Vesicle localization and exocytosis of alpha-synuclein: implications in Parkinson's disease

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Abnormal folding and accumulation of α-synuclein is implicated in several neurological disorders including Parkinson's disease. α-synuclein is a classical cytosolic protein and it was generally assumed that the pathogenic effects of the protein were limited to the cytoplasm of single cells. However, we have recently demonstrated that a portion of cellular α-synuclein translocates into the lumen of vesicles and is secreted from cells via unconventional, ER/Golgi-independnet exocytosis, raising the possibility that the protein exerts extracellular pathogenic actions as well. Subsequent studies with various deletion and point mutations suggest that the vesicle entry of this protein might not be determined by a specific sequence. Rather, it appears that conformational defects might be the signal for vesicle entry and subsequent secretion of this protein. Consistent with this interpretation, the level of vesicular and extracellular α-synuclein (and aggregates) is increased by protein conformational stresses, such as oxidative stress and proteasomal inhibition. Incorporation of amino acid analogs, which induces the production of defective proteins, also increased α-synuclein levels in vesicles and in the conditioned medium. Given these results, we propose that the conformation properties, not the specific sequence, of α-synuclein are the determining factors for translocation into vesicles and secretion. We speculate that this may represent a novel clearing mechanism to remove defective α-synuclein from cells.

Extracellular α-synuclein aggregates have been shown to be neurotoxic, posing a challenge to any cell exposed to them. We show that extracellular α-synuclein aggregates can be removed by receptor-mediated endocytosis and subsequent proteolytic degradation. The rate of aggregate uptake and degradation is much greater in microglia than neuronal cells, suggesting microglia are the major scavenging cells. This clearance mechanism is dependent on the extracellular α-synuclein assembly state; only aggregated forms are removed by endocytosis, monomers freely diffuse across the plasma membrane. These results suggest that cell-mediated uptake and degradation of α-synuclein aggregates, especially in microglia, is a major mechanism for clearance of these aggregates from the extracellular space, maintaining an aggregate-free microenvironment.

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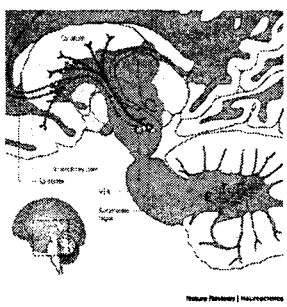
Parkinson's Disease (PD)



Primary manifestations
Resting tremor
Rigidity
Akinesia (hypokinesia, bradykinesia)
Gait disorder

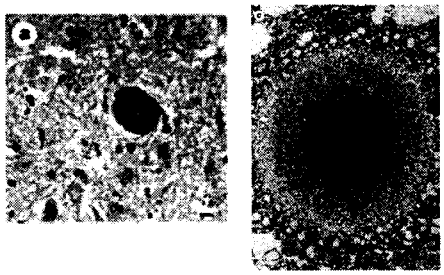
Secondary manifestations
Depression
Cognitive dysfunction and dementia
Autonomic dysfunction: constipation, dysphagia, etc.
Sensory symptoms: paresthesia, loss of olfactory sense, etc.
Sleep disturbance
Speech and voice

It's is estimated that approximately 1.5 million are affected by the disease in US.

Parkinson's Disease (PD)

Pathological feature

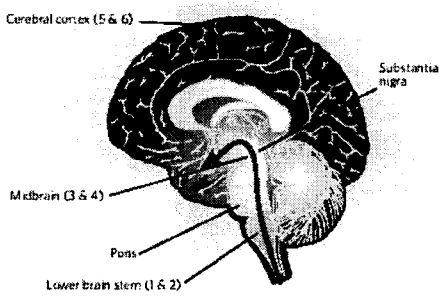
Selective loss of nigrostriatal dopaminergic neurons
Proteinateous inclusion bodies: Lewy bodies and Lewy neurites



Neuropathology of PD is *not* restricted to substantia nigra:
Locus coeruleus, raphe nuclei, nucleus basalis of Meynert, cingulate gyrus, etc.

The loss of motor control in PD may be the late-stage symptoms of a long-term degenerative condition.

