Supporting Information

Molecular mechanism of substrate recognition and specificity of $tRNA^{His}$ guanylyltransferase during nucleotide addition in the 3′-5′ direction

Akiyoshi Nakamura[1], Daole Wang[2], and Yasuo Komatsu[1, 2]*

[1] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

[2] Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan

* To whom correspondence should be addressed. Tel: +81-11-857-8437; Fax: +81-11-857-8954; Email: komatsu-yasuo@aist.go.jp

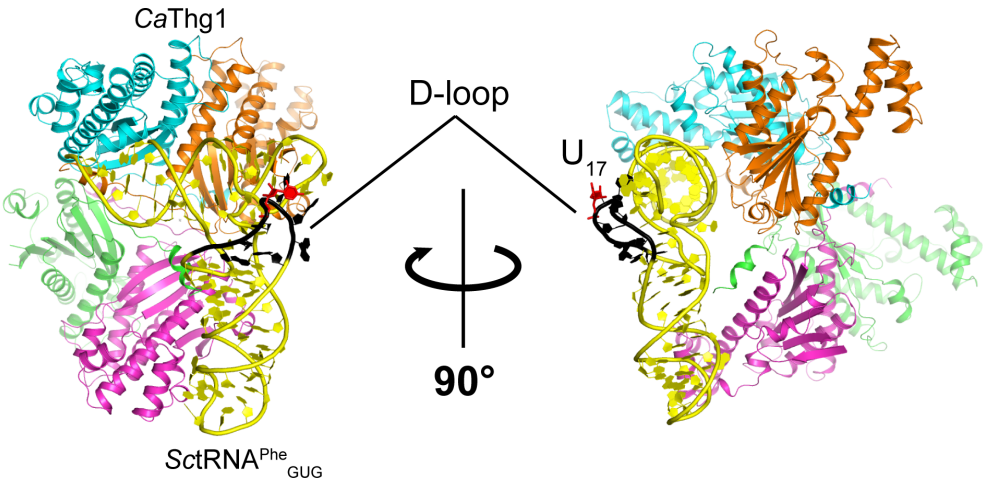

Figure S1: Structure of the *Candida albicans* Thg1 (*Ca*Thg1) and *S. cerevisiae* $tRNA^{Phe}_{GUG}$ ($Sc tRNA^{Phe}_{GUG}$) complex (PDBID: 3WC2). The tetrameric structure of *Ca*Thg1 is shown as ribbon models (cyan, orange, green, and magenta). $Sc tRNA^{Phe}_{GUG}$ is shown as yellow ribbon, and its D-loop is indicated in black. The U_{17} (red stick model) is flipped out from the tertiary core region of the tRNA.

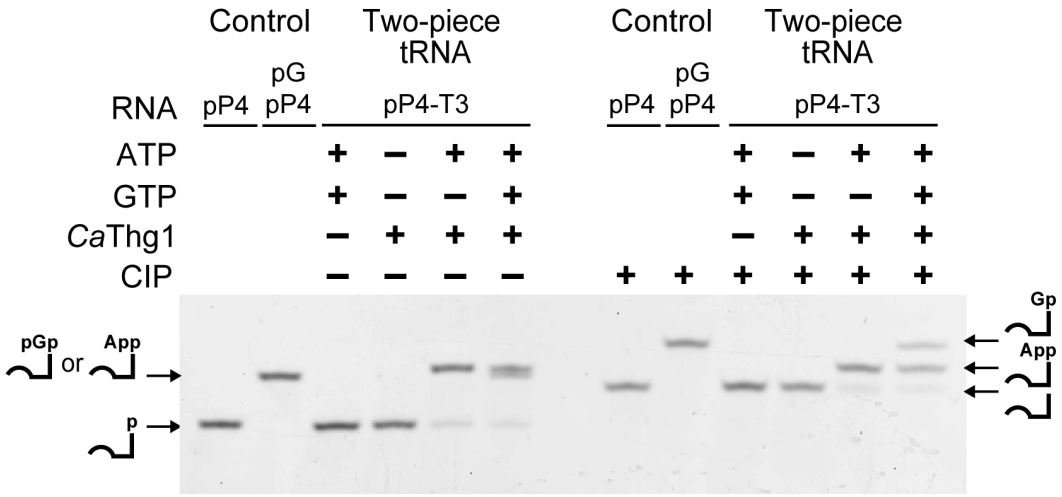

Figure S2: Separation of adenylylation and G_{-1} addition reaction products with/without phosphatase (Calf Intestinal Alkaline Phosphatase; CIP) treatment by a denaturing PAGE. Two-piece tRNA composed of primer (pP4) and template (T3) fragments was incubated with 10 μM *Ca*Thg1 and with/without ATP and GTP for 30 min. Reaction mixture was incubated with CIP, and then loaded on a 20% Urea-PAGE gel. Chemical synthetic pP4 and pP4 with added G_{-1} (pGpP4) were used as control samples.

