Isolation of Nucleic Acid Copuritant from Putative tRNA-modifying Enzymes

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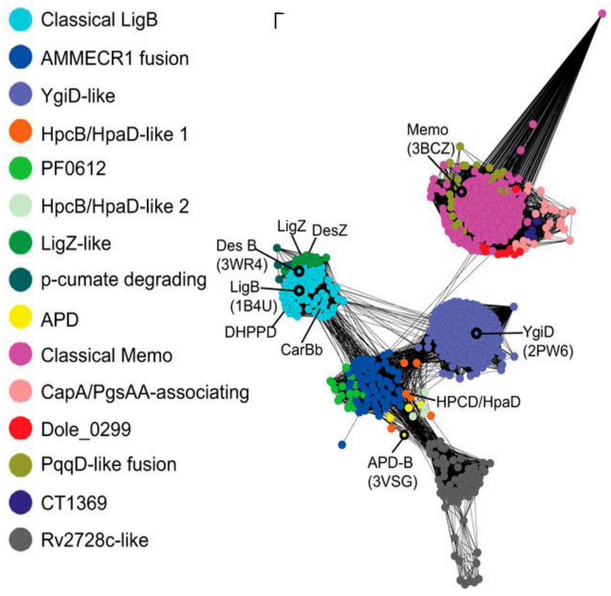
ABSTRACT

Transfer RNAs (tRNAs) are essential adapter molecules of protein synthesis, mediating the translation of the genetic codes into amino acid sequences. To be fully active, tRNAs undergo an extensive range of post-transcriptional modifications that not only maintain the molecule's structural stability but also ensure translational competency and promote cellular viability, especially in response to cellular stress factors. Due to the importance of tRNA modifications on the survival of bacteria, tRNA modification enzymes hold promise to be a novel antimicrobial drug target. Recently, we discovered a set of unstudied bacterial enzymes found in pathogenic organisms including *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, and *Clostridium innocuum*. Based on the gene context, these enzymes are hypothesized to be involved in tRNA modification pathways and hence could potentially be potent antibiotic targets against the aforementioned pathogens. First, the enzyme from *M. smegmatis* has been expressed in *E. coli* and purified. The absorbance spectra we obtained from UV-Vis spectroscopic analysis of the purified protein exhibits a leftward shift from 280 nm to 260 nm, indicating the presence of nucleic acids, supporting the hypothesis that this enzyme may modify tRNA. Attempts at extracting the nucleic acid copuritant for identification has been unsuccessful due to possible RNases contamination during the purification process. With more precautions employed, we hope to isolate the nucleic acids in near future to characterize the enzyme and its nucleic acid substrates of *M. smegmatis* and the other two bacterial strains.

PCAD–MEMO PROTEIN SUPERFAMILY



Sequence similarity network of representative PCAD-Memo superfamily domains. *Nodes* represent groups of sequences that share <50% identity, and edges are shown if the pair-wise BLAST e-value is <e –10 or <e –30. Enzymatically and structurally characterized proteins are labeled. Nodes are colored according to family, as determined by BLASTCLUST analysis.

The newly discovered PCAD–Memo superfamily comprises fifteen clearly-delineable families, with a fundamental divide between the the protocatchuate dioxygenases (PCAD) and the Memo clades despite close structural and sequence similarities. While PCADs are largely known for enzymatic cleavage of extradiol bonds of stable aromatic rings, Memo proteins are reported to serve as general regulators of cell motility-related pathways, such as actin reorganization, microtuble capture, and tumor migration. Although limited data has been collected on Memo enzymatic activities, analyses of the conserve gene-neighborhoods and domain architecture associations for the protein class point to an enzymatic role in the modification of nucleic acid bases.

GENOMIC CONTEXT OF tRNA-MODIFYING ENZYMES

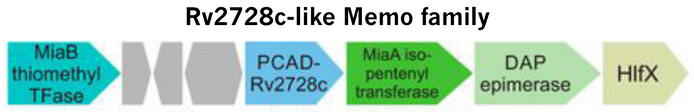
AMMECR1 fusion PCAD proteins

Clostridium acetobutylicum, NP_348048.1
Clostridium difficile

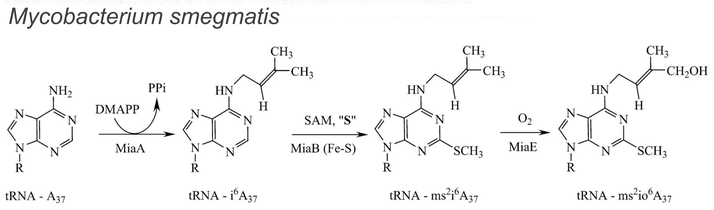
▲ The direct fusion with a AMMECR1 domain and the distal association with a radical SAM enzyme suggests involvement of the PCAD enzyme in a nucleotide or nucleotide-base modification reaction.

