric neurons. LAG3 receptors have been shown to preferentially mediate the uptake of fibrillar forms of α-synuclein.32 From the early endosome α-synuclein is then trafficked to the lysosome. How α-synuclein might escape lysosomal degradation is unknown, but it could be through the ability of α-synuclein fibrils to disrupt lipid bilayer structures.108

α-Synuclein fibrils could also be taken up by cells in the gut via micropinocytosis.109 Cellular uptake of exogenous α-synuclein fibrils by micropinocytosis has been shown to be mediated by cell surface heparin sulfate proteoglycans108 which are ubiquitously expressed in most cell-types throughout the body. Heparin sulfate proteoglycans are crucial for maintaining the intestinal epithelial barrier.109 micropinocytosis.109 Cellular uptake of exogenous α-synuclein is then trafficked to the lysosome.

Dietary sources of α-synuclein

Nearly all of the vertebrate species we eat including Bos taurus (cow), Gallus gallus (chicken), Sus scrofa (pig), and a variety of fish species express α-synuclein.115,116 α-Synuclein protein is highly conserved with 97.9%, 94.3%, and 86.7% sequence homology to the human protein for pig, cow, and chicken, respectively. α-Synuclein is most abundantly expressed in the brain, but is also expressed in many tissues throughout the body with the exception of the liver.7,117-119 Food products containing α-synuclein are most commonly meat products comprised of skeletal muscle.120 Dairy products such as milk may contain trace amounts of α-synuclein, although the unambiguous identification of α-synuclein in the milk proteome is lacking.121 Several less commonly consumed food products such as calves’ brain (i.e., sweet breads) and bone marrow from cows are a rich source of α-synuclein because of the abundant α-synuclein expression in neurons,122,123 and hemopoietic cells/megakaryocytes,124 respectively.

Skeletal muscle of the cow is a source of α-synuclein commonly found in the human diet.125 In such tissue, α-synuclein is most abundantly expressed in neurons, but is also expressed in lower abundance in other cell types, including hemopoietic cells and myocytes.126 The abundant expression of α-synuclein in hemopoietic cells likely accounts for the observed abundance of α-synuclein in various blood cells125 (erythrocytes and platelets). Neurons, blood cells, and myocytes are all found in the skeletal muscle tissues eaten by humans. Therefore, several cell types found within muscle contain α-synuclein and contribute to the total amount of ingested α-synuclein. Peripheral neurons abundantly express α-synuclein126 and therefore peripheral motor axon projections to myocytes119 also contribute to α-synuclein in meat. Erythrocytes are another potential source of α-synuclein125 in meat products as an estimated 2–9 mL blood/kg meat remains following slaughter.127 The presence of alpha-synuclein in the animal food products consumed by humans begs the question: is a potential source for prion-like α-synuclein from the food that we eat?

Vertebrate food products may contain disease-associated α-synuclein

All α-synuclein in dietary meat products contains threonine at the amino acid 53 position, while human α-synuclein has alanine at this position. The A53T mutation of human α-synuclein was the first identified to be associated with PD.128,129 Patients who carry the A53T mutation develop early onset PD. A53T α-synuclein shows impaired lipid binding and enhanced aggregation properties.130-134 The threonine at amino acid 53 of α-synuclein is conserved across most vertebrate species (except several primates), including those animals that are commonly consumed in the human diet135 (Fig. 1). Cows, chickens, and pigs all have threonine at the 53 position of α-synuclein.136 Because A53T human α-synuclein increases aggregation134 and is disease causing in humans,136 the ingestion of a close homolog through diet could increase the likelihood of seeding pathology in the gut (described further in Fig. 1). In support of this idea, α-synuclein from vertebrate species other than human are more prone to rapid fibrillation.137 Alternatively, α-synuclein from other species may be particularly poor, even inhibitory, for the formation of aggregates.137,138 Recent findings utilizing several chimeric α-synuclein proteins suggest an even more complex relationship; where single amino acid sequence differences between α-synuclein fibril seeds and α-synuclein expressed by the host species determine resulting pathology.136-138 Surprisingly, sequence homology between the seed and substrate do not exclusively correspond with pathogenicity139 suggesting pathogenic seeds can cross species. Together, divergence of α-synuclein at amino acid 53 in vertebrate species may have relevance for subsequent cross-species aggregation seeding.

