Physiological role of endosomal Microautophagy in *Drosophila*

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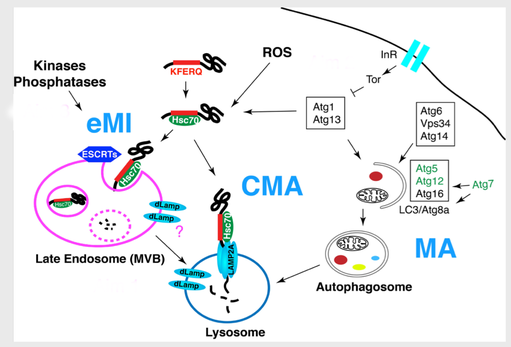
Abstract

Autophagy is a catabolic process which is induced in response to various stresses like starvation, damaged organelle, accumulated misfolded proteins, oxidative stress and DNA damage. Also, basal autophagy is essential for cellular homeostasis and intracellular degradation of damaged organelles and aggregate prone proteins. As such, it counteracts various human diseases, and its reduction leads to aging like phenotypes.

We have developed a genetic model in *Drosophila* for eMI, a form of autophagy during which substrates are taken up into multivesicular bodies for degradation. Using a KFERQ-tagged fluorescent biosensor, we have identified an eMI-like pathway in the genetically easily tractable model organism *Drosophila melanogaster*. Firstly, we are characterizing the physiological role of eMI with a focus on what types of cellular stress activate eMI. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response in an Hsc70-4 and ESCRT machinery dependent manner, implying a selectivity of the process. Further, we are trying to understand the mechanism of stress induced eMI by identifying novel regulators of eMI pathway. In particular we have found one such regulator which when misregulated results in early induction of starvation induced eMI. Since, targeting autophagic pathway might be a promising treatment strategy particularly as protein quality control is critical for non-dividing neurons, we are also testing eMI candidate regulators as a possible modifier of human neurodegenerative diseases. In future it will be interesting to elucidate how eMI affects neurodegeneration with anticipation that such processes are conserved in humans.



eMI-ESCRT machinery dependent
CMA-LAMP2A dependent
MA- ATG5, ATG12, ATG7 dependent

Fig. 1. Schematic of different form of Autophagy

Understanding eMI regulation under nutritional stress condition

Generating endogenous KFERQ containing substrate based sensor to monitor eMI

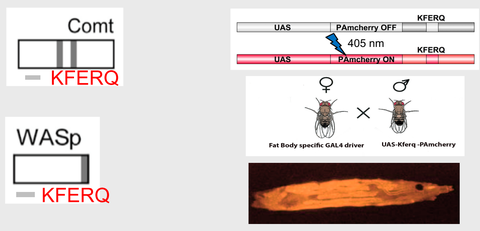


Fig 2. Synaptic proteins ComT and WASp are known eMI substrate which harbor KFERQ motifs. We constructed Photoactivable mCherry tagged ComT and WASp fusion protein downstream to UAS and generated transgenic flies carrying these construct. We crossed these flies with Fat body specific GAL4 driver lines and photoactivated larvae to pulse chase mCherry localization under starved and fed condition.
Starvation-20% Sucrose ,Fed- 20% Sucrose supplemented with Yeast.

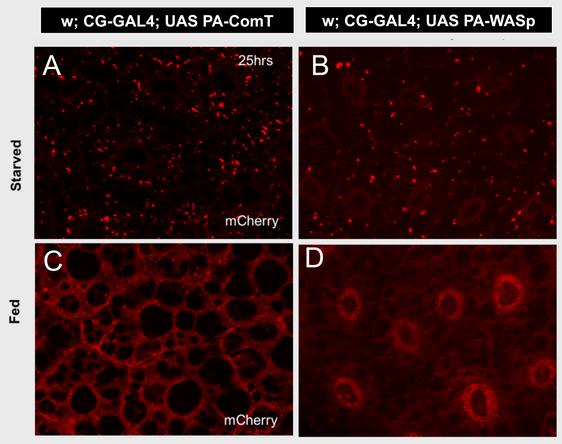


Fig 3. eMI substrate WASp and ComT having endogenous KFERQ motifs were used to generate a biosensor to monitor eMI. Both ComT and WASp sensor when expressed in *Drosophila* fat body showed puncta formation 25h post starvation (A,B) while a diffuse cytoplasmic staining observed in fed larvae (C,D).

WASp/ComT biosensor puncta formation occurs in an ESCRT dependent manner

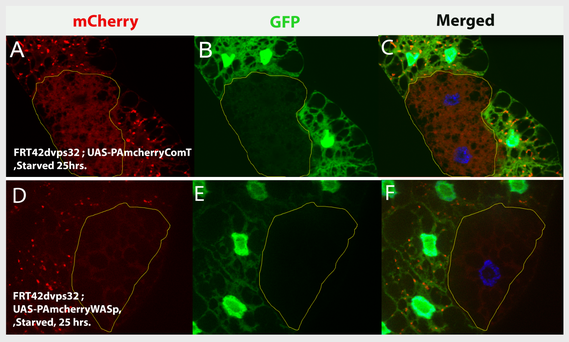


Fig. 4. Flp-Frt based generation of ESCRT III(vps32 in Drosophila) clonal cells. Upon 25h starvation mutant cells (GFP-) lack sensor puncta formation while biosensor puncta can be seen in wild type surrounding cells (GFP+). Both ComT and WASp puncta formation needed ESCRT machinery