▲ The newly identified actino-bacteria-specific Rv2728c-like Memo family is observed in the conserved neighborhoods with MiaA, MiaB, and occasionally with a distal HflX GTPase, altogether implying a function in the synthesis or modification of an adenine nucleotide, possibly of rRNA.

Rv2728c-like Memo family



Mycobacterium tuberculosis, WP_003909733.1
Mycobacterium smegmatis

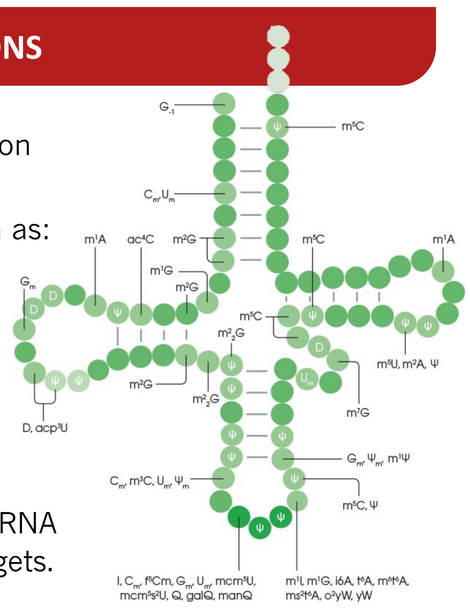


tRNA MODIFICATIONS

▲ tRNA is the most extensively modified RNA with, on average, 13 modifications per molecule.

▲ tRNA modifications play multi-faceted roles, such as:
- increase protein synthesis fidelity
- prevent frameshift mutations
- promote production of certain proteins
- allow rapid response to environmental stress
- enable tRNA isoacceptors to read multiple synonymous codons

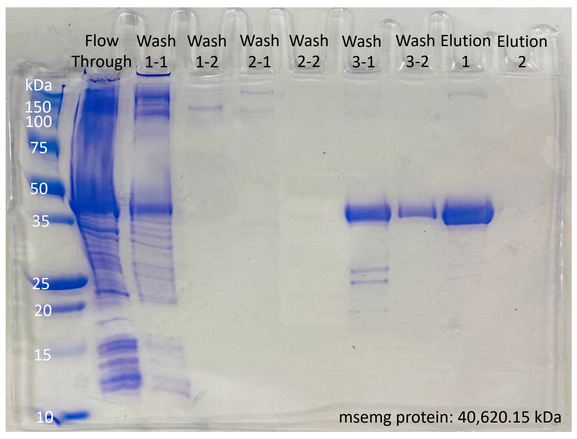
▲ Due to its importance to the cell functionality, tRNA modifying enzymes may be potent antimicrobial targets.

