

E07-08-0827 Lee

Soper et al., online supplemental material index:

Supplementary Figure S1

Supplementary Movies 1-4

Supplementary Figure Legends:

Supplementary Figure S1. α -Syn-EGFP is localized to both the plasma membrane and cytosol in yeast. Membrane fractionation was performed on cells expressing α -syn-EGFP or EGFP alone. α -Syn-EGFP is localized to the plasma membrane fractions (F5, F6), which was confirmed by immunoblotting with an antibody to the plasma membrane marker Pma1. There is also some α -syn-EGFP in the cytosolic fraction (C). EGFP alone localizes only to the cytosolic fraction.

Supplementary Figure S2. Treatment of yeast with DMSO increases α -syn-EGFP accumulation formation. (A) An integrative strain of yeast expressing α -syn-EGFP (p406 Syn-EGFP), grown in normal selective media and in media containing 5% DMSO (B) Quantification of the percentage of cells containing accumulations when cells are grown in different concentrations of DMSO. Error bars represent standard deviation. 0.5% DMSO increases the percentage of cells containing accumulations. Scale bar: 1 μ m.

Supplementary Figure S3. Yeast expressing untagged α -synuclein form vesicular accumulations. (A,B,C) EM of yeast cells expressing untagged α -syn. Vesicular accumulations can be observed forming at the membrane and also in the perinuclear region (arrows), similar to those seen in cells expressing GFP tagged α -syn. (D) EM of a yeast cell expressing EGFP. No vesicular accumulations are observed in cells expressing EGFP. Scale bars: (A,B,D) 500 nm; (C) 100 nm.

Supplementary Figure S4. Expression of Syn-EGFP causes disruption of the Golgi marker Cop1p. (A) Fluorescence microscopy showing disruption of Cop1p caused by expression of Syn-EGFP. Punctate Golgi labeling is observed in cells expressing EGFP alone. (B) Quantification of Cop1p disruption in cells expressing Syn-EGFP and EGFP alone as a percentage increase from control (vector alone). Scale bar: 2 μ m

Supplementary video legends:

Supplementary Video 1: Live imaging of α -syn-EGFP accumulation in yeast. Movie corresponds to still images in Figure 1D. Yeast cells expressing α -syn-EGFP were imaged overnight. Note the α -syn-EGFP membrane localization and subsequent formation of accumulations. Images were taken at 1 frame/10 minutes, elapsed time in hours:minutes is shown on the lower right. Display rate is 5 frames/second.

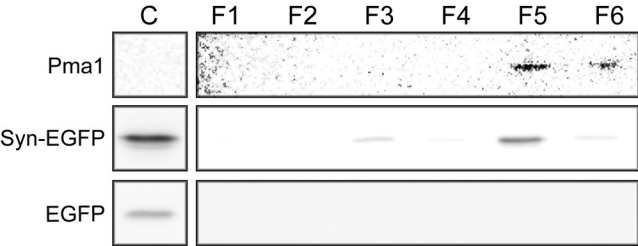
Supplementary Video 2: Live imaging of yeast expressing 58-140-EGFP. Yeast cells expressing 58-140-EGFP were imaged overnight. Note that 58-140-EGFP is localized diffusely throughout the cytoplasm, and there are no punctate accumulations. Images

were taken at 1 frame/10 minutes, elapsed time in hours:minutes is shown on the lower right. Display rate is 5 frames/second.

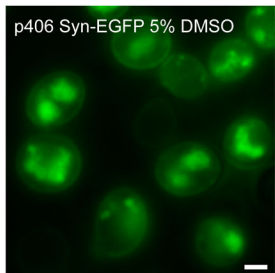
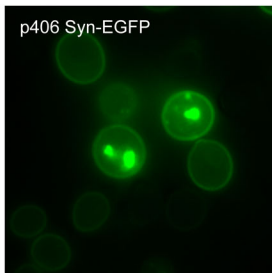
Supplementary Video 3: Live imaging of yeast expressing A30P-EGFP. Yeast cells expressing A30P-EGFP were imaged overnight. Note the initial membrane localization, followed by diffuse cytoplasmic localization. Images were taken at 1 frame/10 minutes, elapsed time in hours:minutes is shown on the lower right. Display rate is 5 frames/second.

Supplementary Video 4: Live imaging of yeast expressing Δ 71-82-EGFP. Yeast cells expressing Δ 71-82-EGFP were imaged overnight. Note the membrane localization and lack of punctate accumulations. Only a small number of cells expressing Δ 71-82-EGFP contained accumulations. Images were taken at 1 frame/10 minutes, elapsed time in hours:minutes is shown on the lower right. Display rate is 5 frames/second.

