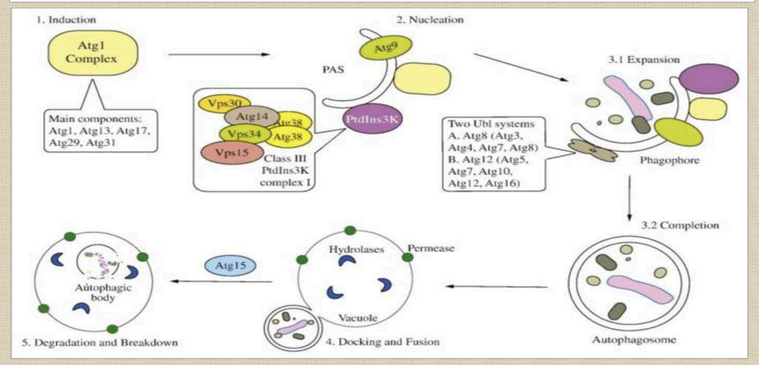
Clearance of mutant huntingtin inclusion bodies is dependent on selective autophagy receptors

Karina Frey, Austin Folger, and Dr. Yanchang Wang
Department of Biomedical Sciences

Abstract

Huntington's disease is an inherited neurodegenerative disorder characterized by personality changes, movement disorders, and cognitive decline. It is caused by the expansion of a polyglutamine tract to more than 39 copies of CAG in exon 1 of the Htt gene. This mutated huntingtin protein (mHtt) tends to form inclusion bodies in cells, which are cytotoxic. Cellular processes exist to mitigate this toxicity, one of which is autophagy. Autophagy is a process by which cells can envelop and recycle cellular contents. This can be either be non-specific, as in starvation autophagy, or specific as in selective autophagy. This experiment aims to elucidate the autophagy machinery involved in the clearance of mHtt inclusion bodies. We used various Atg deletion mutants in both the starvation and selective autophagy pathways to determine if they are required for inclusion body autophagy and vacuolar localization. We also used deletion mutants of selective autophagy receptors to determine which are required for inclusion body degradation. We have found that selective autophagy and a few selective autophagy receptors play a key role in the degradation of inclusion bodies in cells expressing mHtt.

Overview of Autophagy



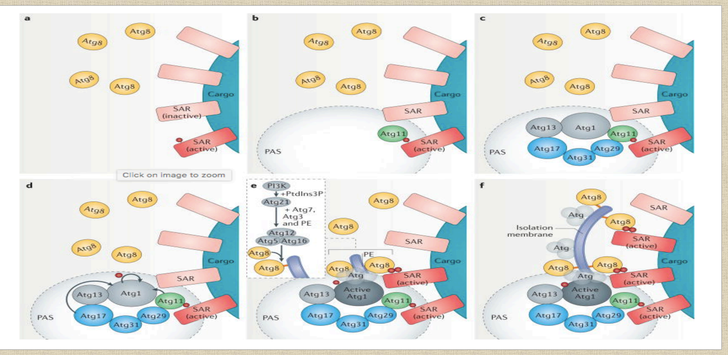
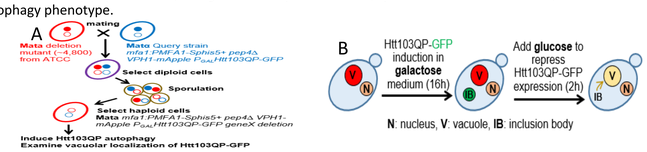


Figure 1. The generalized process of starvation and selective autophagy; highlighting the roles of Atg8, Atg11, and SARs as autophagy machinery.

Strain construction and induction/repression of Htt103QP protein. A) Strain construction carried out using strains from the ATCC Saccharomyces Genome Deletion Project along with a query strain we constructed. The query strain contains mApple-tagged and GFP tag, all under a galactose promoter, referred to Htt103QP. After mating an ATCC deletion strain with strain, we are then able to select haploid cells containing genotype of both strain and ATCC deletion strain. **B)** Induce Htt103QP through use of an inducible/repressible galactose promoter to induce Htt103QP production, which aggregates into an inclusion body (IB). After 16h, add glucose to repress Htt103QP production. After two hours, we are able to see the autophagy phenotype.



Results

Plasmid PCR on mApple plasmid
-Atg8 F/R Primers
-Atg11 F/R Primers
→ Transformation into 3419-1-1 (Htt103QP-GFP)
→ Grow on His⁻ selective plate for 2 days
→ Streak successful colonies on new plate
→ Colony PCR for confirmation

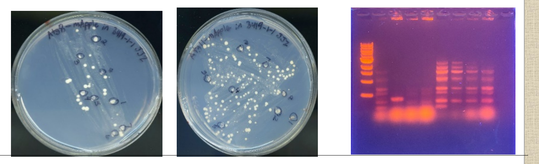
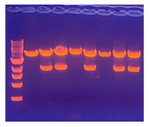
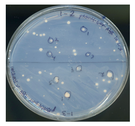
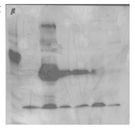


