SUPPLEMENTARY MATERIALS AND METHODS
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Mass spectrometry analysis of the specificity of L/F-transferase

The reaction mixtures (20 μl) contained 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 10 mM Mg(OAc)₂, 1 mM DTT, 2 mM ATP, 56 A₂₆₀ units/ml tRNA mix derived from E. coli (Roche), 0.1 mM each Leu, Met, Phe, and Trp, 10 μM α-casein fragment peptide (RYLGYL; Sigma), an excess amount of leucyl-tRNA synthetase, methionyl-tRNA synthetase, phenylalanyl-tRNA synthetase, and tryptophanyl-tRNA synthetase, and 0.12 μM L/F-transferase. After a 30 min incubation at 37 °C, the peptides, in 5 μl reaction mixtures, were purified and desalted by a NuTip C-18 (Glygen Corp.) and were eluted with 2 μl of a 50% acetonitrile, 0.1% TFA solution saturated with the matrix α-cyano-4-hydroxycinnamic acid. Mass measurements were performed using MALDI-TOF (Voyager, Applied Biosystems) in the reflector mode, and were calibrated with des-arg1-bradykinin (m/z 904.4), angiotensin I (m/z 1296.7), glu1-fibrinopeptide B (m/z 1570.7), and neurotensin (m/z 1672.9) as standards.

UV-cross linking of aminoacyl-tRNAs to L/F-transferase and EF-Tu

Internally [³²P]-labeled tRNA^{Phe} and tRNA^{Leu} transcripts were prepared as described (Okabe et al., 2003). The [³²P]-labeled tRNA transcripts were aminoacylated by the cognate aminoacyl-tRNA synthetases, and the aminoacylated tRNAs were phenol-extracted and precipitated by ethanol under acidic conditions. E. coli EF-Tu was prepared as described (Shimizu et al., 2001) and the purified EF-Tu was converted to the EF-Tu:GTP form as described (Shimizu & Ueda, 2006).

For the UV-cross linking, the solutions, containing 50 mM Hepes-KOH, pH 7.6, 60 mM NH₄Cl, 1 mM DTT, 7 mM MgCl₂, 5 μM EF-Tu:GTP, 3 nM [³²P]-labeled aminoacyl-tRNA (40000 cpm) and various amounts of L/F-transferase (0-10 μM), were irradiated by a UV-lamp (254 nm) for 25 min on ice. After irradiation, SDS stop solution was added to the mixture, and the samples were fractionated by 8% (w/v) SDS PAGE. The gels were dried and analyzed by a BAS5000 analyzer (Fuji Film).

Competitive reactions were carried out in the presence of various amounts of EF-Tu:GTP at 37 °C. A solution (50 μl) containing 50 mM Tris-Cl, pH 8.0, 150 mM
KCl, 10 mM MgCl₂, 1 mM DTT, 10 µM α-casein, 0.5 µM [¹⁴C]-Leu-tRNA<sub>Leu</sub> (or 1.0 µM [¹⁴C]-Phe-tRNA<sub>Phe</sub>) and 25 nM L/F-transferase was incubated at 37 °C. Aliquots of the solution (10 µl) were withdrawn at the indicated times, precipitated by 10 % (w/v) TCA and incubated at 95 °C for 20 min to deacylate the aminoacyl-tRNAs. The amount of [¹⁴C] incorporated into α-casein was quantified by liquid scintillation counting.

REFERENCES

