

Addendum

Ubiquitination of α -synuclein and autophagy in Parkinson's disease

Simone Engelender

Department of Pharmacology; The B. Rappaport Faculty of Medicine and Institute of Medical Research; Technion—Israel Institute of Technology; Haifa, Israel

Key words: Parkinson's disease, Lewy bodies, α -synuclein, autophagy, monoubiquitination, inclusion bodies, synphilin-1

α -synuclein is mutated in Parkinson's disease (PD) and is found in cytosolic inclusions, called Lewy bodies, in sporadic forms of the disease. A fraction of α -synuclein purified from Lewy bodies is monoubiquitinated, but the role of this monoubiquitination has been obscure. We now review recent data indicating a role of α -synuclein monoubiquitination in Lewy body formation and implicating the autophagic pathway in regulating these processes. The E3 ubiquitin-ligase SIAH is present in Lewy bodies and monoubiquitinates α -synuclein at the same lysines that are monoubiquitinated in Lewy bodies. Monoubiquitination by SIAH promotes the aggregation of α -synuclein into amorphous aggregates and increases the formation of inclusions within dopaminergic cells. Such effect is observed even at low monoubiquitination levels, suggesting that monoubiquitinated α -synuclein may work as a seed for aggregation. Accumulation of monoubiquitinated α -synuclein and formation of cytosolic inclusions is promoted by autophagy inhibition and to a lesser extent by proteasomal and lysosomal inhibition. Monoubiquitinated α -synuclein inclusions are toxic to cells and recruit PD-related proteins, such as synphilin-1 and UCH-L1. Altogether, the new data indicate that monoubiquitination might play an important role in Lewy body formation. Decreasing α -synuclein monoubiquitination, by preventing SIAH function or by stimulating autophagy, constitutes a new therapeutic strategy for Parkinson's disease.

Lewy Bodies and Parkinson's Disease

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. It is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra and accumulation of cytoplasmic inclusions termed Lewy bodies in surviving neurons.¹ Mutations found in familial forms of PD place α -synuclein as a major player in the disease.² Although α -synuclein has also been recognized as the major component of Lewy bodies in the sporadic

form of the disease,³ little is known about the events that trigger their formation as well as their role in the death of dopaminergic neurons.

The ability of α -synuclein to promote Lewy body formation is supported by its propensity to aggregate and fibrillate in vitro.⁴ Nonetheless, most live models that overexpress α -synuclein do not develop classical Lewy bodies,⁵ indicating that specific post-translational modification(s) or interaction with other proteins is required for triggering α -synuclein aggregation into more organized inclusions. In agreement, Lewy bodies contain abundant phosphorylated as well as monoubiquitinated and nitrated α -synuclein.⁶⁻¹⁰ Also, Lewy bodies contain additional proteins, including UCH-L1, Cdk5 and the α -synuclein interacting proteins, synphilin-1 and 1A.¹¹⁻¹⁴ Understanding the mechanisms that promote the aggregation of α -synuclein into Lewy bodies will shed light on the mechanisms involved in triggering PD.

Role of Ubiquitin in α -Synuclein Aggregation

Different reports have shown that part of α -synuclein purified from Lewy bodies is monoubiquitinated.⁶⁻⁸ Yet, how monoubiquitination modulates α -synuclein aggregation and the E3 ubiquitin-ligase responsible for α -synuclein monoubiquitination has remained elusive. We have previously shown that the E3 ubiquitin-ligase SIAH interacts with and monoubiquitinates α -synuclein in vitro.¹⁵ In addition, we have shown the presence of SIAH in Lewy bodies of PD patients,¹⁵ indicating that SIAH may represent a novel component of the ubiquitin-proteasome system involved in PD.

It has been recently reported that SIAH interacts with and monoubiquitinates α -synuclein in vivo as well.^{16,17} Mass spectrometry analysis reveals that SIAH monoubiquitinates α -synuclein at several lysines, including 12, 21 and 23,¹⁶ which were previously found to be monoubiquitinated in purified Lewy bodies.⁸

A reason for skepticism regarding a possible role of α -synuclein ubiquitination in Lewy body formation comes from the observation that only a small fraction (about 10%) of α -synuclein is ubiquitinated in Lewy bodies.⁶ We recently raised the possibility that monoubiquitination of α -synuclein by SIAH may work as a seed for further protein aggregation. Accordingly, we found that monoubiquitination promoted by SIAH leads to a marked increase in the in vitro and in vivo aggregation of α -synuclein. At the electron microscopy level, monoubiquitination of α -synuclein induces robust formation of amorphous α -synuclein aggregates (Fig. 1).¹⁶ In dopaminergic cells, monoubiquitination of α -synuclein leads to significant formation of inclusions,¹⁶ suggesting that monoubiquitinated α -synuclein might play a primary role in the formation of Lewy bodies.

