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Mycobacteriphages produce similar tRNAs to their bacterial host when isolated in the host.

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Some clusters of bacteriophages, including the C1, M, and A1, encode for their own transfer ribonucleic acids (tRNAs) for protein synthesis. The presence of tRNAs in bacteriophage genomes is intriguing given that phages use host cellular machinery to replicate and the majority of phage clusters lack tRNAs. GlutenPhree, a C1 phage isolated using *M. smegmatis* mc2155, has 33 tRNAs, annotated using PECAAN, Aragorn, and tRNAScanSE. One explanation for the function of phage tRNAs is that phage encodes for tRNAs using codons the host lacks. We created codon bias tables using DNA Master for GlutenPhree and its host to test this. However, codons preferred by GlutenPhree and M. smegmatis were the same. Genomic sequencing information disproved the alternative hypothesis that bacteriophage tRNAs code for amino acids the host produces minimally. From our initial codon bias analysis of GlutenPhree, we found a GC content of 91.3%. However, when isolated in the host bacteria, the content was lower, 64.7% and similar to that of *M. smegmatis*, 67.4%. We therefore explored whether other phages with tRNAs also have similar GC content to their host. We created codon tables for phages Audrick (C1), Bongo (M1), and HanHannaconda (J) and compared codons to their host, *M. smegmatis*. Our results indicate that phages produce similar tRNAs as their hosts when isolated in the host, since GC content of all of the phages analyzed were similar to that of *M. smegmatis*. We expect this pattern to be consistent across phages containing tRNAs. The similarities in codon preference could suggest that during infection, deactivation of host tRNAs leads phages to produce identical tRNAs to compensate for the temporary lack in host or to increase the effectiveness of infection. Our results contradict the codon and amino acid bias hypotheses and support a novel function of tRNAs in bacteriophages. Our findings could prove useful in the advancement of phage therapy, since the presence of tRNAs could increase the effectiveness of bacteriophage infection.