



Title	Biochemical analysis of human tRNA(His) guanylyltransferase in mitochondrial tRNA(His) maturation
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Supporting Information

**Biochemical analysis of Human tRNA^{His} guanylyltransferase
in mitochondrial tRNA^{His} maturation**

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Table S1. Primers used in this study

Target	Primer	Sequence
hThg1ΔMTS	Thg1_S	GGAATTCCATATGGCAAAAAGCAAGTTCGAGTACGTGAGGG
	Thg1_AS	CCGCTCGAGTCAGCTGTCTTCATCTAGAATCTCTGG
hmtRNA ^{His}	hm_Bam_S	GGAATTGGATCCTAATACGACTCACTATAGTAAATATAGTTTAAC CAAAACATCAGA
	hm_M	AACCAAAACATCAGATTGTGAATCTGACAACAGAGGCTTACGACC
	hm_Hind_AS	ATTCTAAGCTTCCTGGGGTAAATAAGGGGTCGTAAGCCTCTGT
	hm_AS_me	<u>T</u> GGGGTAAATAAGGGGTCGTAAGCCTCTGT
hmtRNA ^{His} -A	hm-A_Hind_AS	ATTCTAAGCTTCCTGGTGTAATAAGGGGTCGTAAGCCTCTGT
	hm-A_AS_me	<u>T</u> GGTGTAATAAGGGGTCGTAAGCCTCTGT
hmtRNA ^{His} _{GAA}	hm-GAA_M	ATTCTAAGCTTCCTGGAATAATAAGGGGTCGTAAGCCTCTGT
hctRNA ^{His}	hc_Bam_S	GGAATTGGATCCTAATACGACTCACTATAGCCGTGATCGTATAGT GGTTAGTACTCTGCGTT
	hc_M	AGTGGTTAGTACTCTGCGTTGTGGCCGCAGCAACCTCGGTTCGAA TCCGA
	hc_Hind_AS	ATTCTAAGCTTCCTGGTGCCGTGACTCGGATTCGAACCGAGGT
	hc_AS_me	<u>T</u> GGTGCCGTGACTCGGATTCGAACCGAGGT
hctRNA ^{His} -C	hc-A_Hind_AS	ATTCTAAGCTTCCTGGGGCCGTGACTCGGATTCGAACCGAGGT
	hc-A_AS_me	<u>T</u> GGGGCCGTGACTCGGATTCGAACCGAGGT
hctRNA ^{His} _{GAA}	hc-GAA_M	AGTGGTTAGTACTCTGCGTTGAAGCCGCAGCAACCTCGGTTCGAA TCCGA

Antisense primers to generate transcription templates were contained 2'O-methyl-guanosine (bold, underline) at the second position from the 5'-terminus.