Oxidation in meat products could further promote the formation of prion α-synuclein particles. Numerous oxidative biological products are found in high abundance in PD brains,142-144 suggesting their involvement in PD. Oxidized proteins often form intra/intermolecular covalent bonds with other molecules, including other proteins. α-Synuclein, specifically, can be modified via oxidation to form covalently linked cytotoxic oligomers.145-148 Specifically, dityrosine covalent linkages between α-synuclein molecules form low weight oligomers/protodbrils that have been
shown to serve as a template to seed α-synuclein aggregation. In meat products, proteins become extensively oxidized in a complex process, dependent both on time and environmental catalysts (i.e., exposure to oxygen, enzymes, etc.). Oxidative products from meat products have been implicated in several human age-dependent diseases, including atherosclerosis and several cancers. Homology of α-synuclein protein sequence between human (Homo Sapiens), pig (Sus Scrofa), cow (Bos Taurus), and chicken (Gallus Gallus). Reference bar 4% divergence in sequence homology. Specific amino acid sequences for α-synuclein of all species compared. Red denotes variable positions adjacent to the NAC domain that greatly influence cross-species seeding. Blue denotes variable amino acid positions compared to human α-synuclein

The species barrier
The zoonotic transfer of prions is considered rare because of the “species barrier.” A major factor influencing the species barrier is the degree of sequence homology between the PrP and the host species. Greater sequence homology between the donor and host species PrP increases the likelihood of infectivity. There is a high degree of sequence homology between human and bovine for PrP (86%), as well as for α-synuclein (94.3%; Fig. 1). It is now clear that PrP can be transmitted between species, including from bovine to humans, most likely via consumption of contaminated meat. It is possible for α-synuclein fibrils to seed pathology across species, although the species barrier does limit this process. Similar to PrP, sequence homology between α-synuclein seed and the host protein (i.e., substrate) is proportional to the seeding initiation rate. For example, fibrils grown from full-length human α-synuclein potently seed aggregation of monomeric human α-synuclein, while being relatively ineffective at seeding the aggregation of monomeric mouse α-synuclein. In vivo there is good evidence that mouse α-synuclein actually inhibits the pathogenic fibrillation of human α-synuclein. Therefore, a crucial question arises whether a zoonotic α-synuclein fibril conformer exists, and from what conditions it can be generated. Indeed, several fibrils raised from chimeric mutant α-synuclein proteins have been found to potently seed pathology. Two residues of α-synuclein, namely amino acids 53 and 87, seem to be critical for cross-species seeding of pathology. A single substitution at either of these positions (i.e., to greater resemble the host protein) restores templating characteristics of the fibril in the host species. Correspondingly, pathology is only observed in rodents overexpressing A53T, and not full length, human α-synuclein. It is an interesting idea that humans carrying the A53T mutation may be susceptible to templating from exogenous α-synuclein of other species. However, sequence homology is likely not the only factor driving α-synuclein pathology, because then pathology would be expected to spontaneously arise within any organism expressing α-synuclein. For example, α-synuclein concentration in human saliva has been measured as ~100 pg/mL.
and humans can produce ~500 mL of saliva everyday,172 and therefore, we readily ingest ~50 ng of our own α-synuclein each day. Certainly there are unknown cellular factors besides sequence homology that are required for initiation and formation of pathogenic seeds.139

Epidemiological evidence for a dietary role in PD
Prion disease involving PrPSc, such as CJD or vCJD, can progress rapidly following the onset of initial symptoms, resulting in death within months. In contrast, PD typically progresses slowly over a period of many years, and therefore, the prion properties of α-synuclein are “slow” when compared with PrPSc. The difference in the rates of prion spread makes it difficult to test the hypothesis of a “slow” prion-like α-synuclein, which may not produce clinical symptoms for many years following exposure. Even a “fast” prion like PrPSc doesn’t always cause disease immediately and can remain dormant for prolonged incubation periods, sometimes exceeding as much as 50 years.173 Patients that received contaminated dura mater tissue grafts may not show symptoms anywhere from 1–30 years.174,175 This prolonged incubation makes tracking all prion infections exceedingly difficult. Even if the α-synuclein prion was known, and a patient had been exposed to this prion, it would be difficult to predict the progression and age of onset of the disease.

