Supporting material

Synthesis of Biocytin-tRNA Suppressor

1. Synthesis of NVOC-Biocytin Cyanomethyl Ester

Biocytin (100 mg, 0.26 mmol, Molecular Probes) and sodium carbonate (56 mg, 0.54 mmol) were dissolved in a mixture of water (15 ml) and THF (10 ml). A solution of 6-nitroveratryloxycarbonyl chloride (NVOC-Cl) (74 mg, 0.26 mmol, Sigma) in 10 ml THF was added slowly. After 3 hours, solvents were removed *in vacuo*. Then, 3 ml of dry DMF and 3 ml of chloroacetonitrile, as well as 800 µl triethylamine was added into remaining residues. After overnight stirring, solvents were removed *in vacuo* and the remaining solid was purified by flash chromatography (silica gel, 10% MeOH in CH_2Cl_2).

2. Preparation of Biocytin-dpCpA

Tetrabutylammonium salt of dinucleotide (dpCpA) was a gift from Prof. Dennis Dougherty's group (Division of Chemistry and Chemical Engineering, California Institute of Technology). At room temperature, 10 mg dpCpA (8.3 µmol) and 16 mg NVOC-biocytin cyanomethyl ester (25 µmol) was mixed in dry DMF under argon. 20 µl ammonium acetate (25 mM, pH 4.5) was added to quench the reaction after 1 hour stirring. The crude product was purified by reversed phase semi-preparative HPLC using a gradient from 25 mM NH_4OAc (pH 4.5) to CH_3CN. The appropriate fractions were combined and lyophilized. The resulting solid was redissolved in 10 mM acetic acid/CH_3CN and lyophilized to give 3 mg biocytin-dpCpA as a pale yellow solid. The product was confirmed by mass spectrum as it gives a peak ($[M+H]^+$) at MW=1230.4.

3. *In vitro* Transcription of tRNA

THG73 tRNA was synthesized *in vitro* from FokI linearized plasmid harboring THG73 tRNA gene (gift from Prof. Dennis Dougherty's group) using T7 MEGAshortscript kit (Ambion). The product was purified by polyacrylamide gel electrophoresis and dissolved in water.

4. Ligation of Biocytin to THG73 tRNA

The mixture of THG73 tRNA (25 µg in 10 µl water) and HEPES (20 µl, 10 mM, pH 7.5) was heated at 94 ºC for 3 minutes and cooled down to 37 ºC slowly. 8 µl biocytin-dpCpA (3 mM in DMSO), 32 µl 2.5X reaction buffer (25 µl 400 mM pH 7.5 HEPES; 10 µl 100 mM DTT; 25 µl 200 mM $MgCl_2$; 3.75 µl 10 mM ATP; 10 µl 5 mg/ml BSA; 26.25 µl water; 1 µl RNasin (Promega)), 5 µl water, as well as 5 µl T4 RNA ligase (New England Biolabs) was added. After 1 hour incubation at 37 ºC, the reaction mixture was extracted once with an equal volume of phenol (saturated with 300 mM sodium acetate, pH 5.0):$CHCl_3$:isoamyl alcohol (25:24:1), then precipitated with 3 volume of cold ethanol at -20 ºC. The precipitate was washed with cold 70% (v/v) ethanol, dried under vacumm, and resuspended in 5 µl 1mM sodium acetate, pH 5.0. The amount of biocytin-tRNA was quantified by measuring A_{260} and the concentration was adjusted to 1 µg/µl with 1 mM sodium acetate (pH 5.0). Prior to the suppression reaction, the biocytin-tRNA solution was deprotected by xenon lamp equipped with a 315 nm cut-off filter for 5 minutes.

General procedure to make mRNA-peptide fusions

1. Construction of Fusion Template

Synthetic DNA templates including Pep1 (5'-ACTATTTACAACCACCATGGGCCGCC AGGAGATCCACTGGGCCAACGACCTGTGCAAGCCCTTCTGGGTGTACACCTCC-3'), Pep2 (5'-ACTATTTACAACCACCATGGGCTAGCTTGACTACAAGGACGAGGACAAGCG CCAGGAGATCCACTTGGCCAACGACCTGTGCAAGCCCTTCTGGGTGTACACCTCC-3'), Lib1 (5'-ACTATTTACAACCACCATGGGCCGCCAGGAGATCCACNNSGCCAACGACCTG TGCAAGCCCTTCTGGGTGTACACCTCC-3') were purified by preparative polyacrylamide gel electrophoresis. Polymerase chain reaction (PCR) of these templates with two synthetic primers, sd2 (5'-GGATTCTAATACGACTCACTATAGGGACAATTACTATTTACAACCACCATG-3') and sd26 (5'-GCCGCCGCCGCCGGAGGTGTACACCCAGAAG-3'), generates double stranded DNA. mRNA was produced by T7 runoff transcription of these templates in the presence of RNAsecure (Ambion) followed by size exclusion column purification (NAP25 column, Amersham Pharmacia Biotech). The flexible DNA linker containing puromycin, F30P (5'-$dA_{21}[C_9]_3$dACdCP; C_9=triethylene glycol phosphate, Glen Research; P = CPG-puromycin, Glen Research), was synthesized using standard chemistry. The oligonucleotide was chemically phosphorylated using phosphorylation reagent II (Glen Research) and purified by OPC cartridge. Ligation of pF30P to transcribed mRNA was done by mixing mRNA, pF30P, a splint (5'-TTTTTTTTTTTTGCCGCCGCCGCC-3') in a 1:0.5:1.2 ratio with 2 Units of T4 DNA ligase (New England Biolabs) per picomole of template mRNA. After ligation, the fusion template was gel-purified, electroeluted and desalted by ethanol precipitation.

2. Translation and Fusion Formation

The fusion template was translated in reticulocyte lysate (Novagen) using standard conditions (800 nM template) with the addition of ^{35}S-methionine as the labeling reagent. In the case of templates containing UAG stop codon, 2 μg of deprotected biocytin-tRNA suppressor was also added. On completion of translation, fusion formation was stimulated by addition of $MgCl_2$ and KCl to 50 mM and 0.6 M, respectively, and incubated at -20 ºC overnight. The resulting ^{35}S-labeled mRNA peptide fusions were directly loaded to 15% tricine SDS-PAGE for separation. After running, the gel was dried and exposed to phosphor screen (Molecular Dynamics) for several hours. The phosphor screen was then scanned to give image shown on Figure 2B.

Enrichment of UAG Stop Codon by Selection against Streptavidin-Agarose Matrix

1. Template-base (dT) Purification

To isolate fusion, the lysate was diluted in binding buffer (1M NaCl, 20 mM Tris pH 8.0, 1 mM DTT, 10 mM EDTA, 0.2% Trition X-100) and incubated with dT-cellulose at 4 ºC for 1 hour. Bound fusions were washed with washing buffer (0.3M NaCl, 20 mM Tris pH 8.0) and eluted by ddH_2O.

2. Reverse Transcription and Selective Step

Fusion after dT purification was concentrated and used for reverse transcription with Superscript II RNase H^- reverse transcriptase (BRL, life Technologies) following standard conditions recommended by the manufacturer. The reaction mixture (50 µl) was directly added into 1 ml phosphate buffer (50 mM, pH 7) and streptavidin-agarose matrix (Pierce). After a 1-hour incubation at 4 ºC, the matrix was washed with washing buffer (50 mM phosphate pH 8.0, 100 mM NaCl, 0.1% SDS) 500 µl × 6 times. The matrix then was used for PCR amplification with sd2 and sd26. The PCR product was cloned with TOPO Clone kit (Invitrogen) for sequencing.