A FRESH VIEW OF PARKINSON'S DISEASE

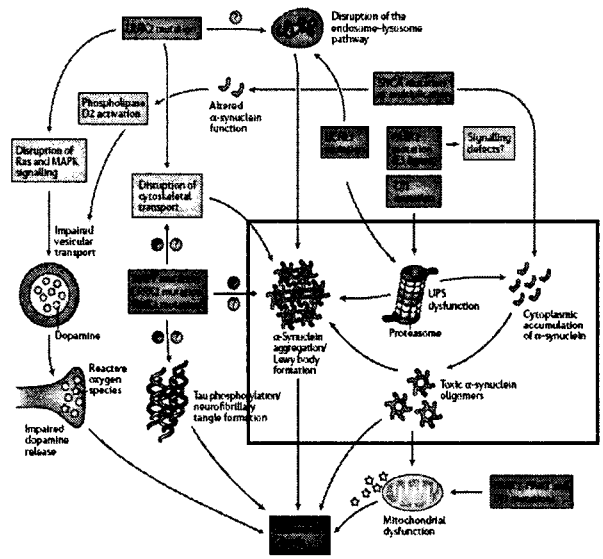


Nature, 437, 1220-1222

Braak et al. (2003) Neurobiol. Aging 24, 197

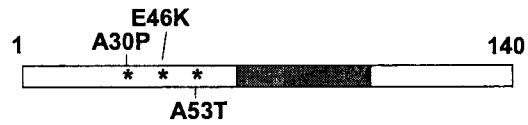
Genes and loci for familial PD

Locus	Position	Protein	Inheritance pattern	Pathology	Clinical features
PARK1	4q21-q23	α-synuclein	AD (high penetrance)	LB Tau pathology	Early onset, dementia Aggressive course Lower prevalence of tremor
PARK2	6q25.2-q27	Parkin	AR	Mostly LB negative Tau pathology	Early or juvenile onset Slow progression
PARK3	2p13	Unknown	AD (incomplete penetrance)	LB β-amyloid tau pathology	Late onset, classical PD Dementia in some cases Rapid progression
PARK5	4p14	UCH-L1	Likely AD	Unknown	Classical PD
PARK6	1p36-p35	PINK1	AR	Unknown	Early onset Slow progression
PARK7	1p36	DJ-1	AR	Unknown	Early onset slow progression
PARK8	12p11.2-q13.1	LRRK2	AD (incomplete penetrance)	LB negetive Tau pathology	Classical PD, dementia and amyotrophy in some cases
PARK9	1p36	Unknown	AR	Unknown	Juvenile onset multisystemic involvement
PARK10	1p32	Unknown	Non-Mendelian	Unknown	Classical PD (Icelandic population)
PARK11	2q36-q37	Unknown	Non-Mendelian	Unknown	Classicla PD (sib pairs study)
Pending	2q22-q23	NURR1	Likely AD	Unknown	Classical PD
Pending	Xq	Unknown	Non-Mendelian	Unknown	Classical PD (sib pairs study)



Genetic linkage between α-synuclein and PD

1. **Missense mutations in α-synuclein have been linked to FPD.**



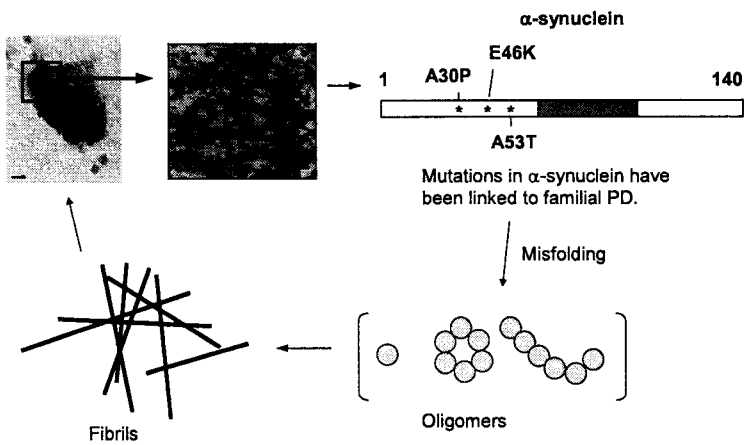
Polymeropoulos *et al.* (1997) *Science*
Kruger *et al.* (1998) *Nature Genetics*
Zarranz *et al.* (2004) *Ann Neurol*

2. **Triplication of the region containing α-synuclein in Iowan kindred.**

   4 functional copies, 2-fold increase in mRNA and protein levels.

Singleton *et al.* (2003) *Science*

Parkinson's disease, Lewy bodies and α-synuclein

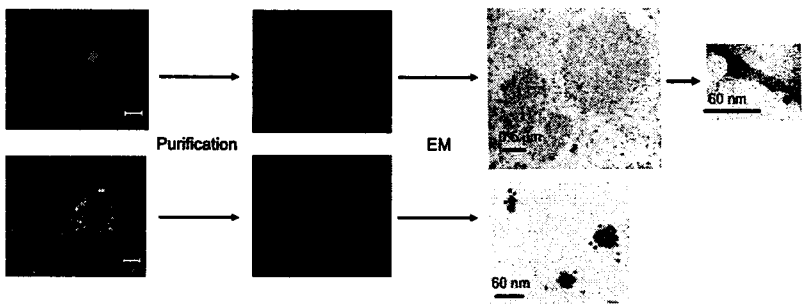


α-Synuclein forms fibrils spontaneously in vitro.

Animal models of α-synucleinopathies.