Correspondence to: Simone Engelender; Department of Pharmacology; The B. Rappaport Faculty of Medicine and Institute of Medical Research; Technion—Israel Institute of Technology; Bat-Galim, Haifa 31096 Israel; Tel.: 972.4.829.5416; Fax: 972.4.829.5419; Email: simone@tx.technion.ac.il

Submitted: 01/10/08; Revised: 01/17/08; Accepted: 01/18/08

Previously published online as an *Autophagy* E-publication:
www.landesbioscience.com/journals/autophagy/article/5604

Addendum to: Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, Liani E, Avraham E, Engelender S. Monoubiquitination of α -synuclein by SIAH promotes its aggregation in dopaminergic cells. *J Biol Chem* 2007; Epub ahead of print.

Monoubiquitination of α -synuclein by SIAH is not altered by the PD-disease mutations A30P, A53T and E46K.¹⁶ However, monoubiquitinated α -synuclein A53T mutant aggregates more than the wild-type protein within dopaminergic cells,¹⁶ which is in accordance with the increased ability of the α -synuclein A53T mutant to aggregate.⁴ Thus, it is probable that monoubiquitination works as a common mechanism to increase α -synuclein aggregation, either in the absence or in the presence of mutations. In agreement, Nonaka et al showed similar levels of α -synuclein monoubiquitination by an unspecified E3 ubiquitin-ligase in both wild-type and disease mutants.¹⁸ On the other hand, Liu et al recently found that the α -synuclein A30P mutant was not efficiently ubiquitinated by SIAH.¹⁷ The reason for this apparent discrepancy is not clear.

Are Inclusions Toxic or Neuroprotective?

Different PD cell models are shown to develop inclusions that are cytoprotective.¹⁹ For instance, the α -synuclein binding protein synphilin-1 forms cytosolic inclusions, in the absence or in the presence of overexpressed α -synuclein, that protect cells from death.^{15,20} Synphilin-1A, an isoform of synphilin-1, also forms neuroprotective inclusions.¹² On the other hand, inclusions formed mainly by monoubiquitinated α -synuclein are toxic to cells (Fig. 1).¹⁶ This finding could be related to the amorphous nature of the monoubiquitinated α -synuclein aggregates we observed.¹⁶ In agreement, oligomeric or protofibrillar forms rather than fibrillar forms of α -synuclein are believed to play a toxic role in the disease.²¹ The presence of amorphous aggregated proteins in the core of Lewy bodies and fibrils at their periphery²² imply that Lewy bodies may be toxic to cells at their initial stages of formation. In fact, soluble aggregated α -synuclein was recently shown to mediate dopaminergic neurotoxicity in *Drosophila*.²³ An interesting possibility is that inclusions containing aggregated monoubiquitinated α -synuclein are toxic to cells and that coaggregation of additional PD-related proteins, such as synphilin isoforms, or the accumulation of fibrillar forms of α -synuclein, may counteract this toxicity. Thus, it is possible that Lewy bodies may promote either cell death or protection, depending on their stage of maturation.

Monoubiquitination and Autophagy

Several studies support an involvement of proteasomal, lysosomal and autophagic pathways in PD. The proteasome activity is decreased in the substantia nigra of PD patients²⁴ and Gaucher disease (a lysosomal storage disease) is associated with a higher incidence of PD.²⁵ Also, different lysosomal storage diseases promote autophagic dysfunction and accumulation of ubiquitinated protein inclusions.²⁶ Moreover, expression of the α -synuclein A53T mutant induces autophagic cell death.²⁷ Regarding α -synuclein degradation, there is still no consensus about the pathway responsible for its degradation, whether it depends on the proteasome, chaperone-mediated autophagy or macroautophagy.²⁸⁻³⁰ We recently found that all three of these proteolytic pathways seem to be involved in the degradation of α -synuclein.¹⁶ Nevertheless, inhibition of autophagy was more effective in preventing α -synuclein degradation than the proteasomal and lysosomal pathways,¹⁶ suggesting that autophagy may be the