Despite the challenges of detecting causal prion exposures and a limited understanding of the cross-species capacity of animal α-synuclein, there have been some dietary studies linking intake of animal products with PD risk.176 Heterocyclic amines are toxic compounds found in cooked meat, and there is some evidence that these compounds are elevated in the brains of PD patients.177 Intake of animal fats in adulthood (i.e., 20–30 years prior to onset of the disease) has been associated with a higher PD risk.178 Some studies found that animal fat consumption strongly increased PD risk (2–9-fold higher PD risk), although this remains controversial.179–181 Furthermore, consumption of dairy products has been found to increase the risk of PD, particularly for men.182 A recent study found that in a middle-aged Hawaiian population, milk consumption significantly increased the risk of PD.183 A meta-analysis confirmed a positive correlation between milk intake and risk of PD.184,185 However, several separate studies failed to show a relationship between milk consumption and PD.186 Dietary habits vary geographically, but the incidence of PD is relatively constant when controlling for age,187,188 which suggests, in part, that the disease is non-infectious. Furthermore, there have been documented cases of CJD involving PrPSc in northern India, which is a primarily vegetarian culture,189 and in patients who had limited contact with meat products.190 Therefore, investigation of vegetarian cultures may not be a perfect test of dietary origins of prion-like α-synuclein. Regardless, large-scale epidemiological studies and meta-analyses of dietary contributions to PD may help resolve the controversies related to dietary risk factors for PD.

Causal genes have been identified for PD,191–195 but approximately 70% of PD cases are sporadic,196 and therefore have no clear monogenic cause. However, evidence for oligogenic contributions to PD risk have been determined.194 Furthermore, a combination of risk factors such as sex, age, genetics, and anosmia, when considered together, have a high predictive value for PD.195 Together these results seem to suggest that environmental factors contribute little to PD. Alternatively, these risk factors could mediate susceptibility to environmental factors, such as increasing the exposure duration to prion particles. For example, genetic risk factors for PD particularly affect genes involved in the autophagy and lysosomal pathway, which is responsible for protein clearance and degradation.194,196,197 α-Synuclein aggregates are cleared by lysosomal degradation,198 suggesting that PD pathogenesis involves an aberrant accumulation of misfolded α-synuclein. Similarly, evidence suggests that infection and spread of PrPSc may be augmented by dysfunctional lysosomes.199,200 Presumably genetic factors that impair the clearance of exogenous α-synuclein particles that are entering through the gut and that are capable of seeding aggregated forms will consequently increase the likelihood of subsequent pathology. Therefore, genetics, age, and other PD risk factors could “prime” certain individuals to be more receptive to environmentally-derived α-synuclein pathology. Autoimmune disease and viral infections also show oligogenic predispositions in the immune system that control the host’s response to “invading” pathogens.201,202 Analogously, genetic predisposition to impaired lysosomal clearance of protein buildup may favor the accumulation of an exogenous α-synuclein prion-like protein.

Possible risk factors
Many people regularly consume meat and dairy products, but only a small fraction of the general population will develop PD. Therefore, it is unlikely that eating meat products is an independent cause of PD. The accompaniment of certain risk factors, such as inflammation, aging, genetic and epigenetic factors, may provide an opportunity for unwanted dietary α-synuclein to enter the host, and initiate disease.

Much research has shown that the immune system and inflammation are likely involved in PD.195,203–207 Systemic inflammation is known to enhance infectivity of PrPSc, and subsequently, inflammation may also trigger and/or accelerate the infection and spread of prion α-synuclein.208,209 Specifically, gut inflammation may allow toxic luminal contents to diffuse across intestinal epithelial tight junctions,210 increase aggregation,209 and promote the spread of aggregates in the gut.209 An imbalance in gut microbiome may be involved in PD.211,212,213 Fecal transplants from PD patients enhance the α-synuclein pathology and motor dysfunction seen in A53T mutant mice.71 The effect of the microbiome on PD pathology and neurological health/function has been shown to involve alterations in the intestinal barrier and activation of immune cells, including microglia.212,214,215 Numerous other neurological disorders and neurodegenerative disorders have been linked to imbalances in the microbiome.216 Alterations in short-chain fatty acids produced by specific gut microbes can have pro-inflammatory properties in the gut, reduce gastric motility, and increase permeability through intestinal epithelial cell tight junctions,217,218 effects that could promote entry of dietary α-synuclein particles from the lumen.