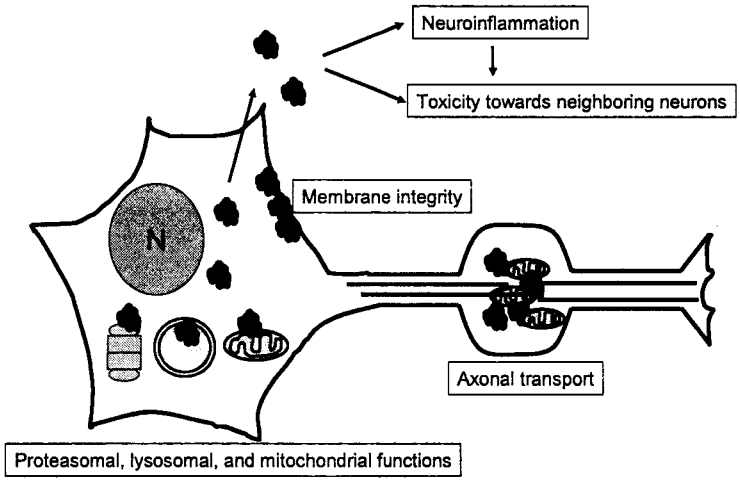
Model	*Expression method*	*Neuron loss/DA*	*Inclusions*	*Behavioral symptoms*
Mice	Transgenic	+/-	+	+
Rats	Viral vector	+/+	+	+
Non-human primates	Viral vector	+/+	+	+
Drosophila	Transgenic	+/+	+	+
C.elegans	Transgenic	+/+	+	+

Cellular α-synuclein aggregates

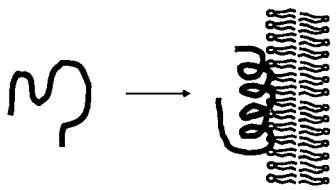


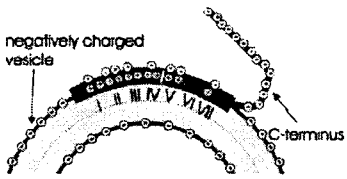
Lee and Lee (2002) J. Biol. Chem.

How does protein aggregation cause neurodegeneration?



α-Syn binds synthetic phospholipid liposomes in helical form.



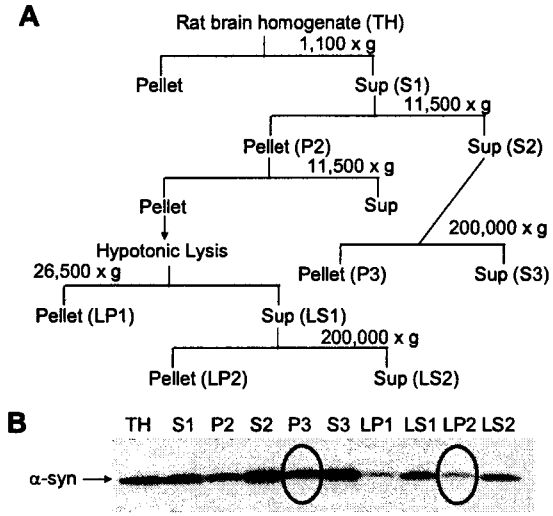


Jao et al. (2004) PNAS, 101, 8331

7
GLSKAKEGVVA
AAEKTKQGVAE
AAGKTKEGVLY
VGSKTKEGVVHGVAT
VAEKTKEQVTN
VGGAVVTGVTA
VAQKTVEGAGS
87

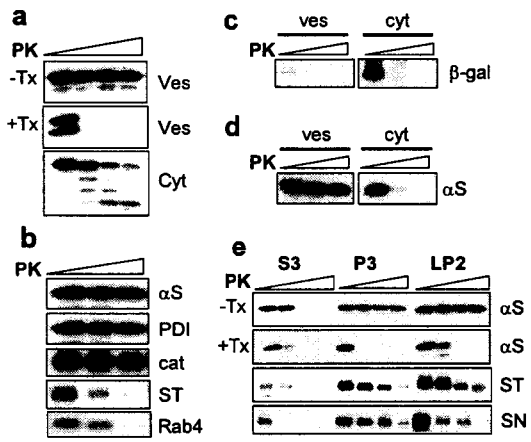
- Extended α-helix formation through the N-terminal repeat region
- Highly stable interaction; very slow dissociation
- Selectivity to negatively charged vesicles; ionic interactions
- Spontaneous interaction; does not require a co-factor

α-Syn is present in both cytosolic and vesicle fractions.



