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Alpha-Synuclein Fails to Form Aggregates in Endocytosis-Defective Fission Yeast Strains, Δ*myo1* and Δ*end4*

Teruaki Takasaki[1], Ryuga Yamada[1], Yoshitaka Sugimoto[1], Reiko Sugiura[1§]

[1]Faculty of Pharmacy, Kindai University

[§]To whom correspondence should be addressed: sugiurar@phar.kindai.ac.jp

Abstract

Alpha-Synuclein (α-Syn) is a soluble neuronal protein whose aggregation is one of the hallmarks of Parkinson's disease (PD). We previously developed a fission yeast model of PD that recapitulates α-Syn aggregation upon high-level expression of human α-Syn. Here, we show that α-Syn aggregate formation in yeast requires Myo1 and End4, proteins essential for the early steps of endocytosis. α-Syn expression levels in Δ*myo1* and Δ*end4* cells were comparable to wild-type cells, suggesting that defects in endocytosis disrupt α-Syn aggregation. These findings highlight the critical role of endocytosis in α-Syn aggregation and PD pathology.

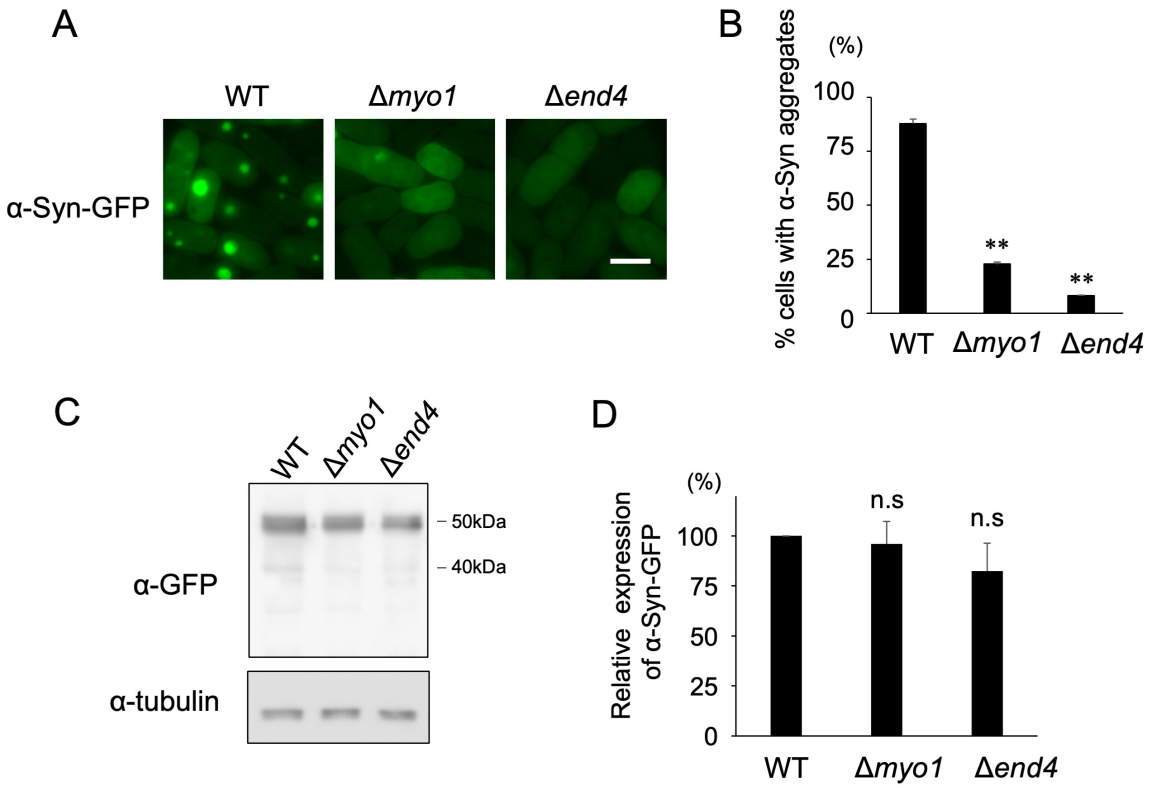

Figure 1. Overexpression of α-Syn leads to aggregate formation in WT cells, but not in Δ*myo1* and Δ*end4* cells.:

A: Representative images of the wild-type (WT), Δ*myo1* and Δ*end4* cells transformed with the plasmid containing α-Syn-GFP gene under the *adh1* promoter. Cells were incubated at 27˚C for 18 h. Scale bar: 5 µm

B: Percent cells with α-Syn-GFP aggregates in the cytoplasm are shown. More than 50 cells were counted for each independent experiment. Graphs show mean ± S.D. (n=2). **P<0.01.