In addition, the gut microbiota may be an exogenous source for prion-like particles to trigger α-synuclein aggregation.72,219 Several prion-like peptides expressed by gut microbiota have been identified and found to enhance α-synuclein aggregation in enteric neurons.72 However, it still remains unclear if the bacterial peptides are produced in sufficient quantities and the microbiome can induce PD in human beings.

Aging is the most important risk factor for PD, and may increase the likelihood of invasion of harmful antigens in the GI tract.176 Age-related GI changes such as slowed gastric emptying, decreased GI tract motility, reductions in protective GI mucous, reductions in luminal digestive enzymes would increase the time of exposure to dietary α-synuclein.216 The intestinal epithelial barrier may also become progressively more permeable with age.217 Epigenetic regulation of gene structure/function may play a role in PD.218–221 Specifically, dysregulation of PD genes may be controlled by epigenetic mechanisms.222,223 In contrast to somatic mutations and copy number variations,192,224–226 the epigenome is partially dynamic such that it is modifiable by environmental factors and with aging.227 Age-dependent epigenetic alterations have been shown accumulate more rapidly in PD patients.228 Furthermore, several PD-associated genes, including parkin (PARK2)226,229 and α-synuclein230–232 exhibit dysregulated
activity in PD. Transcript expression of both of these PD genes has been shown to be regulated by the epigenetic mark DNA methylation, and the microbiome and inflammation and aging may affect enteric neuron susceptibility to α-synuclein pathology. In addition, the upregulation of α-synuclein expression in enteric neurons by viral infections may also involve epigenetic alterations. Consequently, epigenetic misregulation of PD risk genes may increase the likelihood that dietary α-synuclein sources could seed pathological α-synuclein aggregation in the GI tract.

CONCLUSION

Emphasis has been placed on the aggregation of endogenous α-synuclein in the gut as an initiating step in PD. However, if α-synuclein spreads from cell-to-cell in a prion-type fashion then the possibility of an exogenous source α-synuclein seeding aggregation is at least plausible. Correspondingly, it seems intuitive that dietary α-synuclein could seed aggregation in the gut. Active sampling of dietary α-synuclein or passive invasion via leaky gut may provide ample opportunity for the entry of misfolded α-synuclein. Exogenous α-synuclein, once it has entered the host tissue, could interact with several receptors important for the spread of α-synuclein pathology, namely LAG3, which are expressed on immune cells and/or neurons of the GI tract. Together, there is a potential prion source, mechanisms for uptake, and a possible explanation for the spread of α-synuclein in the gut (Fig. 2).

Dietary factors are only weakly associated with PD risk, and therefore, if dietary α-synuclein could seed aggregation, other factors (e.g., inflammation, aging and gut permeability) would likely be important co-mediators of the process. Indeed, many questions remain as to whether dietary α-synuclein could initiate PD pathology in the gut. However, it is difficult to ignore the fact that humans ingest α-synuclein whose receptors are expressed in cells responsible for antigen sampling in the gut and possibly in enteric neurons that are linked to the brain. The hypothesis could be directly tested by the oral administration of α-synuclein prion material to α-synuclein overexpressing mice, similarly to what has been done in studies of PrPsc. This study would be a necessary first step in addressing questions regarding the seeding capability of dietary α-synuclein.
REFERENCES

24. Volpicelli-Daley, L. A. et al. Exogenous alpha-synuclein.

22. Roberti, M. J. et al. Imaging nanometer-sized alpha-synuclein aggregates by superresolution microscopy.

20. Deletrier, E. et al. Imaging alpha-synuclein dynamics in live mice.

18. Luk, K. C. et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice.

19. Abounit, S. et al. Tunneling nanotubes spread pathological alpha-synuclein into the intracellular space.

17. Lashuel, H. A., Overk, C. R., Oueslati, A. & Masliah, E. The many faces of alpha-synuclein: from structure and toxicity to therapeutic target.

15. Robert, H. L. & Brown, D. R. Seeking a mechanism for the toxicity of oligomeric alpha-synuclein.

14. Dettmer, U., Newman, A. J., Luth, E. S., Bartels, T. & Selkoe, D. In vivo cross-linking reveals principally oligomeric forms of alpha-synuclein and beta-synuclein in neurons and non-neuronal cells.

13. Noda, K. et al. Pathological alpha-synuclein transmission is mediated by tunneling nanotubes to non-neuronal cells.

12. Lashuel, H. A., Overk, C. R., Oueslati, A. & Masliah, E. The many faces of alpha-synuclein: from structure and toxicity to therapeutic target.