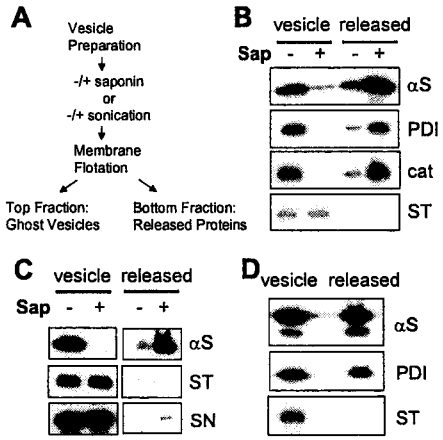
Lee et al. (2002) JBC 277, 671

α-Synuclein is present in the lumen of vesicles. (I)



Lee et al. (2005) J. Neurosci. 25, 6016

α-Synuclein is present in the lumen of vesicles. (II)

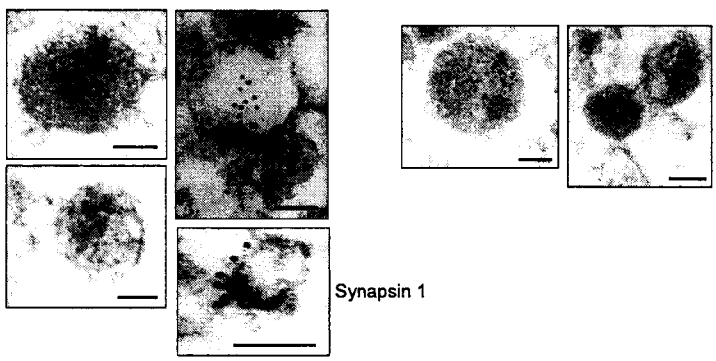


Lee et al. (2005) J. Neurosci. 25, 6016

α-Synuclein is present in the lumen of vesicles. (III)

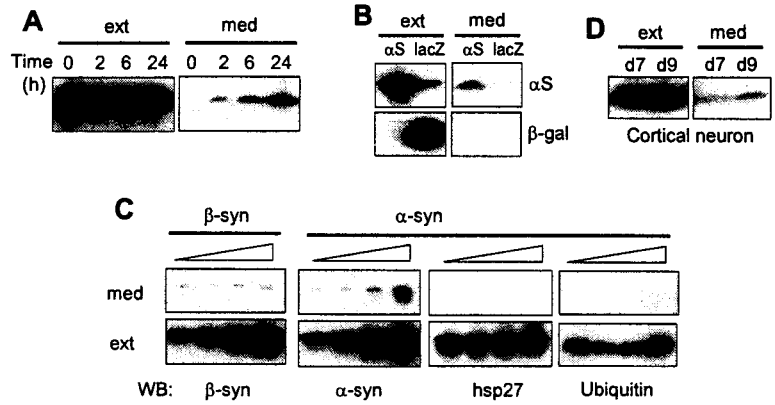
Rat Brain Microsomes

Human Neuroblastoma Microsomes



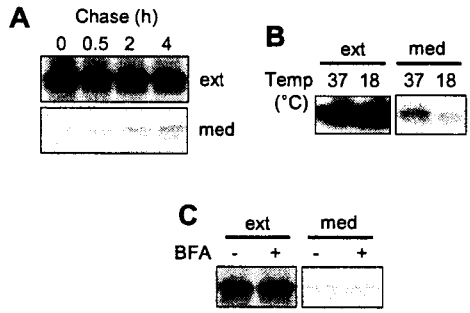
Lee et al. (2005) J. Neurosci. 25, 6016

α-Synuclein is released from cells.



Lee et al. (2005) J. Neurosci. 25, 6016

α-Synuclein is released from cells via vesicle-mediated exocytosis.

