X-linked myotubular myopathy is a rare recessive X-linked disorder leads to early muscle weakness and decreased muscle mass. Severe weakness and joint deformities lead to a curved spine, abnormal gait, and extreme respiratory difficulty, usually leading to early fatality [1]. One gene associated with this disease is MTM1, which is a dual specificity protein tyrosine phosphatase that regulates lipid metabolism. How MTM1 regulates membrane trafficking during muscle formation is unclear. **I hypothesize that** MTM1 alters lipid metabolism and mediates cell fusion events necessary for proper muscle formation. **The overall goal** is to understand if MTM1 plays a role in membrane trafficking events necessary for muscle cell fusion. **A long term goa**l is to determine how MTM1 mediates muscle cell formation during development. For this, *Caenorhabditis elegans* will be my primary model organism. *C. elegans* has been extensively studied in muscle cell biology and is a great organism to study vesicle trafficking and cell fusion. The homologous biology and ease of use for visualization make this an excellent model for these aims.

**Aim 1: Determine conserved amino acids in MTM1 that mediate vesicular trafficking in muscle cells**

**Approach**: To identify conserved amino acids necessary for vesicle trafficking and muscle cell fusion I will first use PFAM, SMART and ClustalOmega. I will then create domain and conserve amino acid knockouts using CRISPR/cas9 in *C. elegans* . MTM-1 mutants would be screened for the inability to clear cell corpses in the epidermis. Those that lack the ability to clear cells, and thus cell fusion, will then be screened for their ability to vesicle trafficking. Vesicles expressing a GFP fusion will show localization within the cell. **Rationale**: MTM-1 has two protein domains and a low complexity region between the two, it is unclear which domain or uncharacterized motifs may be important for vesicle trafficking and muscle cell fusion. **Hypothesis**: The ph-GRAM domain may be important for mediating muscle cell fusion and vesicle trafficking because GRAM domains are common motifs in proteins associated with membrane coupled processes and signal transduction [2].

**Aim 2: Characterize the role of MTM1 in muscle cell fusion events**

**Approach**: *Using C. elegans* as a model, RNA will be isolated from muscle tissue from WT and domain analysis MTM1 mutants made in Aim1 and subject to RNA-seq. Data will be sorted using GO analysis to identify vesicle trafficking proteins that are decreased in these MTM-1 mutant animals. These newly identified genes will then be subject to mutation and their role in muscle fusion will be determined by visualizing muscle fusion events in live *C. elegans* worms. **Rationale**: Genes that are up or down regulated in MTM1 domain mutants will provide insight into how MTM1 mediates vesicle trafficking and muscle cell fusion during development. Subsequent mutations will give insight into where human myotubularin-1 lies in the fusion pathway. **Hypothesis**: I hypothesize that the MTM1 ph-GRAM mutant will be important for proper fusion pathways. Further, proteins involved in trafficking and fusion will be dysregulated in MTM1 ph-GRAM mutants.

**Aim 3: Identify novel MTM-1 muscle-specific fusion protein interactors**

**Approach:** An analysis of protein interactions will be done by TAP-MS performed on whole worms obtained from WT *C. elegans* and MTM-1 mutant animals. Different interactions occurring between the WT and MTM-1 mutant animals will be subject to GO analysis to sort for function, then immunoprecipitation to determine physiological protein interactions and fixed by Western blotting to observe changes in protein interaction complexes. Proteins that function in muscle fusion will be identified after knocking them down via CRISPR. **Rationale**: Since myotubularin is lowly expressed in healthy muscle, identifying novel protein interactors will elucidate why its mutations affect muscle development so severely. **Hypothesis**: Certain dysregulated proteins found in aim 2 with GO analysis involved in vesicle fusion will only be found in muscle tissue. These interactions lead to the severity of mtm1 mutations.