C: Protein expression levels of α-Syn-GFP were analyzed by immunoblotting with anti-GFP and anti-tubulin antibodies.

D: Relative expression levels of α-Syn were measured as the ratio between the intensities of the α-Syn-GFP and tubulin, and normalized to WT cells. Graphs show mean ± S.D. (n=3). n.s.: not significant.

Description

Alpha-synuclein (α-Syn) is a small 140-amino acid protein that is abundant in human brain (Maroteaux et al. 1988; Burré et al. 2018) . Under physiological conditions, α-Syn is intrinsically disordered and soluble; however, postmortem samples from the basal ganglia and limbic cortex of Parkinson's disease patients show increased insoluble α-Syn compared to age-matched controls (Mamais et al. 2013). Aggregation of α-Syn has been implicated in the pathogenesis of several neurodegenerative disorders, including Parkinson's disease and dementia with Lewy bodies, collectively termed synucleinopathies (Goedert 2001; Srinivasan et al. 2021) . However, the mechanisms that induce α-Syn aggregation are not fully elucidated.

Previously, we have generated a fission yeast Parkinson's disease model that recapitulates α-Syn aggregate formation by forcing the cells to express human α-Syn at high levels (Sugimoto et al. 2025). Using this yeast model, we found that rapamycin and Torin1, inhibitors of mammalian target of rapamycin (mTOR), potently inhibit the formation of α-Syn aggregates without altering α-Syn expression (Sugimoto et al. 2025). This discovery prompted us to hypothesize that mTOR function(s) other than regulation of protein synthesis is required for α-Syn aggregate formation. mTOR is a conserved protein kinase that regulates a variety of fundamental cellular processes, including protein synthesis, nutrient sensing, autophagy, endocytosis, and cell proliferation (Grahammer et al. 2017; Goul et al. 2023; Panwar et al. 2023). Among mTOR's many roles, in this study, we directly tested whether endocytosis is involved in α-Syn aggregate formation using a genetic approach.

We first confirmed that overexpression of the α-Syn-GFP fusion protein leads to aggregate formation in wild-type cells, as reported in a previous study (Sugimoto et al. 2025) (Fig. 1A). Approximately 80% of the cells exhibited cytoplasmic α-Syn aggregates (Fig. 1B). Next, we overexpressed the α-Syn-GFP fusion protein in the cells lacking *myo1*$^+$ gene (Δ*myo1*). *myo1*$^+$ encodes a type I myosin that regulates the actin assembly/disassembly process and is required for the early steps of endocytosis (Lee et al. 2000; Toya et al. 2001; Petrini et al. 2015) . Notably, α-Syn-GFP failed to form aggregates in Δ*myo1* cells (Fig. 1A, B). We also tested whether similar defects in the α-Syn aggregate formation were observed in the cells lacking *end4*$^+$, which is essential for the internalization process of the endocytic pathway, but not for the later stages of endocytosis, such as vacuolar protein transport (Iwaki et al. 2004). As we predicted, α-Syn aggregates were rarely detected in Δ*end4* cells (Fig. 1A, B).

Given that the expression levels of α-Syn-GFP in Δ*myo1* and Δ*end4* cells were comparable to those in wild-type cells (Fig. 1C, D), the failure in α-Syn aggregate formation is likely due to defects in the early step of endocytosis. The early step of endocytosis involves the internalization of a portion of the plasma membrane to incorporate membrane bound proteins, lipids, and extracellular components (Kumari et al. 2010) . As α-Syn inclusions are known to contain a high amount of lipid membrane (Shahmoradian et al. 2019) , the step of internalizing cell membrane components might be required to provide a scaffold to form α-Syn aggregates. Alternatively, the process of endocytosis may contribute more directly to the α-Syn aggregate formation, for example, by internalizing and accumulating membrane-bound α-Syn through the endocytic pathways. Our findings contribute to a deeper understanding of the mechanisms underlying the α-Syn aggregate formation and the Parkinson's disease pathology.

Methods

Standard yeast culture and genetic methods were used except where otherwise noted (Sabatinos and Forsburg 2010) . Yeast strains harboring the plasmid pKD4557 [*Padh1-α-syn-GFP*] (Sugimoto et al. 2025) were grown in Edinburgh minimal medium (EMM) in conical tubes at 27°C for 18 h. Images were acquired by an All-in-one fluorescence microscope (BZ-X710, KEYENCE, Osaka, Japan).

Reagents

Strain	Genotype	Reference
HM123	*h*$^-$ *leu1-32*	Lab stock

KP1257	*h⁻ leu1 myo1::kanr*	Ma et al. 2011
KP4150	*h⁻ leu1 ura4 arg1 end4::ura4⁺*	Iwaki et al. 2004

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