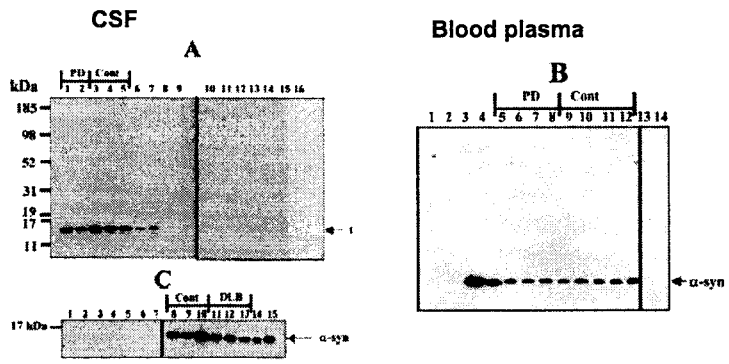


Lee et al. (2005) J. Neurosci. 25, 6016

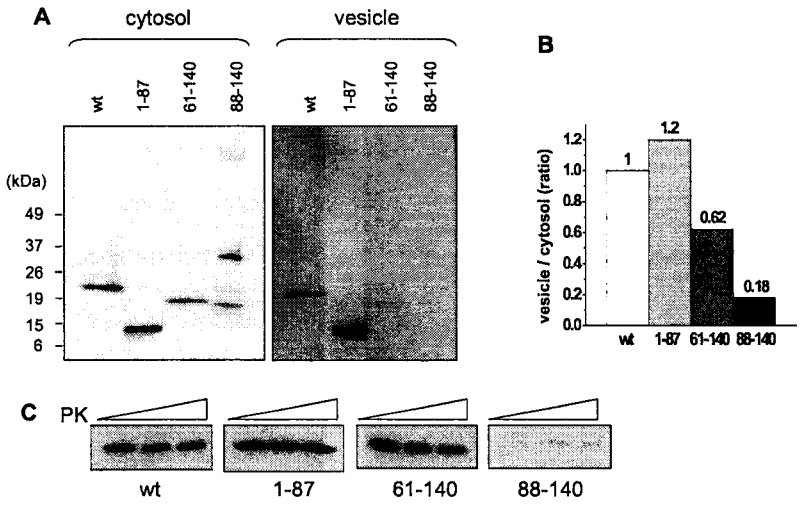
The FASEB Journal express article 10.1096/fj.03-0098fje. Published online August 15, 2003.

α-Synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma

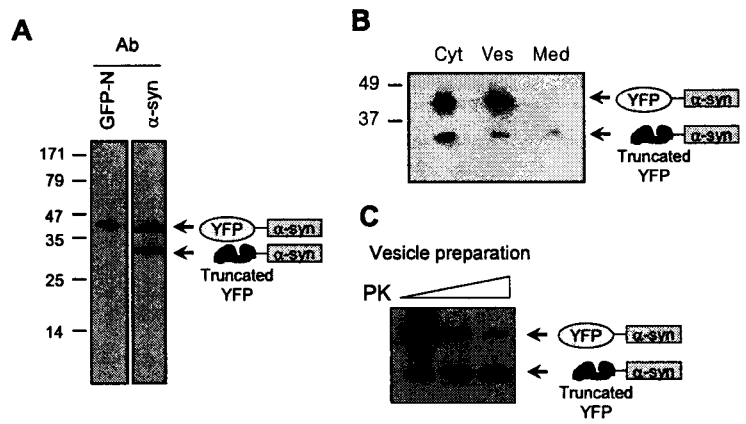
Omar M. A. El-Agnaf,*[,††] Sultan A. Salem,*[,††] Katerina E. Paleologou,* Leanne J. Cooper,* Nigel J. Fullwood,* Mark J. Gibson,[†] Martin D. Curran,[‡] Jennifer A. Court,[§] David M. A. Mann,[‖] Shu-ichi Ikeda, Mark R. Cookson,[¶,**] John Hardy,[¶,**] and David Allsop*



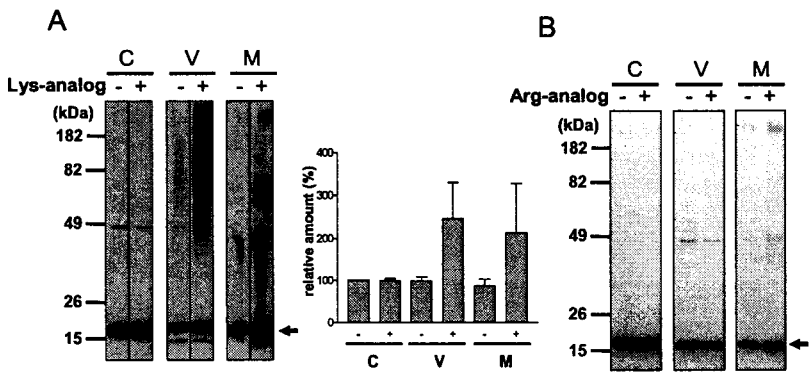
Vesicle entry of various α-syn fragments



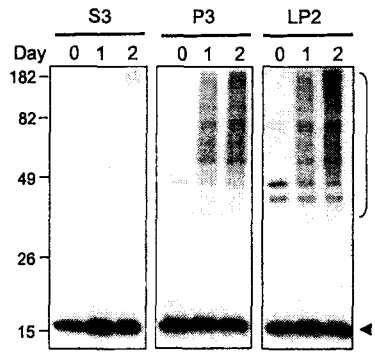
Effects of tagging with a stable globular protein



Effects of misfolding induced by amino acid analog incorporation



α-Synuclein aggregates more rapidly in vesicles than in cytosol.



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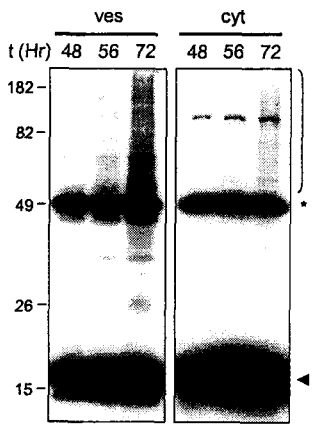
α-Synuclein aggregates more rapidly in vesicles than in cytosol in intact cells.