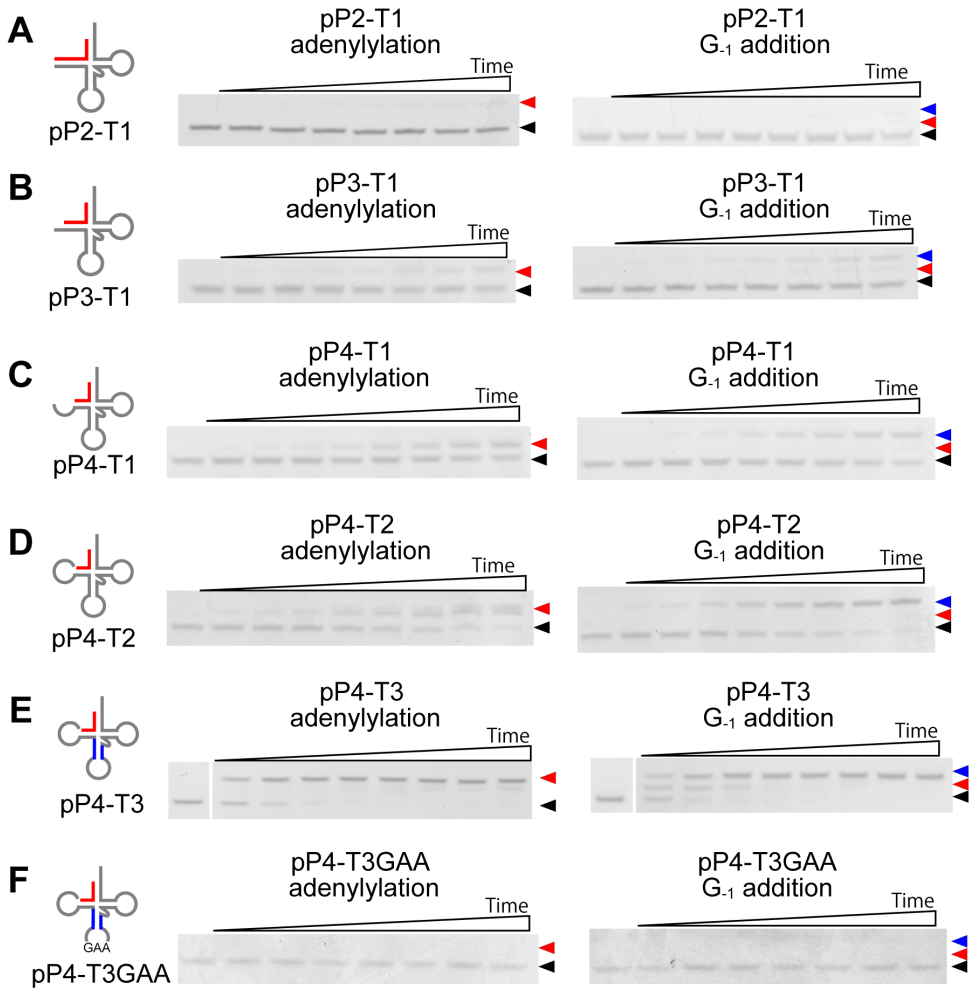

Figure S3: A primer/template assay of adenylylation and nucleotide addition reaction with two-piece tRNAs: pP2-T1 **(A)**, pP3-T1 **(B)**, pP4-T1 **(C)**, pP4-T2 **(D)**, pP4-T3 **(E)**, pP4-T3GAA **(F)**. Black, red, and blue triangles indicate bands of substrate, adenylylated product, and G_{-1} added product, respectively.