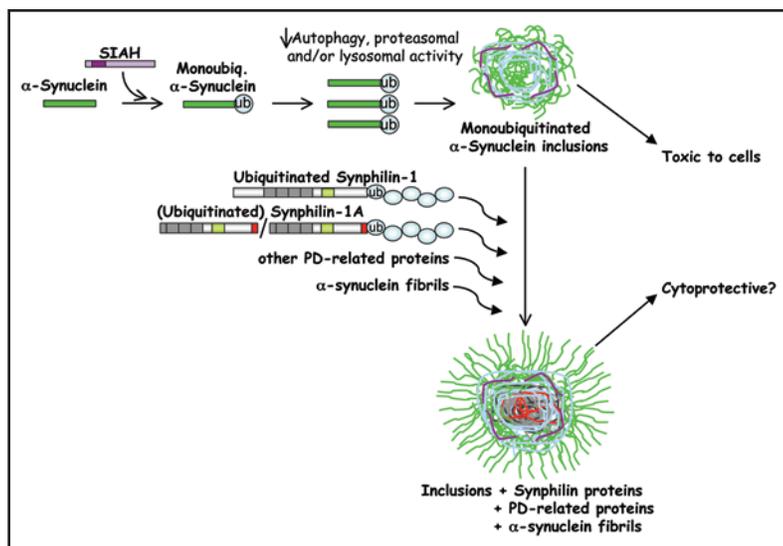


Figure 1. Schematic representation of inclusion body formation in PD cell models. Monoubiquitination of α -synuclein by SIAH leads to the aggregation of α -synuclein and formation of inclusions within dopaminergic cells. Inhibition of autophagic, proteasomal and/or lysosomal pathways promotes further accumulation and aggregation of monoubiquitinated α -synuclein within cells.¹⁶ α -Synuclein inclusions are toxic to cells,¹⁶ compatible with the nonfibrillary nature of monoubiquitinated α -synuclein aggregates we observed. The protective nature of the different PD cell models containing synphilin proteins, suggest that coaggregation with synphilin-1 and -1A, either ubiquitinated or not, may prevent the toxicity mediated by monoubiquitinated α -synuclein inclusions.^{12,15,20} Accumulation of additional PD-related proteins, such as UCH-L1 and parkin, or build up of α -synuclein fibrils may also decrease the toxicity of monoubiquitinated α -synuclein inclusions. We propose that Lewy bodies may promote different extents of cell toxicity depending on their maturation stage.

predominant pathway involved in α -synuclein clearance. Supporting this possibility is the finding that α -synuclein is detected inside vesicles with autophagic morphology, and that the autophagy activator, rapamycin, stimulates its clearance.³⁰ Autophagy inhibition promotes the accumulation of monoubiquitinated forms of the α -synuclein protein and subsequent aggregation.¹⁶ In this framework, we propose that specific disruption of α -synuclein monoubiquitination and/or stimulation of macroautophagy represent promising new strategies to prevent or decrease the progression of PD.

References

- Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci* 2005; 28:57-87.
- Hardy J, Cai H, Cookson MR, Gwinn-Hardy K, Singleton A. Genetics of Parkinson's disease and parkinsonism. *Ann Neurol* 2006; 60:389-98.
- Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. α -Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci USA* 1998; 95:6469-73.
- Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant α -synuclein linked to early-onset Parkinson disease. *Nat Med* 1998; 4:1318-20.
- Lee VM, Trojanowski JQ. Mechanisms of Parkinson's disease linked to pathological α -synuclein: New targets for drug discovery. *Neuron* 2006; 52:33-8.
- Hasegawa M, Fujiwara H, Nonaka T, Wakabayashi K, Takahashi H, Lee VM, Trojanowski JQ, Mann D, Iwatsubo T. Phosphorylated α -synuclein is ubiquitinated in α -synucleinopathy lesions. *J Biol Chem* 2002; 277:49071-6.
- Tofaris GK, Razaq A, Ghetti B, Lilliey KS, Spillantini MG. Ubiquitination of α -synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. *J Biol Chem* 2003; 278:44405-11.
- Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, Barbour R, Huang J, Kling K, Lee M, Diep L, Keim PS, Shen X, Chataway T, Schlossmacher MG, Seubert P, Schenk D, Sinha S, Gai WP, Chilcote TJ. Phosphorylation of Ser-129 is the dominant pathological modification of α -synuclein in familial and sporadic Lewy body disease. *J Biol Chem* 2006; 281:29739-52.

9. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K, Iwatsubo T. α -Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002; 4:160-4.
10. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM. Oxidative damage linked to neurodegeneration by selective α -synuclein nitration in synucleinopathy lesions. *Science* 2000; 290:985-9.
11. Wakabayashi K, Engelender S, Yoshimoto M, Tsuji S, Ross CA, Takahashi H. Synphilin-1 is present in Lewy bodies in Parkinson's disease. *Ann Neurol* 2000; 47:521-3.
12. Eyal A, Szargel R, Avraham E, Liani E, Haskin J, Rott R, Engelender S. Synphilin-1A: An aggregation-prone isoform of synphilin-1 that causes neuronal death and is present in aggregates from α -synucleinopathy patients. *Proc Natl Acad Sci USA* 2006; 103:5917-22.
13. Lowe J, McDermott H, Landon M, Mayer RJ, Wilkinson KD. Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases. *J Pathol* 1990; 161:153-60.
14. Brion JP, Couck AM. Cortical and brainstem-type Lewy bodies are immunoreactive for the cyclin-dependent kinase 5. *Am J Pathol* 1995; 147:1465-76.
15. Liani E, Eyal A, Avraham E, Shemer R, Szargel R, Berg D, Bornemann A, Riess O, Ross CA, Rott R, Engelender S. Ubiquitination of synphilin-1 and α -synuclein by SLAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease. *Proc Natl Acad Sci USA* 2004; 101:5500-5.
16. Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, Liani E, Avraham E, Engelender S. Monoubiquitination of α -synuclein by SLAH promotes its aggregation in dopaminergic cells. *J Biol Chem* 2007.
17. Lee JT, Wheeler TC, Li L, Chin LS. Ubiquitination of [alpha]-synuclein by Siah-1 promotes α -synuclein aggregation and apoptotic cell death. *Hum Mol Genet* 2007.
18. Nonaka T, Iwatsubo T, Hasegawa M. Ubiquitination of α -synuclein. *Biochemistry* 2005; 44:361-8.
19. Ross CA, Poirier MA. Opinion: What is the role of protein aggregation in neurodegeneration? *Nat Rev Mol Cell Biol* 2005; 6:891-8.
20. Tanaka M, Kim YM, Lee G, Junn E, Iwatsubo T, Mouradian MM. Aggregates formed by α -synuclein and synphilin-1 are cytoprotective. *J Biol Chem* 2004; 279:4625-31.
21. Caughey B, Lansbury PT. Protofibrils, pores, fibrils, and neurodegeneration: Separating the responsible protein aggregates from the innocent bystanders. *Annu Rev Neurosci* 2003; 26:267-98.
22. Baba M, Nakajo S, Tu PH, Tomita T, Nakaya K, Lee VM, Trojanowski JQ, Iwatsubo T. Aggregation of α -synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am J Pathol* 1998; 152:879-84.
23. Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated α -synuclein mediates dopaminergic neurotoxicity in vivo. *J Neurosci* 2007; 27:3338-46.
24. McNaught KS, Jenner P. Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci Lett* 2001; 297:191-4.
25. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *N Engl J Med* 2004; 351:1972-7.
26. Settembre C, Fraldi A, Jahress L, Spampinato C, Venturi C, Medina D, de Pablo R, Tacchetti C, Rubinsztein DC, Ballabio A. A block of autophagy in lysosomal storage disorders. *Hum Mol Genet* 2008; 17:119-29.
27. Stefanis L, Larsen KE, Rideout HJ, Sulzer D, Greene LA. Expression of A53T mutant but not wild-type α -synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death. *J Neurosci* 2001; 21:9549-60.
28. Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of α -synuclein by proteasome. *J Biol Chem* 1999; 274:33855-8.
29. Paxinou E, Chen Q, Weisse M, Giasson BI, Norris EH, Rueter SM, Trojanowski JQ, Lee VM, Ischiropoulos H. Induction of α -synuclein aggregation by intracellular nitrate insult. *J Neurosci* 2001; 21:8053-61.
30. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α -Synuclein is degraded by both autophagy and the proteasome. *J Biol Chem* 2003; 278:25009-13.