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Partial Knockdown of Putative MTSA2 Gene Supports Its Role in 6mA Adenine Methylation of the Somatic Genome in *Tetrahymena Thermophila*

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PARTIAL KNOCKDOWN OF PUTATIVE M TSA2
GENE SUPPORTS ITS ROLE IN 6mA ADENINE
METHYLATION OF THE SOMATIC GENOME IN
TETRAHYMENA THERMOPHILA

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DNA methylation can confer heritable epigenetic information critical for proper reading of the genetic code. Methylation on the fifth carbon of cytosine (5mC) has been broadly studied for its roles in gene regulation and epigenetic inheritance. In contrast, much less is known about the importance of methylation on the sixth nitrogen of adenosines (6mA) in DNA or RNA. 6mA is the only DNA modification known in the model organism *Tetrahymena thermophila*, whose nuclear dimorphism provides a unique context for studying epigenetic regulation. In this ciliate, 6mA is found exclusively in the somatic nucleus, which may suggest that it participates in the control of gene expression. The enzyme(s) responsible for this modification remain undiscovered. Methyltransferase targeting position N-Six of Adenine 2 (*MTSA2*) is a putative gene in *T. thermophila* that belongs to a larger family of genes theorized to encode proteins that add 6mA to DNA or RNA. *MTSA2* contains a highly-conserved MT-A70 domain with known function as the S-adenosylmethionine binding subunit of mRNA methyltransferase in eukaryotic mRNA. We examined *MTSA2*'s function using a localization assay of a yellow fluorescent protein construct and assessed relative levels of 6mA by generating an *MTSA2* knockout (KO) strain. We found that *MTSA2* localizes to the macronucleus during growth, supporting its role as a DNA methyltransferase. Moreover, *MTSA2* KO strains showed a substantial decrease in 6mA relative to wild type (WT) strains. Interestingly, it was difficult to generate strains in which all of the copies of the *MTSA2* were knocked out in the polyploid (50N) somatic macronucleus. These KO strains appeared to grow more slowly than strains containing a greater number of WT copies. These results suggest that *MTSA2* appears to be important or even essential for cell growth. Together these data show that *MTSA2* likely functions as a primary 6mA methyltransferase in *T. thermophila*.