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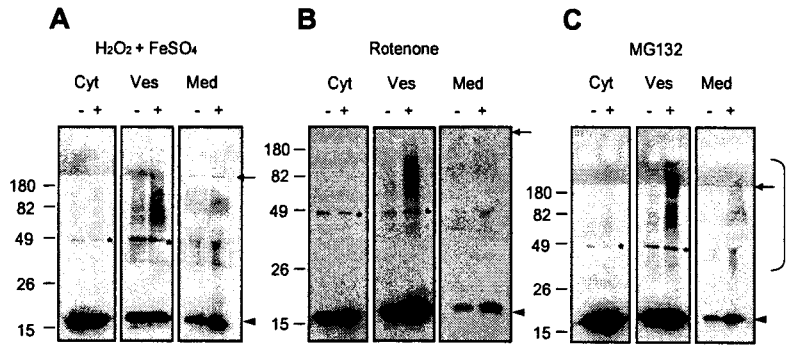
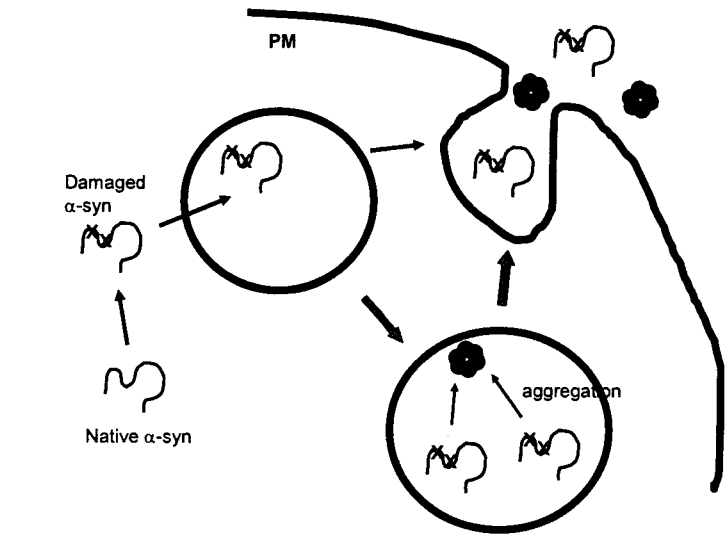
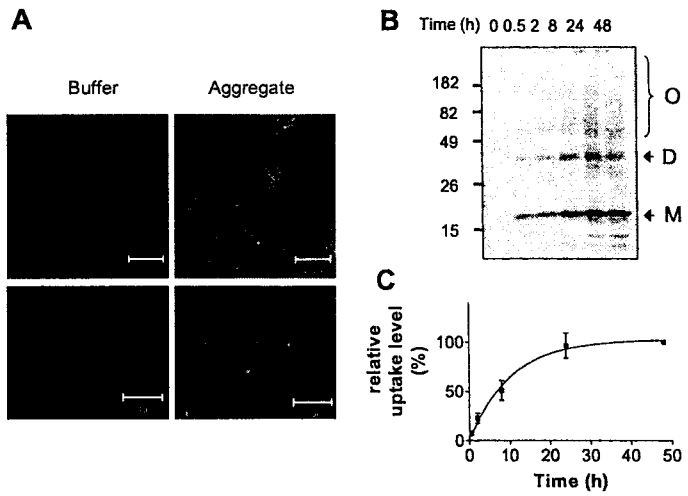
Stress-induced aggregate formation in vesicles and secretion



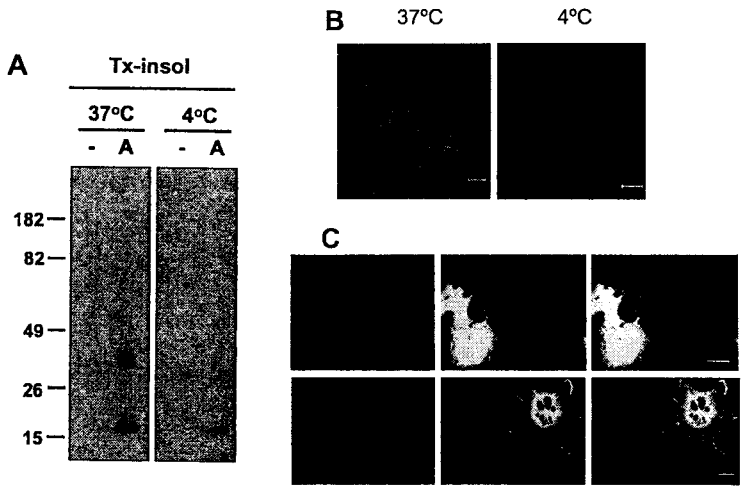
Exocytosis of damaged α-syn as a quality control mechanism?



