CRISPR and Its Use in Determining the Function of the VPS-34 Gene in Caenorhabditis elegans

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The VPS-34 gene in C. elegans is required for vesicular trafficking, including endocytosis, though the exact function of the protein is still unknown. My project involves identifying the specific function of the VPS-34 protein in C. Elegans, a nematode. I hoped to attach a ubiquitin-dependent degron to the C-terminus of VPS-34 to have more control over gene expression and thereby witness the exact function of the gene. When a ubiquitin-dependent degron, a specific brief sequence of nucleotides at the c-terminus of a gene, comes in contact with Ubiquitin protein, it will be targeted for degradation. All one must then do is increase and decrease the concentration of Ubiquitin protein to witness how nerve cells communicate when the VPS-34 gene is active or suppressed. A degron can be attached to the c-terminus by creating a two strand break in the DNA at that specific locus. This can be accomplished using CRISPR Cas-9 with a strand of guide RNA that matches the given region. However, most techniques for introducing a gene into the genome are inefficient and can cause mutations in other regions of the genome. In order to avoid such random inaccuracies, a more efficient gene editing technique must be used, specifically a SAP-Trap system wherewith the CRISPR gene, guide RNA, selectable markers, and desired degron flanked by homology arms are located on the same plasmid or extrachromosomal array. After raising and lowing Ubiquitin concentration I witnessed a slowing of nerve communication. I am yet to freeze fracture nerve tissue to solidify my theory, but it seems as though when VPS-34 is suppressed, nerve cells cease the 30-millisecond ultrafast synaptic vesicle endocytosis and revert back to 3-4-second Clathrin-mediated endocytosis.