**Using RNA interference to identify the acp3U tRNA modification enzyme in higher eukaryotes**

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Cellular enzymes form dozens of post-transcriptional tRNA modifications to increase tRNA stability and function. tRNA modification defects are linked to various diseases, including intellectual disability and cancer. The enzyme responsible for the 3-(3-amino-3-carboxypropyl) uridine (acp3U) tRNA modification, which is found in animals and plants, but not in yeast, is unknown. Because of its high conservation among plants and animals, acp3U is likely to be important for tRNA function. To identify the acp3U enzyme, we are silencing candidate genes in cultured *Drosophila melanogaster* cells by RNA interference (RNAi), and then analyzing acp3U levels on tRNA from treated cells using primer extension. Candidate genes are identified through BLAST searches of predicted human methyltransferase genes of unknown function that are also found in *D. melanogaster* and plants, but not in yeast. To date, we have identified 20 candidate genes, treated cells with double stranded RNA (dsRNA) to induce RNAi for over half of these genes, and then analyzed tRNA from treated cells for the presence of acp3U. Because the acp3U modification blocks Watson-Crick base-pairing, its presence causes a primer extension stop which can be detected by polyacrylamide gel electrophoresis. Thus, primer extension of tRNA from cells treated with dsRNA to the gene required for the acp3U modification would result in the absence of the primer extension block seen in wild type cells. Identification of the acp3U gene in animals, including humans, will increase our understanding of the link between tRNA modifications and disease.