Electronic Supplementary Information

For

Investigation of end processing and degradation of premature tRNAs and their application to stabilization of *in vitro* transcripts in wheat germ extract

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CONTENTS:

Preparation of DNA templates. DNA templates for *in vitro* transcription were prepared with standard polymerase chain reactions (PCRs) by using PrimeSTAR Max DNA Polymerase, which has extremely high fidelity, from Takara Bio (Ohtsu, Japan). The YPet-encoding plasmid named pHis-TAG-RY-YPet, which has the amber codon in the N-terminal region of the open reading frame, was used as a PCR template for **amber-mRNA**.[1] A PCR template for **opal-mRNA** was prepared by ligation (and the subsequent final PCR) between the 5' segment and the 3' segment, both of which were PCR-amplified from pHis-TAG-RY-YPet by using a primer with an *Spe*I site. PCR templates for RNAs other than mRNAs were synthesized by Life Technologies (Tokyo, Japan). Normal primers for PCRs were purchased from Life Technologies or Operon Biotechnologies (Tokyo, Japan). Reverse primers modified with 2'-methoxy-G (2'-OMe-G) at the second base from the 5' terminus, from Tsukuba Oligo Service (Tsukuba, Japan), were used in the final PCR for RNAs with the 3' mature tRNA structure to reduce the non-templated 3' nucleotide addition.[2] The sequences of templates and primers are summarized below.

Sequences of primers and templates for PCR.

(written from 5' (left) to 3' (right); underlined: an *Spe*I site; X: 2'-OMe-G.)

Forward primer in the 5' segment amplification for **opal-mRNA**

CCGGCGCCAGATGGCTAGACA

Reverse primer in the 5' segment amplification for **opal-mRNA**

GACACTACTAGTGATATCTTGGTGATGTAGA

Forward primer in the 3' segment amplification for **opal-mRNA**

GACACTACTAGTATGGCCCATCACCATCACCATCATTGAAGATACAGCAGCGGCCT

Reverse primer in the 3' segment amplification for **opal-mRNA**

AGCTGTTTGCGCGTCTGAAAG

Forward primer in the final PCR for **opal-mRNA** and **amber-mRNA**

CATACGATTTAGGTGACACT

Reverse primer in the final PCR for **opal-mRNA** and **amber-mRNA**

TTAGCGGCTTTATTGATTGC

Template for **t86**, **5pt**, **3pt**, **3pt2**, **dpt**, **5SL-5pt**, **5SL-dpt**, and **5SL-5L-GGCt86**

GGAGAGATGGCTGAGTGGTTGATAGCTGCGGTCTCTAAAACCGCTATAGTTCTAGGAACTA
TCGAGGGTTCGAATCCCT

Template for **opal-t86**

GGAGAGATGGCTGAGTGGTTGATAGCTGCGGTCTTCAAAACCGCTATAGTTCTAGGAACTA
TCGAGGGTTCGAATCCCT

Forward primer for **t86**, **3pt**, **3pt2**, and **opal-t86**

GAAATTAATACGACTCACTATAGGAGAGATGGCTGAGTG

Forward primer for **5pt** and **dpt**

GTAATACGACTCACTATAGGATTAGGCTGTGGAGAGATGGCTGAGTG

Forward primer for **5SL-5pt** and **5SL-dpt**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTGGATTAGGCTGTGGAGA
GATGGCTGAGTG

Forward primer for **5SL-5L-GGCt86**

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTGGATTAGGCTGTGGGCG
GAGAGATGGCTGAGTG

Reverse primer for **t86**, **5pt**, **5SL-5pt**, **5SL-5L-GGCt86**, and **opal-t86**

TXGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for **3pt**, **dpt**, and **5SL-dpt**

AAGCAAACGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Reverse primer for **3pt2**

CXGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Template for **apt12**, **5SL-apt12**, **5SL-apt12-3SL**, and **5SL-apt12-GGCt86** (in the 1st PCR for the last)

GGGAGCTCAGAATAAACGCTCAAGTACCTGAAAATGGGAAGCAGAGCGAGCCTTTCGACA
TGAGACACGGATCCTGC

Forward primer for **apt12**

GAAATTAATACGACTCACTATAGGGAGCTCAGAATAAACGCTCA

Forward primer for **5SL-apt12**, **5SL-apt12-3SL**, and **5SL-apt12-GGCt86** (in the 1st, 2nd and final PCR for the last)

GAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTGGGAGCTCAGAATAAA
CGCTC

Reverse primer for **apt12** and **5SL-apt12**

GCAGGATCCGTGTCTCATGTCG

Reverse primer for **5SL-apt12-3SL**

CCCTCTGGGTTGTCCAAAGGGAGCAGGATCCGTGTCTCATGTCG

Reverse primer in the 1st PCR for **5SL-apt12-GGCt86**

TATAGCGGTTTTAGAGACCGCAGCTATCAACCACTCAGCCATCTCTCCGCCGCAGGATCCG
TGTCTCATGTCG

Reverse primer in the 2nd PCR for **5SL-apt12-GGCt86**

TGGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTCCTAGAACTATAGCGGTTTTAGAGAC
CGCAGCTATC

Reverse primer in the final PCR for **5SL-apt12-GGCt86**

TXGCGGAGAGAGAGGGATTCGAACCCTCGATAGTTC

Preparation of mRNAs. Run-off transcription of the obtained DNA templates for mRNAs was performed with an AmpliScribe SP6 High Yield Transcription Kit (CellScript, Madison, WI) according to the manufacturer's protocol. The transcribed mRNA was purified with an RNeasy MinElute Cleanup Kit (QIAGEN, Tokyo, Japan) and quantified by the absorbance at 260 nm.

Preparation of other RNAs. **t86**, **opal-t86**, pre-tRNAs, **apt12**, and its derivatives were constructed by *in vitro* run-off transcription of the DNA templates including the T7 promoter sequence with an AmpliScribe T7 High Yield Transcription Kit (CellScript) according to the manufacturer's protocol. The transcribed RNAs were purified with a QIAquick Nucleotide Removal Kit (QIAGEN) and quantified by the absorbance at 260 nm.

Evaluation of the end processing and degradation of *in vitro* transcripts in WGE. A high-quality wheat germ extract (WGE) in a WEPRO1240 Expression Kit (CellFree Sciences, Matsuyama, Japan), which is optimized for cell-free translation, was used for evaluating the end processing and the degradation of *in vitro* transcripts. A mixture (10 μL) of an *in vitro* transcribed RNA analyte (pre-tRNA, **apt12**, or its derivative; 10 pmol for translation or 50 pmol for gel analyses), **amber-mRNA** (3 pmol for translation or no mRNA for gel analyses), WEPRO1240 (WGE, 2 μL), creatine kinase (final concentration: 40 ng/μL), and SUB-AMIX (final concentration: 1×), the latter three of which were included in the kit, was incubated at 26°C. In the amber suppression experiments, the fluorescence intensity of the translated YPet was measured after 1-h incubation as previously described.[1] Tha background fluorescence in the absence of any tRNA was subtracted from each value. In the gel analyses, an aliquot (3.75 μL) of the reaction solution was resolved by 15% SDS-PAGE and stained with ethidium bromide. The amount of RNAs on the gel was estimated from their band intensity with Image J software (NIH) by referring to a calibration curve drawn with serially-diluted non-incubated RNA.

Kinetic analyses. The rate constant k of the removal of the 5' leader or the 3' trailer, the CCA addition, and the degradation were calculated by fitting the data from gel analyses to a single-exponential function

of the form $y(t) = A(1 - e^{-kt})$ with the least squares method by using the solver function of Microsoft Excel.

Evaluation of the function of stabilized eRF1-binding aptamers. Cell-free translation was carried out, as described above, with slight modifications. A mixture (10 μL) of **opal-mRNA** (3 pmol), **opal-t86** (50 pmol), **apt12** or its derivative (10 pmol), WEPRO1240 (2 μL), creatine kinase (final concentration: 40 ng/μL), and SUB-AMIX (final concentration: 1×) was incubated at 26°C for 2 h. After incubation, the fluorescence intensity of the expressed YPet was measured as in the amber suppression experiments.

Figures.