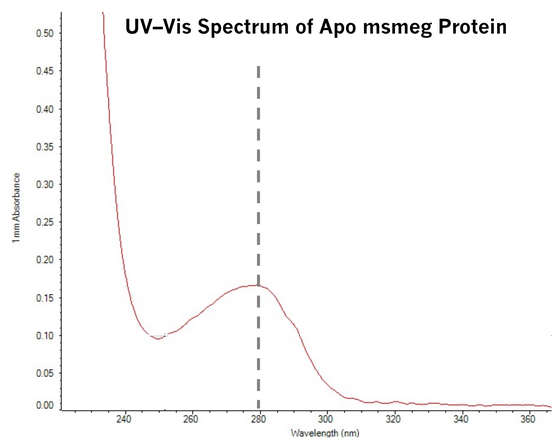


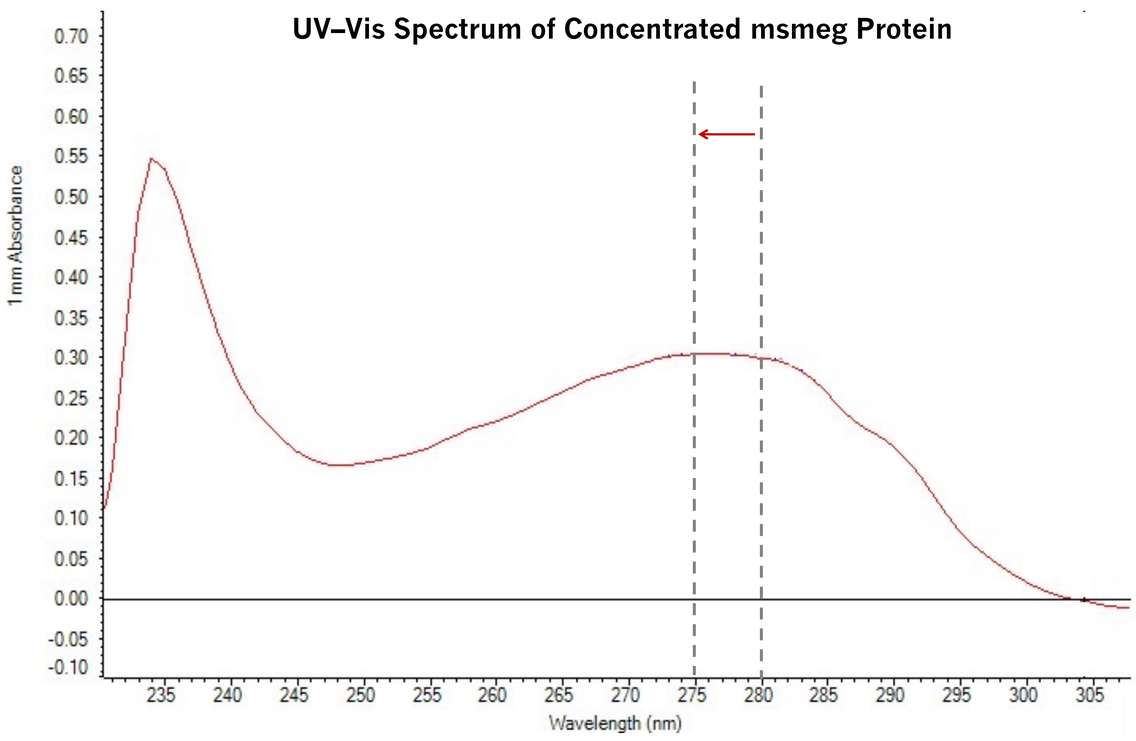
ISOLATION OF PUTATIVE tRNA-MODIFYING PROTEIN

A knockout construct of the *Mycobacterium Smegmatis* PCAD–AMMECR1 gene was created and transformed into BL21–AI *E. coli* chemical competent cells. The 'msmeg' protein was purified using Ni-Affinity Chromatography. The SDS–PAGE gel of purified msmeg protein is shown.

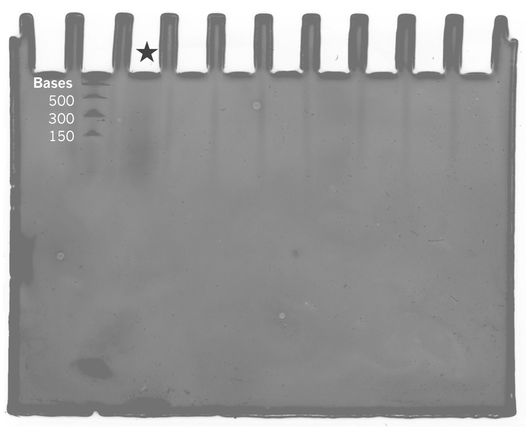
The UV-Vis spectroscopy analysis of the concentrated msmeg protein shows a leftward shift from 280 nm to 260 nm, indicating the presence of nucleic acid.







ISOLATION OF NUCLEIC ACID COPURITANTS



July 28	ng/µL	A260/280	A260/230
Run 1	1167.1	1.65	1.57
Run 2	1166.5	1.65	1.56
Run 3	1159.1	1.65	1.56
Average	1164.4	1.65	1.56

Possibly due to contamination with RNases, attempts at isolation of nucleic acid copuritants of the msmeg protein through phenol extraction was unsuccessful.

FUTURE DIRECTIONS

▲ With more precautions, isolate of nucleic acid copuritants from the hypothetical tRNA binding enzymes of *M. smegmatis* and the remaining two bacteria, *Mycobacterium tuberculosis* and *Clostridium innocuum*

▲ Determine the identity of the nucleic acid copuritants

▲ Structural characterization of these novel enzymes

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