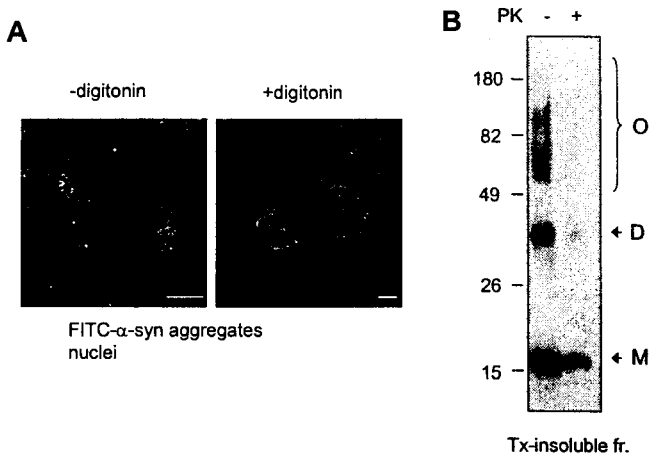
Internalization of extracellular α-syn aggregates into neuronal cells



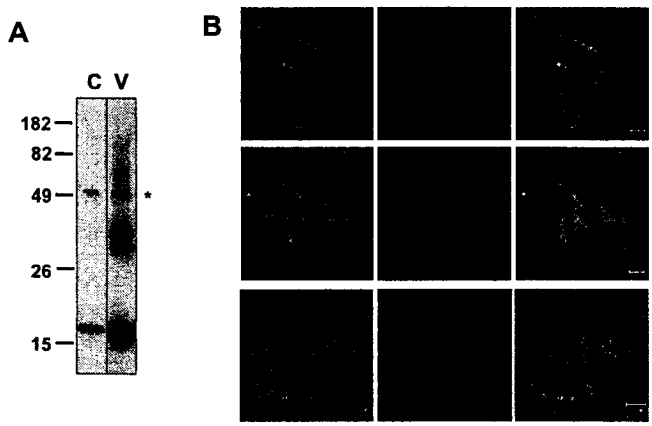
Internalization of α-syn aggregates by endocytosis



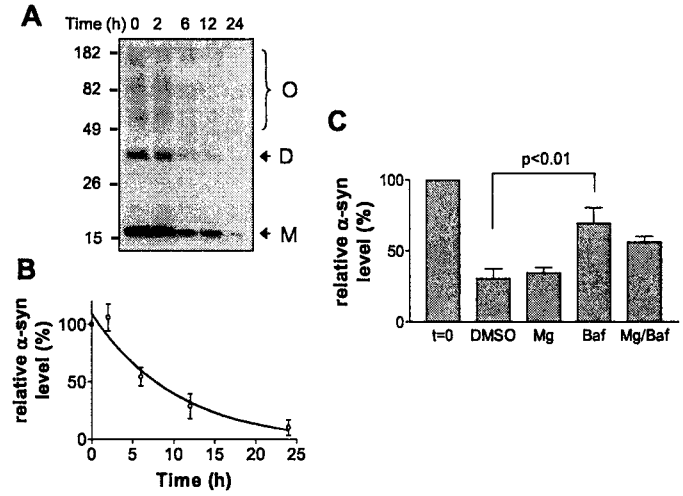
Receptor-mediated endocytosis of α-syn aggregates



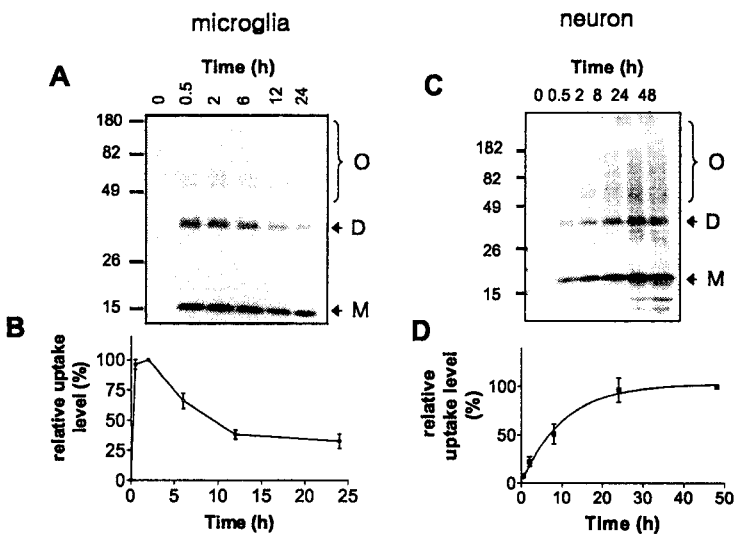
Localization of internalized α-syn aggregates in endosomal compartments



