**TITLE: Identification of tRNA modification enzymes in higher eukaryotes via primer extension**

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Extensive post-transcriptional modification of tRNA is required for translation of the genetic code into protein, and any defects could cause disease. The goal of this study is to develop an approach to identify tRNA modification enzymes in higher eukaryotes, to better understand their impact on human health. To identify genes involved in modification of animal tRNAs, we have knocked down candidate genes using RNA interference in cultured fruit fly cells. Using this technique we identified the fruit fly gene required for the N2,N2-dimethylguanosine (m2,2G) modification at tRNA residue 26. To identify the gene we first found the predicted gene for the m2,2G modification in fruit flies by BLAST search, knocked down expression of the gene by RNA interference, and then detected the modification using a primer extension assay. We are also studying 2’-*O*-methylation modifications, which commonly occur on tRNA, using partial base hydrolysis of tRNA followed by primer extension. Hydrolysis of the RNA occurs via a 2’-*O*-hydroxl group, and primer extension of the partially hydrolyzed sample results in a “ladder” of bands corresponding to each tRNA residue. However, the presence of a 2’-*O*-methyl group blocks cleavage, resulting in a missing band when a 2’-*O*-methylation is present. Using this approach we have detected 2’-*O*-methylations on yeast and animal tRNAs. We are now combining these approaches to identify genes required for important 2’-*O*-methylations found on animal tRNAs to better understand the link between tRNA modifications and their impact on human health.