Glial-specific knockdown of a subunit of the ER membrane complex (EMC) dramatically reduces survival of D. melanogaster

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Background and preliminary results

The endoplasmic reticulum (ER) is involved in the modification, packaging, and insertion of proteins into the membrane [1]. A recently discovered complex called ER membrane protein complex (EMC) is believed to help with many ER functions such as protein biogenesis and degradation [2]. Additionally, it has been linked to the shuttling of misfolded proteins to the ER-associated degradation (ERAD) pathway [2]. This protein complex is highly conserved from yeast to humans, and it is composed of 8-10 subunits that work in tandem [2]. However, there is not much known about, the role this complex plays in multicellular organisms, as most research has been conducted in yeast and cell cultures [3]. Our lab found severe survival phenotypes when we knocked down EMC4 through a glial-specific RNAi KD screen of genes that were found to be differentially expressed following TBI [4]. The flies exhibit delayed developmental time of 1-2 days, strongly impaired locomotion, a shorter lifespan of 5-6 days compared to the normal 50–60-day lifespan of control flies, and increased protein aggregation. The dramatic effect that this glial-specific loss of EMC4 expression has on fruit flies, sheds light on the cell type-specific importance of this subunit to organismal health and protein degradation.

Goals and objective

The role of EMC4 at different stages of development and maintenance of adult organisms have not been studied. Our current system to knock down EMC4 at embryogenesis doesn't allow us to obtain temporal resolution at the functions of EMC4 in glial cells. Therefore, our goal is to use Gal80ts to focus on what leads to the accumulation of protein aggregates seen in adults: loss of glial EMC4 during development or in the adult organism. Gal80ts is temperature temperature-sensitive inhibitor of the Gal4-UAS system. This system uses Gal4, a transcription activator inserted downstream of a promoter, which in this project is repo, a glial-specific promoter. This transcription activator binds to the UAS enhancer region placed upstream of an RNAi construct targeting EMC4 (TRiP line #HMC06642). Using a temperature-sensitive inhibitor of Gal4 expression will allow us to manipulate the knockdown through temperature shifts at any time points we choose. I will use this tool to knock down EMC4 selectively 48 hours post eclosion or during development to discern between developmental and adult EMC4 function in glia. To compare how time-specific glial-EMC4 knockdown affects fruit flies, I will perform Western Blots looking at ubiquitinated proteins as a biomarker of protein aggregation in the fruit fly for both time conditions. The severe decrease in adult fly survival but successful transition to eclosion that Gal4-UAS flies showed in our prior results led me to hypothesize that EMC4 knockdown is acutely lethal when EMC4 is not present only in adult fruit flies. In this study, I propose that adulthood-only glial-EMC-KD flies will show higher protein aggregates at 5 days post-eclosion than development-only glial-EMC-KD, suggesting that the effect of glial EMC4 KD on protein aggregates is acute during adulthood, rather than accumulating during development.

Specific aim

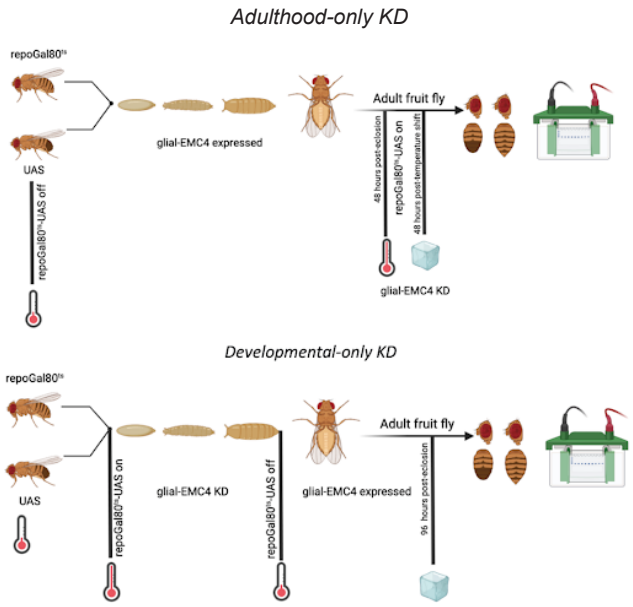
Compare how adulthood-only and developmental-only knockdown of glia EMC4 affect fruit flies by assessing protein aggregation in the heads and bodies of female and male flies.

Research design and methods

Genetic crossing and Gal80ts; Gal4-UAS: Virgin female parents carrying the temperature-sensitive inhibitor and glial-specific driver constructs (Gal80ts; repoGal4) will be mated with males carrying the UAS-RNAi-EMC4 construct at 20ºC. Hatched larvae will be either placed at 29ºC for the developmental-only KD or kept at 20ºC for the adulthood-only KD. On the first day of eclosion, the adulthood-only KD flies will be switched to 29ºC while developmental-only KD flies will be transferred to 20ºC.

Protein extraction and aggregation assay: Heads and bodies will be separated into groups of 50 and 20 respectively in 1% Triton homogenization buffer containing protease inhibitors to avoid protein degradation. The insoluble portion of the protein samples will be extracted and analyzed through a Western blot. The signal will be normalized to total protein (Ponceau). The signal will be detected by primary antibody: Ubiquitin (P4D1) Mouse mAb, secondary antibody: Anti-mouse IgG, HRP-linked, and ECL.

Graphical representation of experimental design



Timeline: Fly husbandry to obtain the Gal80ts; Gal4 and UAS flies is completed. Fly work pertaining to the temperature shifts will be completed by the end of July 2023. Protein extractions and Western Blotting will be carried through the summer and completed by October 2023. The analysis will be finished by the end of November 2023. The culmination of this project will be the completion of my senior thesis in Spring 2024. Presentations at the CSfN Meeting in Chicago and The Allied Genetics Conference (TAGC) in Washington DC in March 2024 will present the findings from this project.

Budget: I request $1000 to purchase reagents and materials such as antibodies, Bovine Serum Albumin, and materials necessary to complete this project. (See separate budget table for full list and costs)

References

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