Several neurodegenerative disorders are characterized by intracellular protein accumulations or inclusions, such as the Lewy bodies of Parkinson's disease. Although little is know about the origin of these accumulations and their specific affect on neurons, it seems likely that as they grow they prove fatal to the cell. Here Michael Brownstein and colleagues speculate on a broad role for alpha synuclein in the development of Lewy bodies and the other protein inclusions associated with neurodegeneration.

Alpha synuclein in neurodegenerative disorders: Murderer or accomplice?

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The recent description of α-synuclein mutations underlying autosomal dominant Parkinson's disease (PD)[1,2] led several groups to wonder[3] whether α-synuclein might be a component of the Lewy body (LB), an intracellular protein inclusion found in affected neurons in PD. Indeed LBs in brains of sporadic PD patients are strikingly synuclein-positive (Fig. 1*a*). When one looks at the masses of LBs and Lewy neurites—neuronal processes engorged with proteinaceous material—in nigral neurons in a PD brain, one is forced to conclude that sooner or later these cells are choked by this accumulation. If true, it seems clear that factors that contribute to the precipitation or aggregation of proteins may initiate or sustain the pathological process. Alternatively, failure of the cell to detect or eliminate waste proteins efficiently could also produce the problem. The make-up of the LB supports these hypotheses. In addition to synuclein, LBs contain ubiquitin, ubiquitin C-terminal hydrolase, and proteasomal subunits—major components of the cellular protein degradation pathway[3–5].

Virtually all misfolded proteins or proteins that have failed to find their partners undergo ubiquitination[6–8]. Proteins tagged with four or more ubiquitin molecules are targeted to the 26S proteasome for destruction. The targeted proteins are then unfolded and threaded through narrow pores into the cylindrical proteolytic chamber of the 20S proteosome. Upon being admitted to this chamber, proteins undergo processive degradation resulting in the production of small peptides, while the ubiquitin tag is recycled.

The fact that members of the ubiquitin/proteasome pathway are found in Lewy bodies, suggests that LB-containing cells may make a futile attempt to dispose of the protein aggregates when they begin to form. Ironically, proteasomal proteins may be incorporated into the LB, adding to the problem rather than solving it. The formation and sequestration of a mass of protein may save the life of the neuron for some time, but so much of the cytosolic space is ultimately filled that the cell can no longer function or, finally, survive. When the neuron dies, its contents must gradually leak out. One wonders whether bits of aggregate are taken up by neighboring cells, spreading the disease.

This brings us to the question posed in the title: What role does α-synuclein play in the hypothetical process outlined above? There are two possibilities. It could be a member of the ubiquitin/proteasome pathway. Then, in sporadic PD, it may be incorporated passively into the Lewy body along with other ubiquitin-related proteins. Alternatively, α-synuclein may never normally interact with the proteasome or its molecular partners except in the course of being turned over. In this event, it may be an innocent bystander that gets incorporated into the growing protein complex, perhaps acting as a molecular "glue" that holds the pathological complex together.

Unfortunately, so little is known about synuclein, that one can only speculate about its physiological role in the nervous system. It is found in nerve terminals, is quite abundant in brain (1% of total protein), seems to be associated with vesicles, and may regulate (i.e., inhibit) phospholipase D2, a phosphoinositide-dependent enzyme that catalyzes the hydrolysis of phosphatidylcholine generating phosphatidic acid[9,10]. Phosphatidic acid, in turn, is thought to be important in controlling vesicular transport and changes in cell morphology. α-synuclein is also known to be a member of a class of "natively unfolded" proteins many of which potentiate protein–protein interactions. Based on this sketchy information, it is tempting to speculate that α-synuclein could be especially prone to self aggregate or to cause the aggregation of other protein species or even intracellular structures. Presumably, these complexes are difficult to dissociate and thread into the proteasome. Thus synuclein—abnormally folded because of a mutation, attracted to another mutant protein, or damaged by oxidation or free radical attack—must be considered a