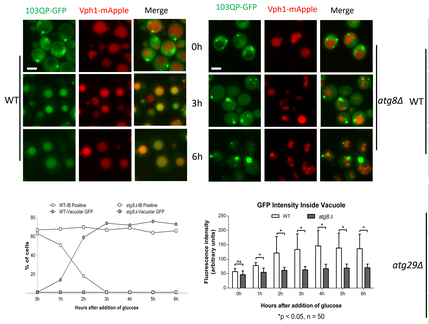
Figure 2: The tagging process of Atg8 and Atg11 with the mApple tag
A) For the Atg8 and Atg11 proteins to be tagged with mApple, the forward and reverse primers needed to be designed. The primers were designed to all have a length of 60 Base Pairs, a Melting Temperature between 70°C and 77.5°C, and a Guanine-Cytosine Content between 40% and 60%. **B)** The yeast cells were grown on His- selective plates as the mApple tag uses a His⁻ marker. The circled and numbered colonies were streaked onto a new plate. **C)** The results of the Colony PCR are depicted in this image. The bands of interest are circled in red. The bands of interest are approximately 450 and 400 base pairs, respectively.

1. Cut pRS414-GFP-Atg8 with Xho1 and SacI to produce pRS414 and GFP-Atg8
→ Incubate GFP-Atg8 fragment with pRS404 in ligase solution.
→ Transformation into RH087#1 (103QP-mApple) and Cry
→ Grow on Trp⁻ selective plate for 2 days and inoculate yeast into Trp⁻ media

A 
B 
C 

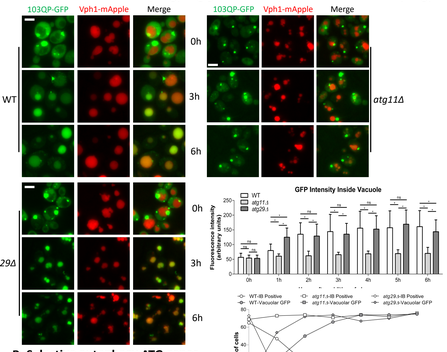
C. Western Blot to confirm presence of GFP-Atg8

Figure 1. Autophagic clearance of mHtt IBs



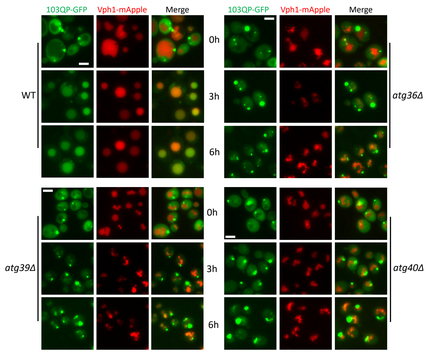
A. The core autophagy machinery is required for mHtt IB vacuolar localization.

Figure 2. Selective autophagy Atg genes are required for mHtt IB autophagy.



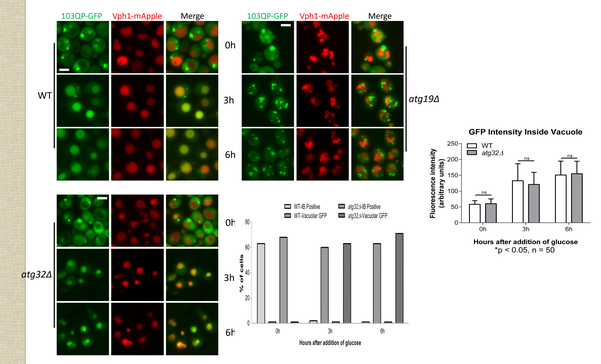
B. Selective autophagy ATG genes are required for vacuole transport of mHtt IBs.

Figure 3. The SAR for mHtt IB autophagy.



C. SARs Atg36, Atg39, and Atg40 are required for vacuolar transport of mHtt IBs.

Figure 3 continued. The SAR for mHtt IB autophagy.



D. Other yeast SARs are not required for mHtt vacuolar localization.

Conclusion

The results suggest that autophagy plays a role in the clearance of Htt103QP inclusion bodies using the selective autophagy pathway, inclusion body autophagy or (IBophagy). These experiments suggest that Htt103QP IBophagy is dependent on selective autophagy receptors, Atg36, Atg39 and Atg40. All of these results support the hypothesis that the clearance Htt103QP inclusion bodies depends on selective autophagy (IBophagy), while the presence of free or small aggregates of Htt103QP inhibits IBophagy.

Acknowledgements

Special thanks to Dr. Yanchang Wang for allowing me to research in his laboratory, Austin Folger for mentoring me throughout the entirety of my research, my lab mates Delaney Sherwin, and Emily Gutierrez-Morton, and Marie-Helene Kabbaj, and the College of Medicine at Florida State University.

References

Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annual review of biochemistry*, *75*, 333–366.
Forum on Neuroscience and Nervous System Disorders. (2013). *Protein aggregation*. Neurodegeneration: Exploring Commonalities Across Diseases: Workshop Summary.
Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *The Journal of pathology*, *221*(1), 3–12.
Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., & Mizushima, N. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*, *441*(7095), 885–889.
Mason, R. P., & Giorgini, F. (2011). Modeling Huntington disease in yeast: perspectives and future directions. *Prion*, *5*(4), 269–276.
U.S. National Library of Medicine. (2021). *What are proteins and what do they do?: Medlineplus genetics*. MedlinePlus.