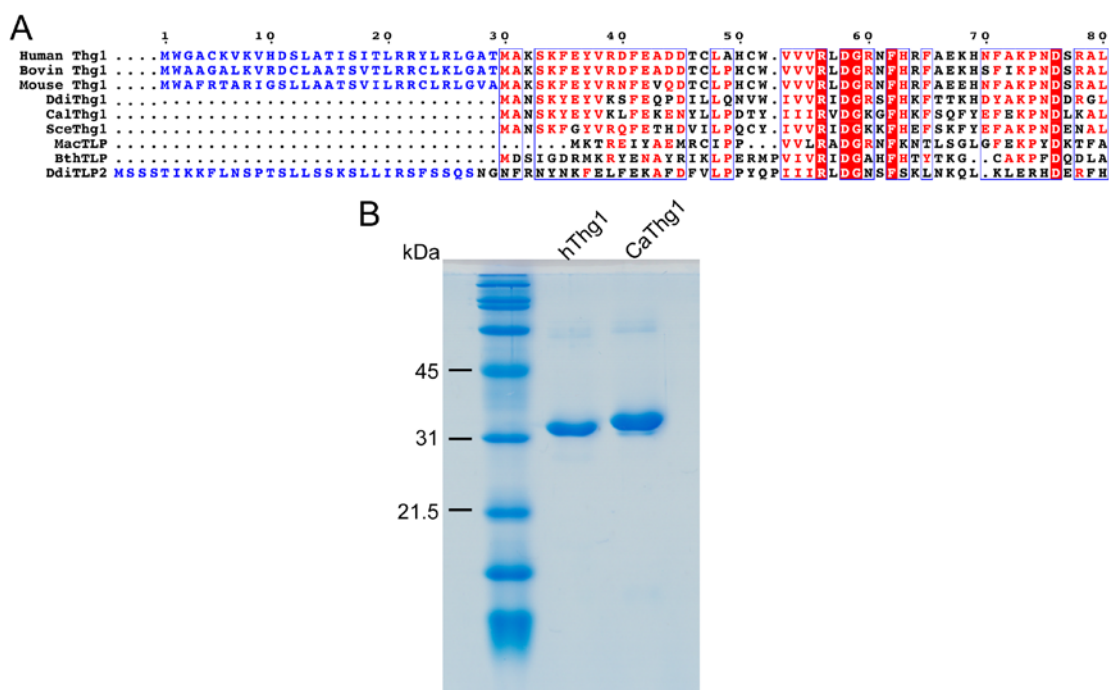


Figure S1:

(A) Multiple sequence alignment of Thg1 and TLP enzymes. Residues highlighted in blue are putative N-terminal mitochondrial targeting sequences predicted by MitoProt [1]. The species aligned are as follows: *Human Thg1*, *Bovin Thg1*, *Mouse Thg1*, *D. discoideum*; *DdiThg1*, *C. albicans* Thg1; *CalThg1*, *S. cerevisiae*; *SceThg1*, *D. discoideum*; *DdiThg1*, *M. acetivorans*; *MacTLP*, *B. thuringiensis*; *BthTLP*, *D. discoideum*; *DdiTLP2*. The figure was prepared using ESPrpt [2]

(B) SDS–PAGE of purified hThg1 without the MTS and CaThg1. The gel was stained with Coomassie Brilliant Blue (CBB).

- [1] M.G. Claros, MitoProt, a Macintosh application for studying mitochondrial proteins. *Comput Appl. Biosci.*, 11 (1995) 441-447.
- [2] X. Robert, P. Gouet, Deciphering key features in protein structures with the new ENDscript server, *Nucleic Acids Res.*, **42**, (2014) W320-W324.

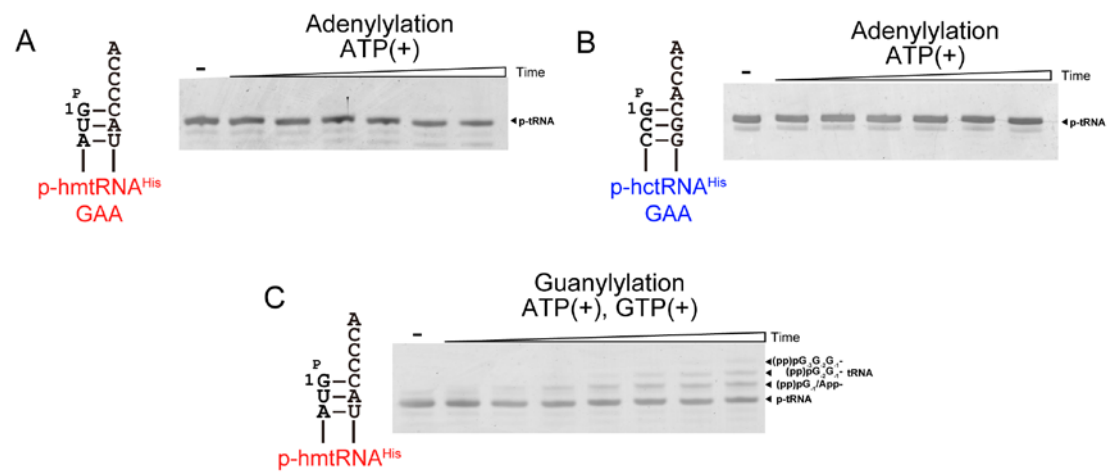


Figure S2:

(A-B) Adenylation reactions with anticodon variants of $\text{hmtRNA}^{\text{His}}$ and $\text{hctRNA}^{\text{His}}$, in which the GUG anticodon were replaced with the GAA anticodon ($\text{hmtRNA}^{\text{His}}_{\text{GAA}}$ and $\text{hctRNA}^{\text{His}}_{\text{GAA}}$, respectively)

(C) Guanylation reactions with $p\text{-hmtRNA}^{\text{His}}$ in the presence both ATP and GTP.

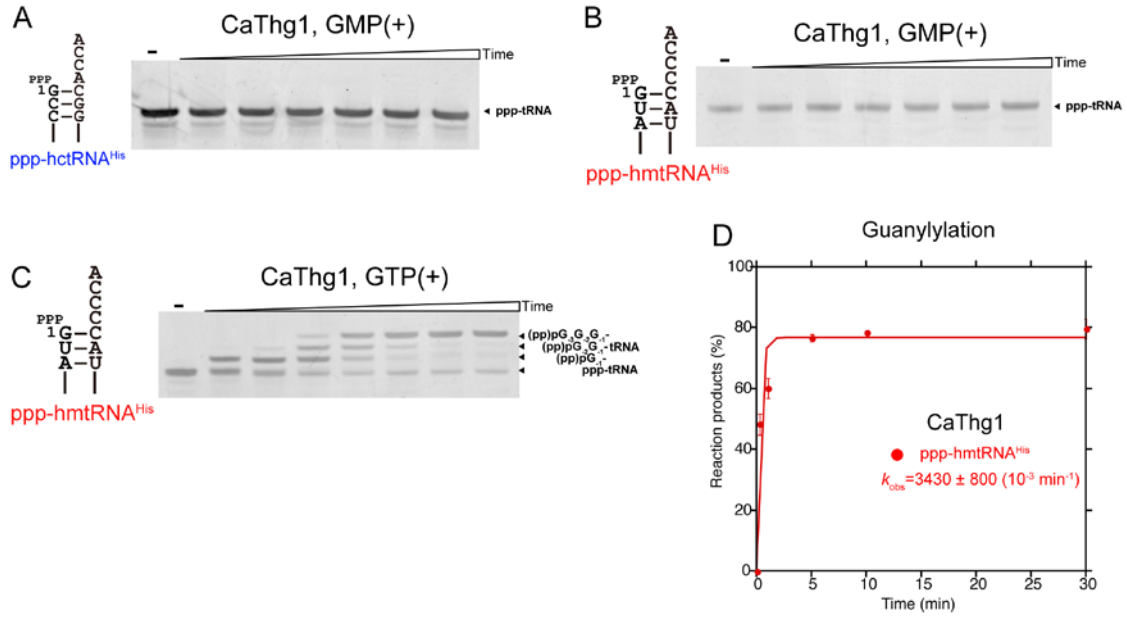


Figure S3:

(A-C) Guanylylation and GMP addition reaction to ppp-hmtRNA^{His} by using *C. albicans* Thg1 (CaThg1). CaThg1 could not use GMP as a substrate for guanylylation on both hctRNA^{His} (A) and hmtRNA^{His} (B), but catalyzed consecutive GTP addition to ppp-hmtRNA^{His} (C).

(D) Time course experiments of guanylylation of ppp-hmtRNA^{His} using CaThg1. Lines represent fits of each time course to a single-exponential equation (eq. 1) to yield k_{obs} in Material and Methods section. The bars in the graphs are SD of more than two independent experiments.