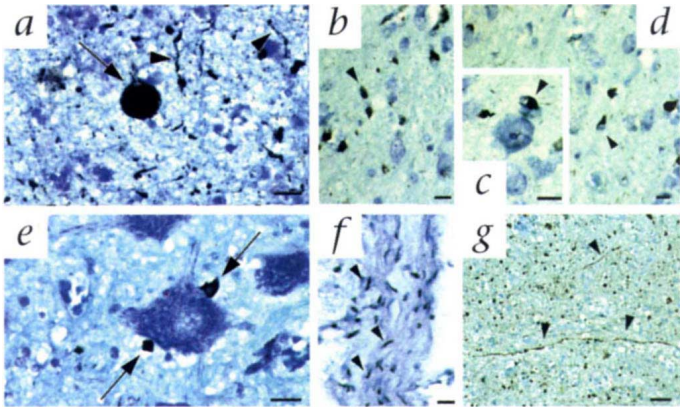

Fig. 1 ***a***, Parkinson's disease; substantia nigra. Many synuclein-containing swollen neurites (arrow heads) and a large Lewy body (arrow) are shown. ***b–d***, Synuclein-positive triangle-shaped inclusion bodies (arrow heads) are common in oligodendrocytes in the frontal cortex of a patient with multiple system atrophy. In *c*, a synuclein containing oligodenrocyte is seen lying next to a small neuron. ***e*** and ***f***, Synuclein-stained glial cells in tissue from an ALS patient. In *e* a large motor neuron is in close contact with two synuclein-positive glial cells (arrows). *f* shows a portion of the spinal nerve, where many of the Schwann cells are synuclein stained (arrow heads). ***g***, Synuclein-positive "neuritic threads" (arrow heads) in the hippocampus of a patient with Alzheimer's disease. Scale bars: *a*, 25 μm; *b*, 20 μm; *c–f*, 10 μm and *g*, 50 μm.

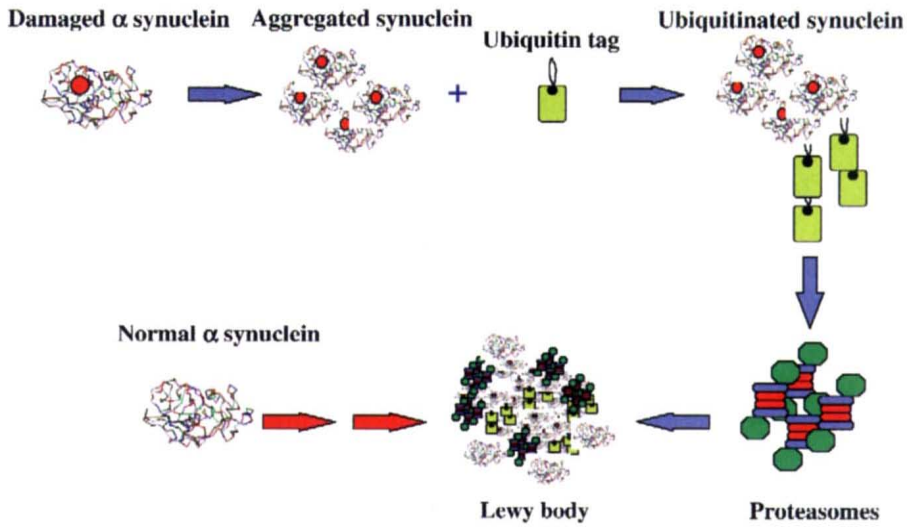

Fig. 2 Schematic representation of an hypothesis to explain the observed accumulation of α-synuclein in Lewy bodies. Damaged α-synuclein molecules adopt an abnormal conformation and aggregate. The aggregates resist destruction by the 26S proteasome and form a seed around which other proteins accumulate. Normal α-synuclein molecules are recruited into the developing protein complex.

prime suspect in the case of the Lewy body (Fig. 2). Looking for factors that cause α-synuclein particles to begin to develop in cultured cells could be an important step towards understanding PD. The list of such "factors" must include mutations in proteins in the ubiquitin/proteasome pathway. These could cause molecules like synuclein to begin to accumulate in the cytosol.

It is possible that other neurodegenerative disorders characterized by intracellular inclusion bodies are caused by synuclein aggregation. Multiple system atrophy (MSA), is a sporadic, progressive illness with an average age of onset in the fifth decade of life[11,12]. As its name suggests, neuronal loss and gliosis in this illness are widespread. The striatum (especially the putamen), substantia nigra, locus coeruleus, inferior olive, pons, cerebellum, and intermediolateral cell columns of the spinal cord and Onuf's nucleus in the spinal cord are preferentially involved. As a result, patients suffer from a variety of symptoms including parkinsonism, cerebellar or pyramidal signs, and autonomic failure. While the spectrum of symptoms varies from person to person, the brains of all affected individuals exhibit abundant oligodendroglial and scattered neuronal intracytoplasmic argyrophilic inclusions[13]. The glial cytoplasmic inclusions (GCIs) found in MSA can be stained with antibodies directed against ubiquitin[14] and, less consistently, tau. While GCIs are found in other illnesses (e.g., corticobasal degeneration, Steele-Richardson-Olszewski syndrome, and SCA1), they are relatively sparce, and their appearance and distribution seem to differ from those seen in MSA.

We used an anti-synuclein antibody to stain sections from brains of MSA patients and found that α-synuclein is a component of the GCI (Fig. 1*b–d*). Rare intraneuronal inclusions were stained as well. The distribution and features of the structures stained with the synuclein antibody were similar to those of the inclusions stained when ubiquitin antibodies[14] or silver impregnation[13] were employed.

