Supporting Information

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Mechanistic Studies of the tRNA-Modifying Enzyme QueA: A Chemical Imperative for the Use of AdoMet as a "Ribosyl" Donor

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***In vitro* transcription of 17-mer RNA Substrate**. Template DNA for the transcription reactions was composed of two synthetic DNA oligomers: T_7-Top 5'-TAATACGACTCACTATA-3' and T_7-Asn 5'-GCGGATTAACAGTCCGCTATAGTGAGTCGTATTA-3'. The Standard transcription reactions were run in 40 mM Tris (pH 8.1), 1 mM spermidine, 5 mM DTT, 50 μg/mL bovine serum albumin, 0.01% Triton X-100, 80 mg/mL polyethylene glycol (6000-7000 MW), 20 mM $MgCl_2$, 4 mM NTPs, 500 nM T_7-Top, 500 nM T_7-Asn, and T_7 RNA polymerase (5 μg). Transcription reactions were typically run for 6 hours at 37 ˚C, followed by the addition of DNase (200 units) and incubation at 37 ˚C for 1 hour. The reactions were then extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with an equal volume of chloroform. The RNA was precipitated after addition of 3 volumes of ethanol and cooling to -20 ˚C, collected by centrifugation for 30 minutes at 15,000 rpm (Sorvall SS-34 rotor), and after air-drying was dissolved in 3 mM citrate buffer (pH 6.3). The RNA was subjected to two additional cycles of ethanol precipitation before use, and stored in 3 mM citrate buffer (pH 6.3) at -90 ˚C until use.

Tgt/QueA Reaction. A typical reaction contained in a final volume of 500 μl 50 mM HEPES (pH 7.5), 2 mM DTT, 10 mM $MgCl_2$, 50 mM KCl, 0.2 mM $preQ_1$, and 20 μM RNA. Reactions were initiated with the addition of 30 μg of recombinant Tgt, and after 1 hour at 30 ˚C, [1'-^{13}C]AdoMet and recombinant GST-QueA were added to final concentrations of 0.2 mM and 0.24 μg/μL (added in 3 portions over 8 hours), respectively. The reactions were terminated by precipitation of the modified RNA with three volumes of ethanol and cooling to -20 ˚C.

Isolation of [^{13}C]oQ. The precipitated RNA from a Tgt/QueA reaction was resuspended in 10 mM ammonium acetate (pH 5.3) and digested with nuclease P1 (8 units) for 2 hours at 42 ˚C.

The pH was made alkaline with the addition of 1 M ammonium carbonate, and the nucleotides dephosphorylated with alkaline phosphatase (30 units, 2 hours at 37 ˚C). The mononucleoside solution was then passed through a Microcon-10 (Amicon) ultrafiltration unit to remove proteins and particulates. The [^{13}C]oQ in the filtrate was then purified via HPLC using a Supelcosil LC-18S column (Supelco) with a mobile phase of 100% 100 mM ammonium acetate (pH 6.0) to 40/60 acetonitrile/water. A representative chromatogram is shown below, along with the UV spectrum of [^{13}C]oQ. HPLC Retention times and the UV spectra of the oQ were compared with authentic material obtained from the digestion of tRNA^{Tyr} obtained from *E. coli* MRE600, which is known to contain oQ instead of Q.

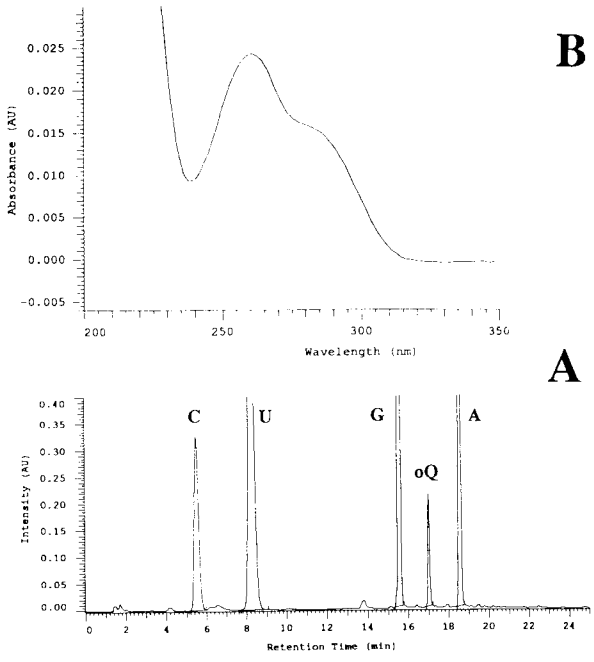

Figure S1. (A) Representative reverse-phase HPLC chromatogram of nucleosides generated after reaction of the RNA oligomer with recombinant Tgt and GST-QueA, nuclease digestion of

the modified RNA, and dephosphorylation of the mononucleotides. **C**, cytidine; **U**, uridine; **G**, guanosine; **oQ**, epoxyqueuosine; **A**, adenosine. HPLC was performed with an Hitachi HPLC system composed of a L7100 pump and L4500A diiode array detector controlled via the D7000 Chromatography software using a Supelcosil LC-18S column with a gradient of 100% 250 mM NH_4OAc (pH 6.0) to 40% CH_3CN/60% H_2O. (B) UV spectrum of the [^{13}C]oQ isolated.