WASp sensor puncta is also induced at 4h post-starvation

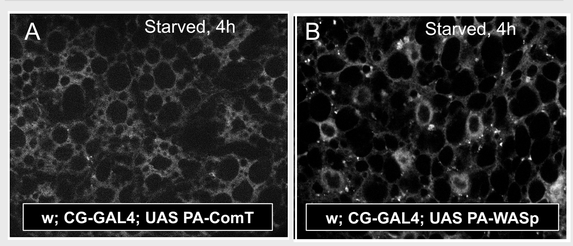
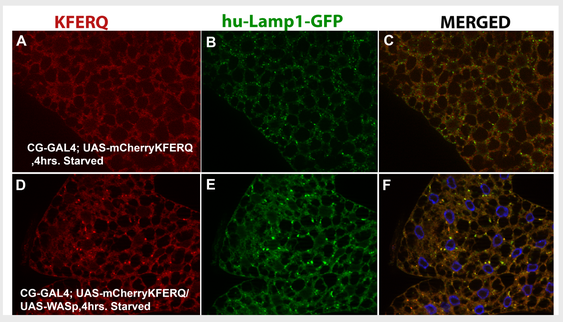


Fig.5. mCherry WASp sensor shows puncta formation even at 4h post starvation (B) while ComT sensor puncta was not observed.

WASp overexpression is sufficient to induce early eMI



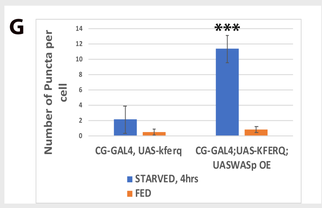


Fig.6. Overexpression of WASp was sufficient to induced eMI as monitored by UAS-KFERQ based sensor post 4h starvation.UAS-KFERQmcherry sensor when expressed alone in fat body doesn't induced eMI while co-expression of WASp results in an early induction of KFERQ sensor

WASp overexpression results in an early eMI induction in an ESCRT machinery dependent manner

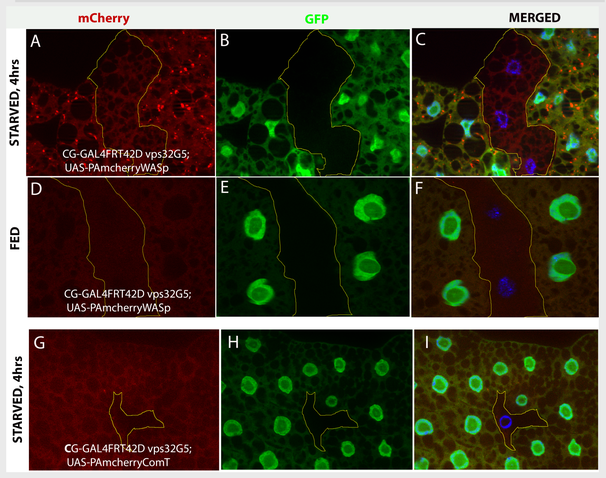
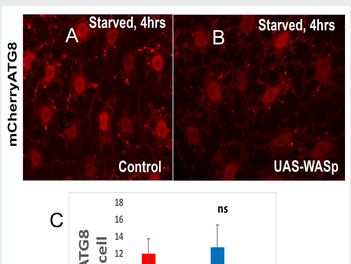


Fig.7 . Flp-Frt based generation of ESCRT III mutant clones (GFP-) and surrounding wild type cells (GFP+ve) under starved (4h) and Fed condition. ComT sensor was used as control. While WASp OE results in early eMI induction upon starvation in an ESCRT dependent manner(A-C). ComT sensor showed no significant induction (G-I).

WASp overexpression doesnot effects on macroautophagy



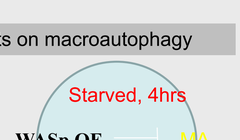


Fig.8. Early eMI induction with WASp O.E could also be to compensate deficient macroautophagy process. To understand the effect of excess WASp protein on macroautophagy process we checked the levels of ATG8a, which is commonly used as autophagy marker. There was no significant difference between mCherry ATG8a alone (A,C) as compared to when co-expressed with WASp (B,C).

DNA and oxidative damage but not ER stress results in eMI induction

Fig.9 Treatment of larvae with the DNA damaging agent Eto for 25 h robustly induces eMI puncta. (C,D) Oxidative stress induced by Paraquat induces eMI. (C) untreated control, (D) fat body of Paraquat treated larvae. E) Quantification of eMI puncta per cell at indicated time points. Grey: untreated, blue: Paraquat, and magenta: Eto treated. (F) Quantification of eMI puncta upon treatment with 50 µM Doxorubicin (magenta). (G) Quantification of eMI puncta upon treatment with indicated concentrations of Menadione bisulfite (blue).

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Fig.10. ER stress doesnot elicit eMI. Treatment of larvae with 1µM Thapsigargin (B, B') or 50 µg/ml Tunicamycin (C, C') does cause the formation of sensor puncta in the fat body. (Endo)lysosomes are marked using GFP-hsLAMP1 (D,E) Quantification of a dose response curve of treatment with Thapsigargin (D) and Tunicamycin (E).

Summary

- We have generated a endogenous substrate based reporter for eMI and we show that ComT and WASp based sensor is inducible upon prolong starvation in an ESCRT machinery dependent manner
- We found WASp protein as a possible regulator of eMI as WASp O.E results in premature eMI induction in an ESCRT machinery dependent manner
- WASp overexpression does not effect the canonical macroautophagy pathway
- Beside Nutritional stress eMI is also induced upon DNA damage and oxidative stress but not ER stress can elicit an eMI response