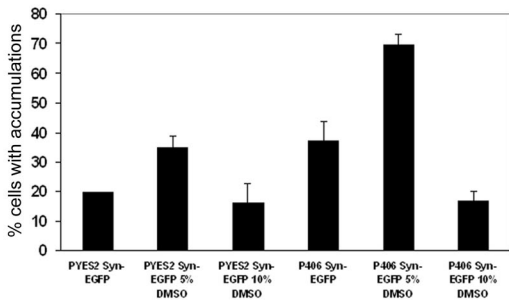
Supplementary Figure S1



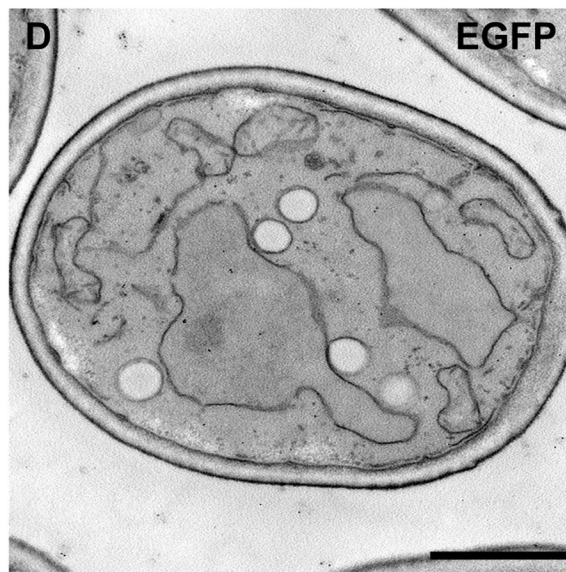
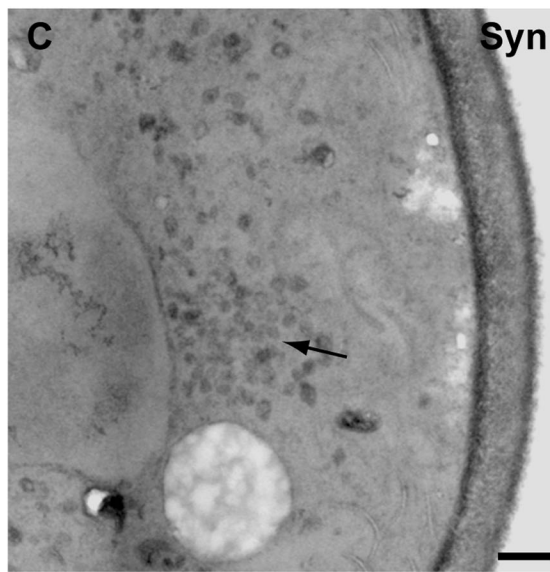
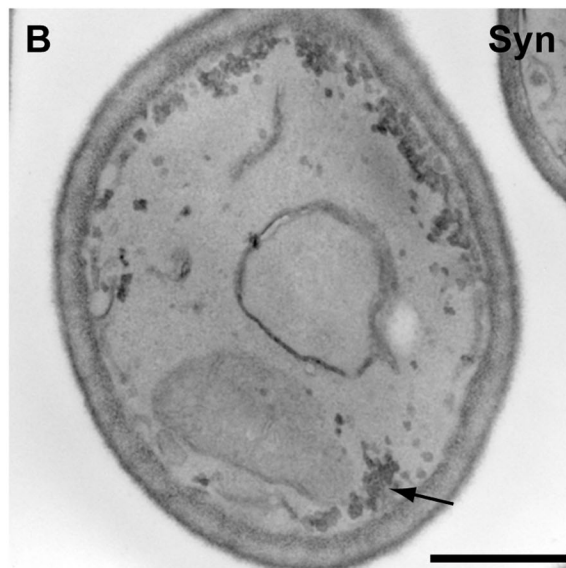
A



B

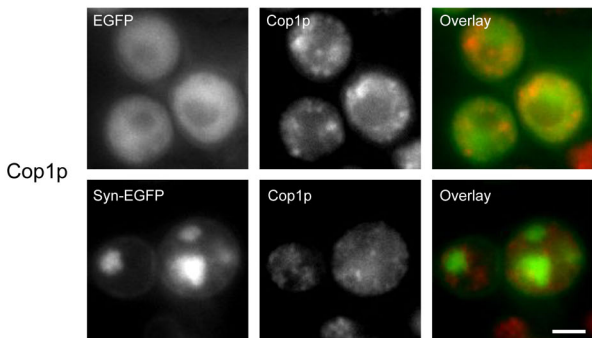


Supplementary Figure S3



Supplementary Figure S4

A



B

