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Hitting the greens: Backcrossing the csn-5 mutant, par for the course, a CSN-5 protein null

Germline development is crucial to all eukaryotic organisms and the survival of their species. Dr. Bennett's laboratory utilizes Caenorhabditis elegans, the free-living soil worm and the Nobel-prize winning model organism, to study cytoplasmic granules called P granules. P granules contain both protein and RNA and are specifically associated with the germ cells. The germline RNA-helicase proteins (GLHs) are necessary components of the P granules; they are essential for fertility in the nematode. Several protein interactors with the GLHs were previously identified in a yeast-two hybrid screen. One of these GLH binding partners is CSN-5, a known component of the COP 9 signalosome, a complex involved in protein stability that is conserved from plants to worms to humans. Recently, a deletion mutant strain, csn-5(vc861), has been isolated by the C. elegans Knockout Consortium. My project involves backcrossing (also called outcrossing) the green GFP balanced csn-5(vc861) strain against wild-type worms to remove other possible mutations arising from the original mutagenesis. The other objective is to determine whether the homozygous non-green mutant is a CSN-5 protein null. To date, four backcrosses are finished with two to be completed before initiating crosses with other pertinent mutant strains. In addition, immunocytochemistry and western blot analyses were done using an anti-CSN-5 antibody we generated. Both of these assays show that csn-5(vc861) does not make any CSN-5 protein and therefore the strain is likely a null.

This project was completed to fulfill a Capstone requirement.