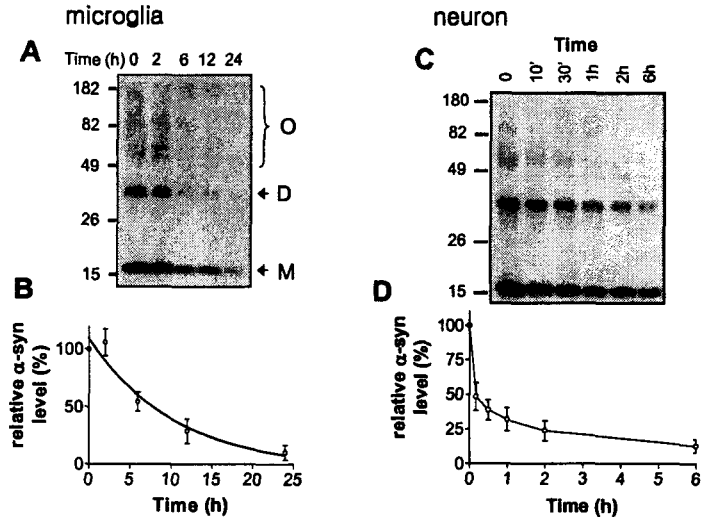
Lysosomal degradation of internalized α-syn aggegates



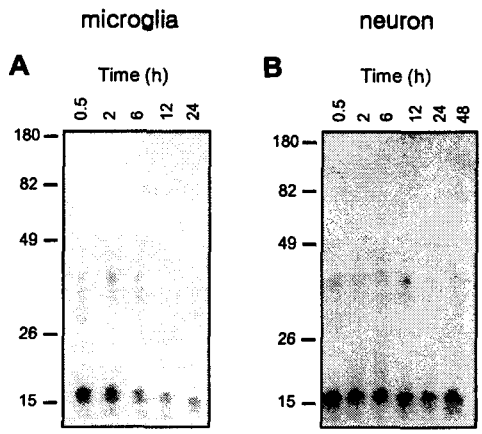
Kinetics of a-syn aggregate uptake in microglia



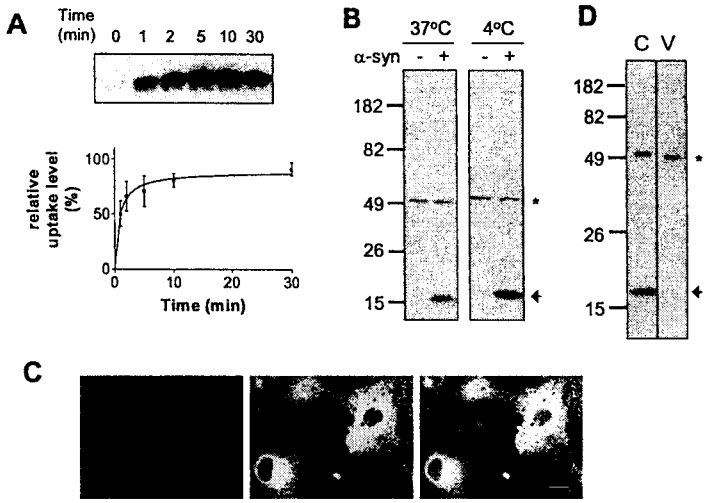
Degradation kinetics of internalized a-syn aggregate in microglia



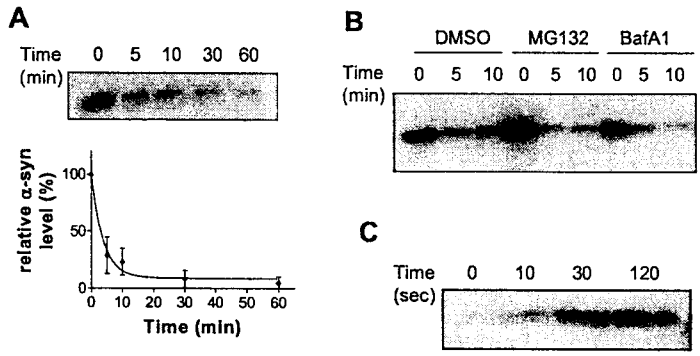
Clearance kinetics of extracellular α-syn aggregates

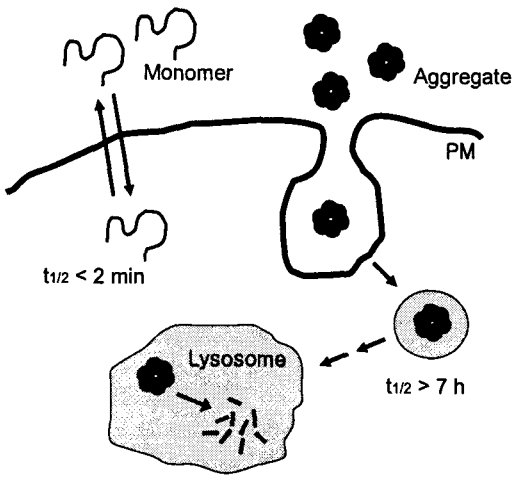


Non-endocytic entry of α-syn monomers



Free diffusion of α-syn monomers across the plasma membrane





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