11. Ljung, A. et al. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells.

10. Li, J. Y. et al. Characterization of Lewy body pathology in 12- and 16-year-old intrastriatal mesencephalic grafts surviving in a patient with Parkinson's disease.

9. Li, J. Y. et al. Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death.

8. Pinotti, D. et al. Direct observation of heterogeneous amyloid fibril growth kinetics via two-color super-resolution microscopy.

7. Volpicelli-Daley, L. A. et al. Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death.

6. Stefanis, L. Alpha-synuclein in Parkinson disease.

5. Goldman, J. G. & Postuma, R. Premotor and nonmotor features of Parkinson's disease.

4. Vesterby, A. et al. Imaging alpha-synuclein transmission in the human brain.

3. Paulus, W. & Jellinger, K. The neuropathologic basis of different clinical subgroups of Parkinson's disease.

2. Goldman, J. G. & Postuma, R. Premotor and nonmotor features of Parkinson's disease.

1. Vidalhiet, M. Movement disorders in 2010: Parkinson disease-symptoms and treatments.

31. Braicu, M., Bousset, L., Bieri, G., Melki, R. & Gitler, A. D. Axonal transport and secretion of fibrillar forms of alpha-synuclein, Abeta42 peptide and HTT exon 1.

30. Mao, X. et al. Pathological alpha-synuclein transmission initiated by binding lymphocyte-activation gene 3.

29. Alvarez-Erviti, L. et al. Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission.

28. Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B. & Olanow, C. W. Lewy body pathology.

27. Peelaerts, W. et al. Alpha-synuclein strains cause distinct synucleinopathies after local and systemic administration.

26. Pinotsi, D. et al. Nanoscopic insights into seeding mechanisms and toxicity of alpha-synuclein species in neurons.

25. Dettmer, U. et al. Parkinson-causing alpha-synuclein missense mutations shift the equilibrium of native tetramers to monomers as a mechanism for disease initiation.

24. Dettmer, U. et al. Parkinson-causing alpha-synuclein misense mutations shift native tetramers to monomers as a mechanism for disease initiation.

23. Prusiner, S. B. Prions.

22. Prusiner, S. B. Biology and genetics of prions causing neurodegeneration.

21. Prusiner, S. B. Biology and genetics of prions causing neurodegeneration.

20. Prusiner, S. B. Prions.

19. Prusiner, S. B. Prions.

18. Prusiner, S. B. Prions.

17. Prusiner, S. B. Prions.

16. Prusiner, S. B. Prions.

15. Prusiner, S. B. Prions.

14. Prusiner, S. B. Prions.

13. Prusiner, S. B. Prions.

12. Prusiner, S. B. Prions.

11. Prusiner, S. B. Prions.

10. Prusiner, S. B. Prions.

9. Prusiner, S. B. Prions.
81. Pan-Montojo, F. et al. Progression of Parkinson's disease: a dual-hit hypothesis. *Neuropharmacology* **33**, 599–614 (2007).

82. Braak, H. & Del Tredici, K. Neuroanatomy and pathology of sporadic Parkinson's disease. *Adv. Anat. Embryol. Cell Biol.* **201**, 1–119 (2009).

83. Schneider, S. A. et al. Can we use peripheral tissue biopsies to diagnose Parkinson's disease? *Nat. Rev. Neurol.* **6**, 267–271 (2010).

84. Bode, L., Murch, S. & Freeze, H. H. Heparan sulfate plays a central role in a fallible sentinel? *J. Comp. Neurol.* **522**, 17062–17079 (2005).

85. Yuan, J. & Zhao, Y. Evolutionary aspects of the synuclein super-family and sub-families based on large-scale phylogenetic and group-discrimination analysis. *Biochem. Biophys. Res. Commun.* **479**, 803–809 (2016).

86. Calabrese, G., Mesner, L. D., Foley, P. L., Roven, C. J. & Farber, C. R. Network analysis implicates alpha-synuclein (snca) in the regulation of ovariectomy-induced bone loss. *Sci. Rep.* **6**, 29475 (2016).

87. Nakai, M. et al. Expression of alpha-synuclein, a presynaptic protein implicated in Parkinson's disease, in erythropoietic lineage. *Biochem. Biophys. Res. Commun.* **358**, 104–110 (2007).
Food products as a source for prion-like α-synuclein
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