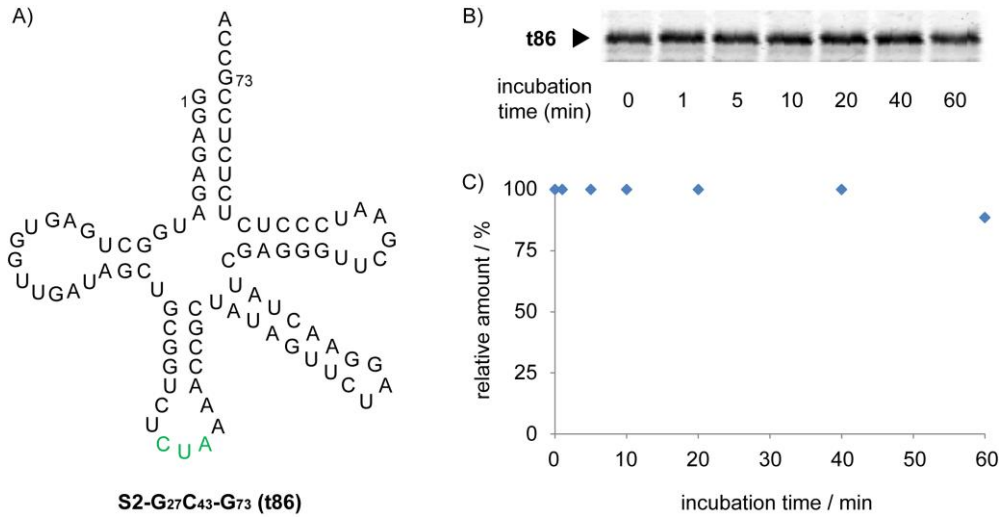

Figure S1. The *in vitro*-transcribed amber suppressor tRNA (**t86**) that exhibits high suppression efficiency and stability in WGE. (A) The secondary structure of **t86**.[1] Three green bases indicate the anticodon for the amber codon. (B) Gel analysis of the stability of **t86** in WGE. (C) Time course of relative amounts of **t86**, calculated from the band intensities.

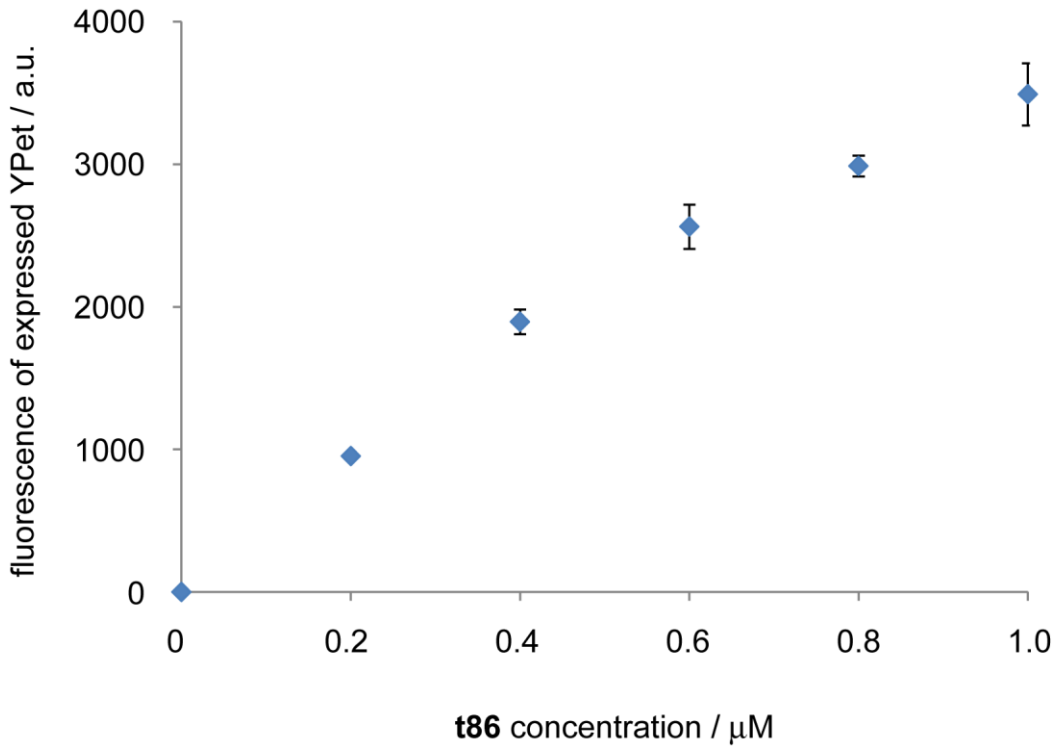

Figure S2. Fluorescence intensities of YPet translated from **amber-mRNA** via amber suppression in the presence of various concentrations of **t86** in WGE.