MSA was not the only disease characterized by synuclein-positive glial inclusions. We also observed them in astrocytes and Schwann cells in the spinal cords of patients who had died from the motor neuron disorder amyotrophic lateral sclerosis (ALS) (Fig. 1*e*&*f*). Symptoms of ALS include weakness in one or two limbs or in the bulbar musculature. As the disease progresses, one sees muscular wasting and fasciculation, and patients typically survive for only two or three years. A dominantly inherited form of ALS is known to be caused by mutations in copper/zinc superoxide dismutase[15]. This probably accounts for fewer than 2% of all cases (20% of familial cases), however. The etiology of the disease in the remainder of pateints is unknown. Our data suggest that the neuronal death seen in ALS (and MSA) could result from impaired function or loss of glial supporting elements as in multiple sclerosis. Alternatively, what we see could be a glial response to neuronal damage. Of further interest, brains of AD patients also exhibited synuclein-positive inclusions—this time in "neuropil threads" or neuritic tangles in layers 5 and 6 of the hippocampus (Fig. 1*g*).

Protein aggregation in MSA, ALS and Alzheimer's disease seems to be a specific consequence of neuronal damage; brains of patients with multi-infarct dementia have no synuclein inclusions[16], and the tau-positive neuronal inclusion bodies in Pick's disease have no synuclein associated with them[16]. Thus, synclein is not an obligatory component of protein bodies. Clearly the ubiquitin-positive intranuclear inclusions seen in Huntington's[17] and Machado-Joseph[18] disease should be examined and these diseases placed on or removed from the list of "synucleinopathies."

Parkinson's disease, multiple system atrophy, amyotropic lateral sclerosis, and Alzheimer's disease affect different areas of the central nervous system and different cell types. Why these cells accumulate synuclein and other cells with inclusion bodies—such as the neurons in Pick's disease—do not, remains to be seen. Certainly there is much work to be done before convicting α–synuclein, but if the glove fits...!

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With the identification of several Alzheimer disease (AD)-associated genes, genetic testing for AD is possible. However, as for some other-late onset disorders, the ethical and social implications of such tests are considerable. In response to the commercial availability and the likely increased use of AD genetic tests, The Stanford Program in Genomics, Ethics, and Society formed a broad-based working group to examine the pros and cons of testing. Here they present their recommendations.

Genetic testing and Alzheimer disease: Has the time come?

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Several genes associated with Alzheimer disease (AD) have been identified, including three—*APP*, *PS1* and *PS2*—in which autosomal dominant, highly penetrant mutations are associated with the early-onset familial form of AD. A susceptibility gene, *APOE*, is associated with an increased risk of AD when one or two e4 alleles (APOE4) are inherited. Several other possible genetic links have been proposed and the search for additional AD genes continues.

Predictive and diagnostic genetic tests for *PS1* mutations and diagnostic tests for *APOE* alleles are already commercially available and other tests are being developed. Thus, genetic testing for AD exists for clinical use and is also likely to be used more often to stratify subjects in AD research, both in trials of preventive agents and in tests of new therapies. Widespread use of these tests raises a number of complex ethical, legal and social questions, many of which are likely to apply to other adult-onset genetic diseases.

APOE testing was initially offered for disease prediction but was subsequently withdrawn from the market[1]. The controversy surrounding this experience, and continuing concern about inappropriate use, prompted several professional groups to publish consensus statements on the clinical application of genetic testing for AD[2–5]. This report expands on previous policy deliberations on AD genetic testing and on genetic testing more broadly[6–7].

The Working Group was multidisciplinary and not dominated by one particular professional perspective. Members included faculty and health professionals from Stanford University and nearby institutions, and representatives from San Francisco Bay area AD and caregiving organizations. The Group met twice monthly for nine months. Initial meetings consisted of presentations by experts in epidemiology, genetics, psychiatry, geriatrics, commercial genetic testing technologies, family and caregiver issues, aging health policy, risk assessment and medical decision making, and cross-cultural understandings of AD. Subgroups were formed to draft sections of the report, which were then debated by the entire Group. Resulting recommendations were presented for comment at a conference at Stanford University in October 1997 (ref. 8), after which the report was finalized based on feedback from participants.

These recommendations are shaped by consideration of the medical and genetic characteristics of AD, the distinct social and political history that accompanied its emergence as a disease entity, and the meanings of dementing illness, aging and death in Western culture. The recommendations that concern when genetic testing for AD is appropriate are particularly complex and merit further explanation.

Fewer than 2% of AD patients are thought to carry one of the highly penetrant mutations associated with the disease[10]. They and their family members may find some value in genetic testing, to help confirm an AD diagnosis or for predictive purposes. For people who test positive, there are no proven measures for the prevention of AD, and available treatments have only marginal value. However, several interventions to delay or prevent the onset of symptoms are under investigation (for example, estrogen replacement, anti–inflammatory drugs and vitamin E) and some treatments of limited benefit are available. There are also a number of non–medical, life-planning steps that the person may want to take. For people who test negative for a mutation, there may be the benefit of psychological relief. When properly counseled, some adults at risk for carrying a high-penetrance mutation will reasonably choose to be tested and others will reasonably decline. The ethical, legal and social dilemmas raised by this kind of testing are similar to problems already faced in the introduction of testing for other late-onset genetic disorders, such as Huntington's disease[11]. Testing programs must include careful pre- and post-test genetic counseling and follow-up care. Governments must provide adequate guarantees of privacy, safeguards against loss of health insurance and employment discrimination, and as-