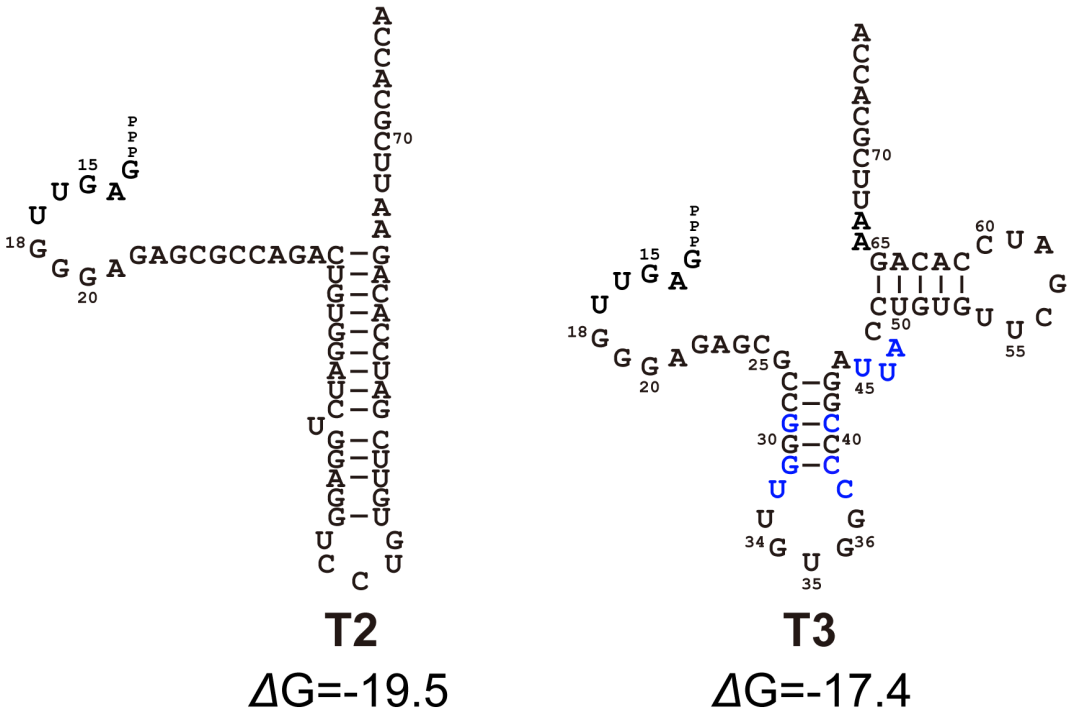

Figure S4: Secondary structure prediction of template RNAs by *Mfold* (1). The altered bases of T3 are indicated in blue.

1. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*, **31**, 3406-3415.