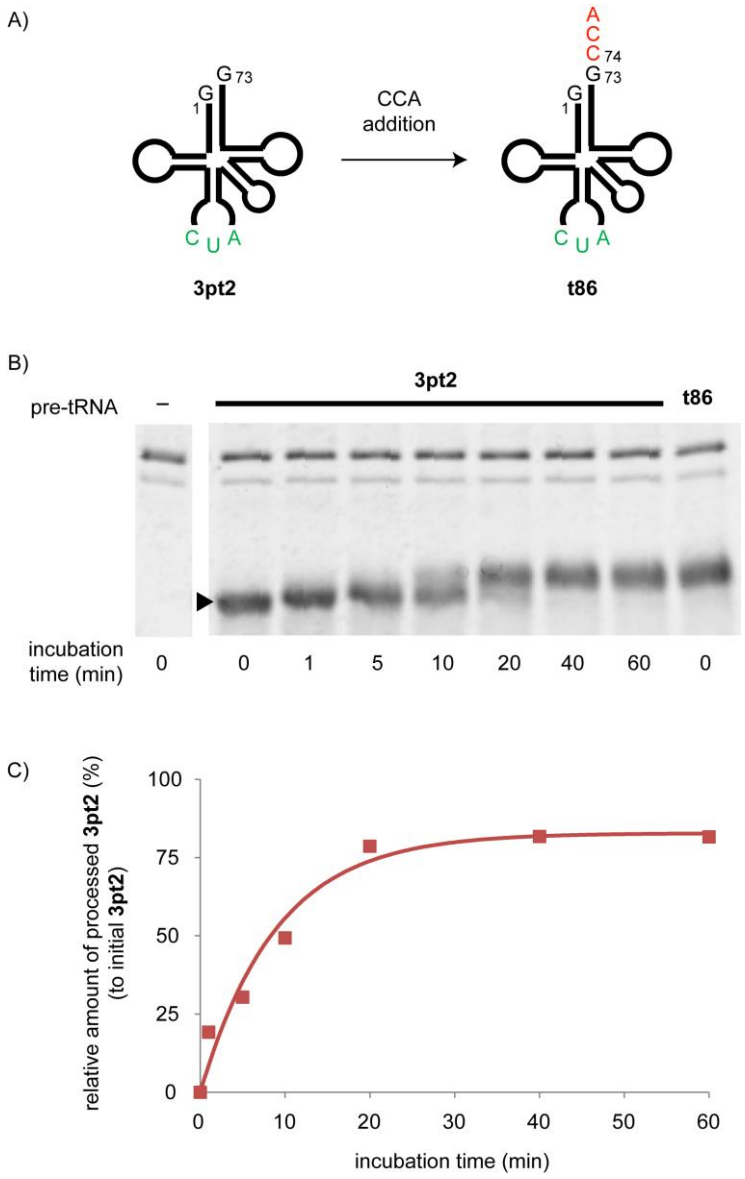

Figure S3. Another 3' pre-tRNA with no 3' trailer (**3pt2**). (A) Schematic illustration of **3pt2** and the CCA addition thereof to **t86**. (B) Gel analysis of the CCA addition of **3pt2**. The arrowhead indicates **3pt2**. (C) Time course of the roughly-determined amount of processed **3pt2** relative to that of initial **3pt2**. The data were fitted to a single-exponential function of the form of $y(t) = A(1 - e^{-kt})$.

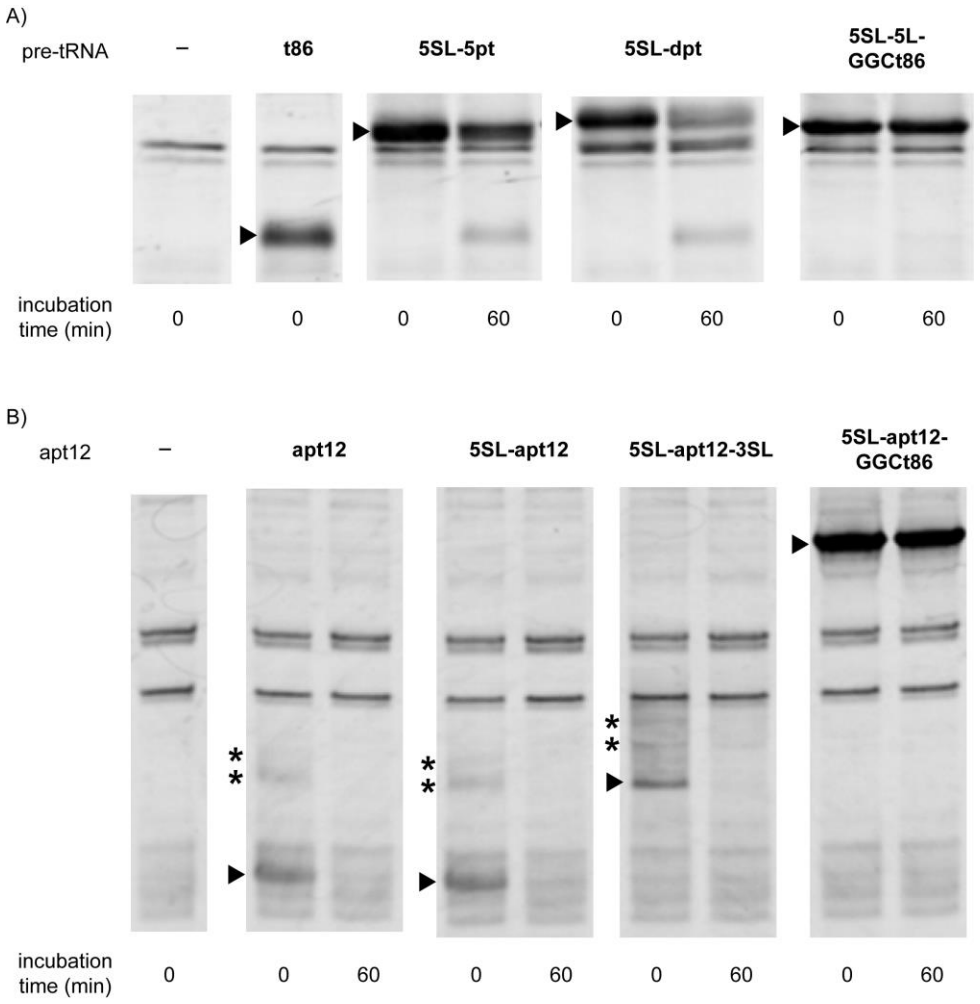

Figure S4. Gel analyses of stabilized pre-tRNAs (A) and **apt12** (B) that were incubated for 0 or 1 h in WGE. The arrowheads represent initial *in vitro* transcripts. **apt12** and its derivatives other than **5SL-apt12-GGCt86** seem to be somewhat folded into some different structures (asterisks).

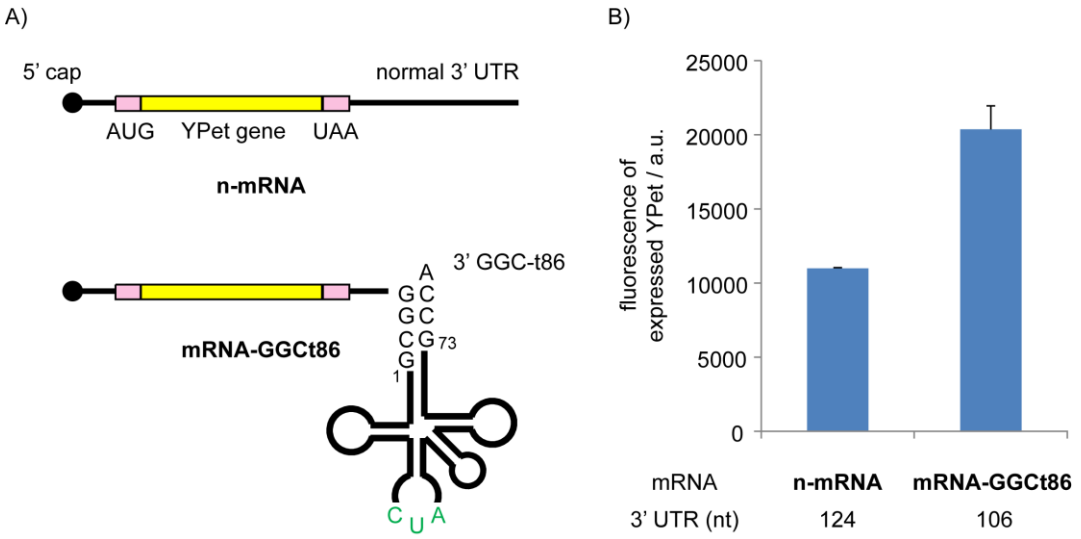

Figure S5. The effect of the 3' end protector on mRNA. (A) Schematic illustration of **n-mRNA** with a normal 3' untranslated region (UTR) derived from the plasmid and **mRNA-GGCt86** with the 3' end protector (3' $G_{-3}G_{-2}C_{-1}$-t86). Both mRNAs have the 5' cap as the 5' protector. (B) Fluorescence intensities of Ypet translated from each mRNA template. Despite the fact that the 3' UTR length of **mRNA-GGCt86** is slightly shorter than that of **n-mRNA**,[3] the former was translated approximately two-fold as efficiently as the latter.

References.

(1) A. Ogawa, Y. Doi and N. Matsushita, *Org. Biomol. Chem.*, 2011, **9**, 8495-8503.

(2) C. Kao, M. Zheng and S. Rüdisser, *RNA* 1999, **5**, 1268-1272.

(3) A. Ogawa, J. Tabuchi and Y. Doi, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3724-3727.