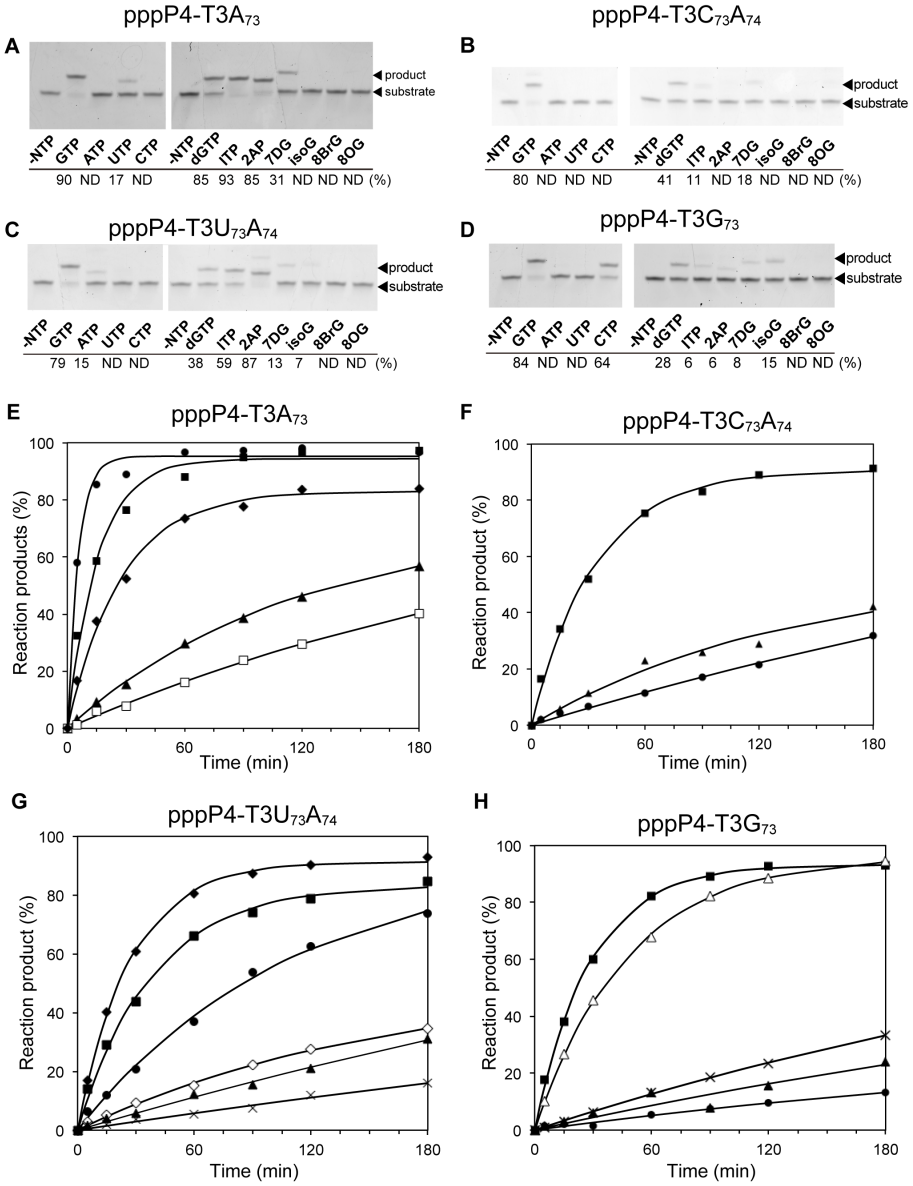

Figure S5: Nucleotide addition reactions for natural NTPs and various GTP analogs into two-piece tRNA variants.

(A-D) The percentage of nucleotide addition products for four natural NTPs and various GTP analogs after 60 min incubation with pppP4-T3 variants, analyzed by Urea-PAGE.

(E-H) Time course experiments of nucleotide addition reactions for various GTP analogs onto pppP4-T3A_{73} **(E)**, -T3$C_{73}A_{74}$ **(F)**, -T3$U_{73}A_{74}$ **(G)**, and -T3G_{73} **(H)**. Lines represent each time course fitted to a single-exponential equation (eq. 1) to yield k_{obs}. The marks indicate as follows; GTP (■), ITP (●), 2AP (◆), 7DG (▲), UTP (□), ATP (◇), CTP (△), isoG (×).

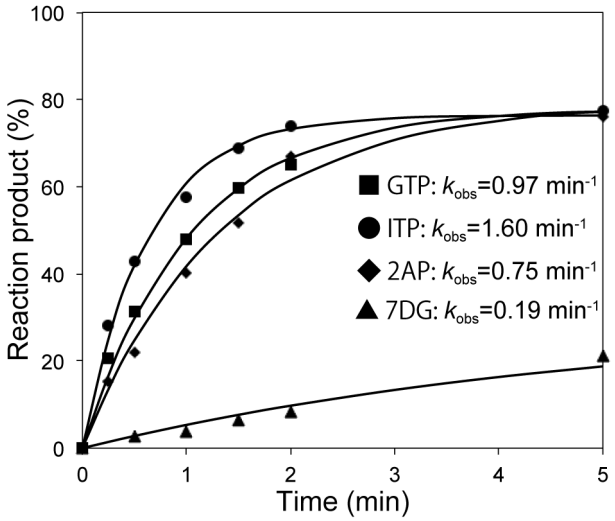

Figure S6: Time course experiments of nucleotide addition reaction for various GTP analogs onto the full-length *Sc*tRNA$^{Phe}_{GUG}$. Lines represent each time course fitted to a single-exponential equation (eq. 1) to yield k_{obs}. The marks indicate as follows; GTP (■), ITP (●), 2AP (◆), 7DG (▲).

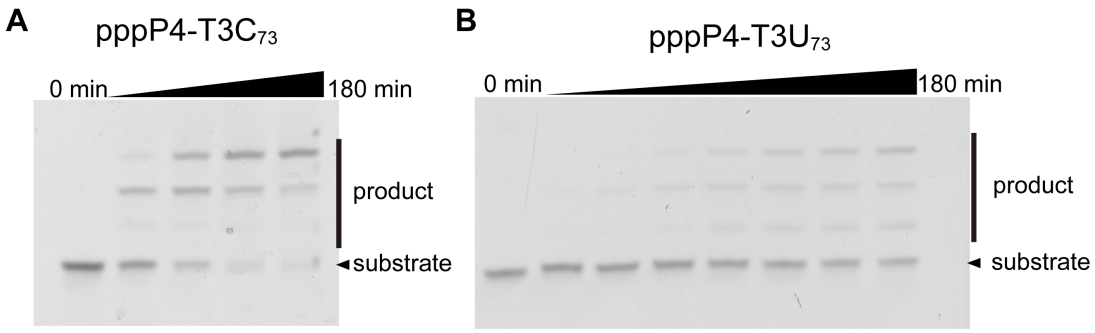

Figure S7: The multiple products formation of GTP addition onto pppP4-T3C_{73} (A), -T3U_{73} (B).