Supplementary information

Potential for Interdependent Development of tRNA Determinants for Aminoacylation and Ribosome Decoding

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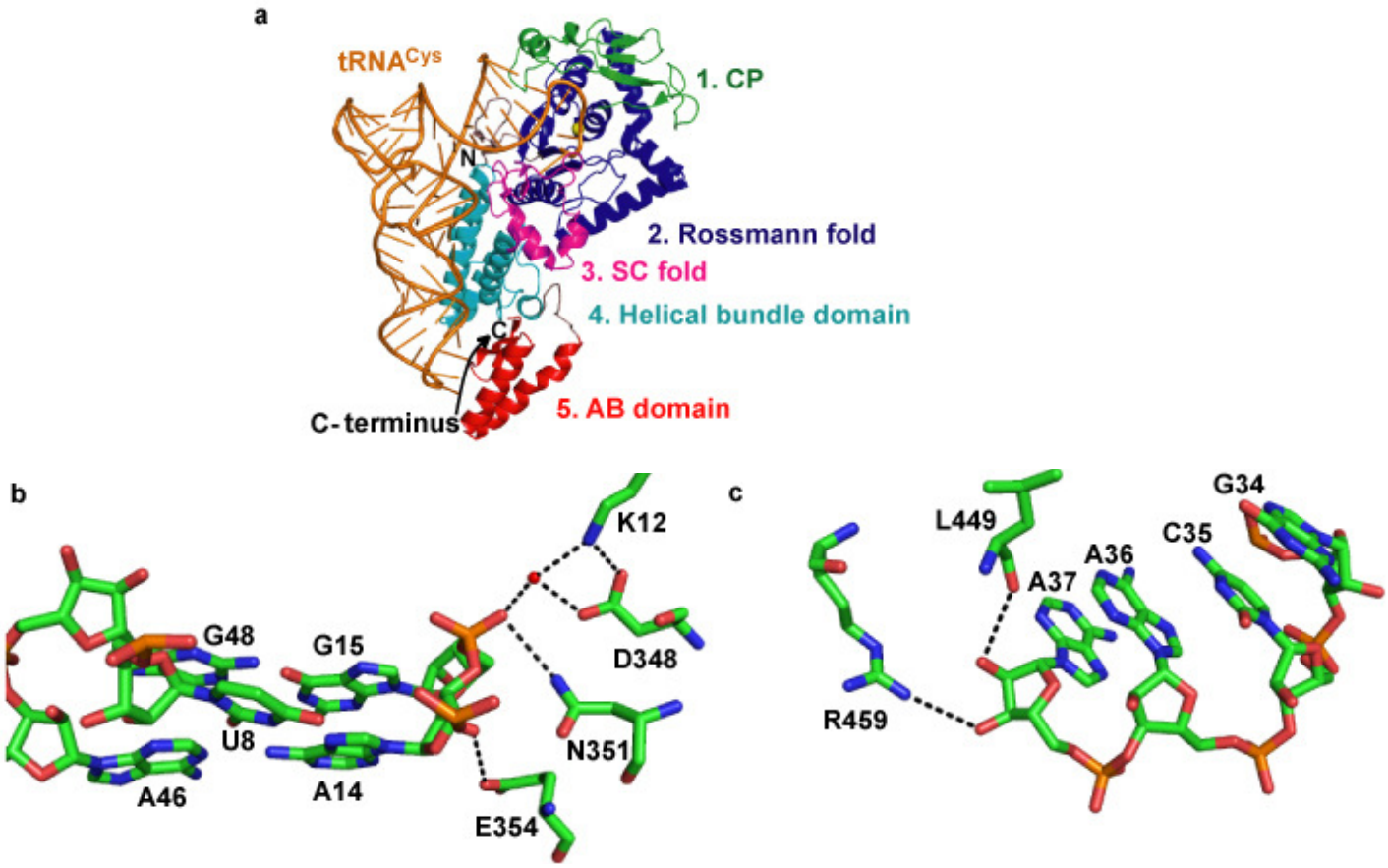
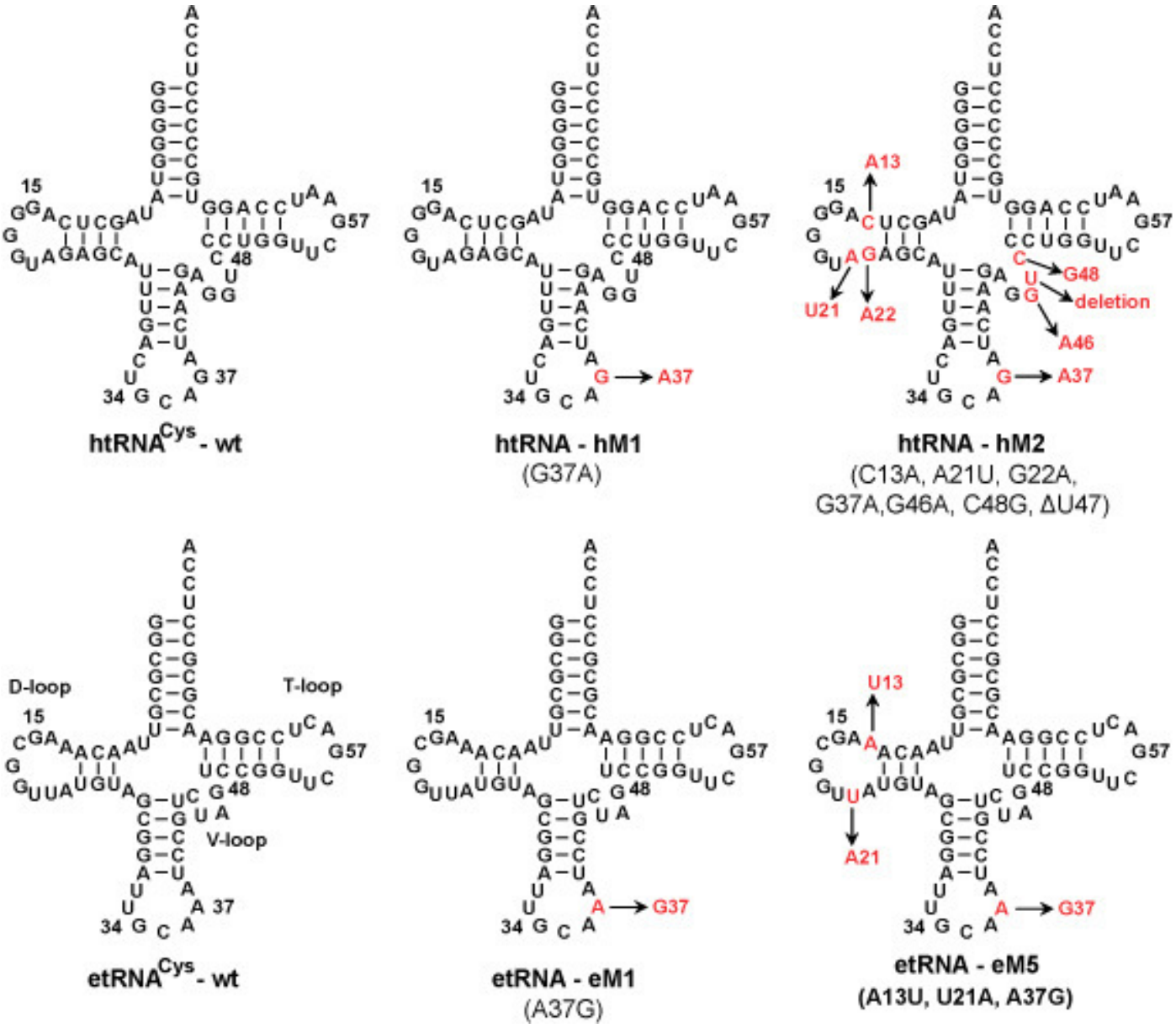
Supplementary Figure S1 (Hou)



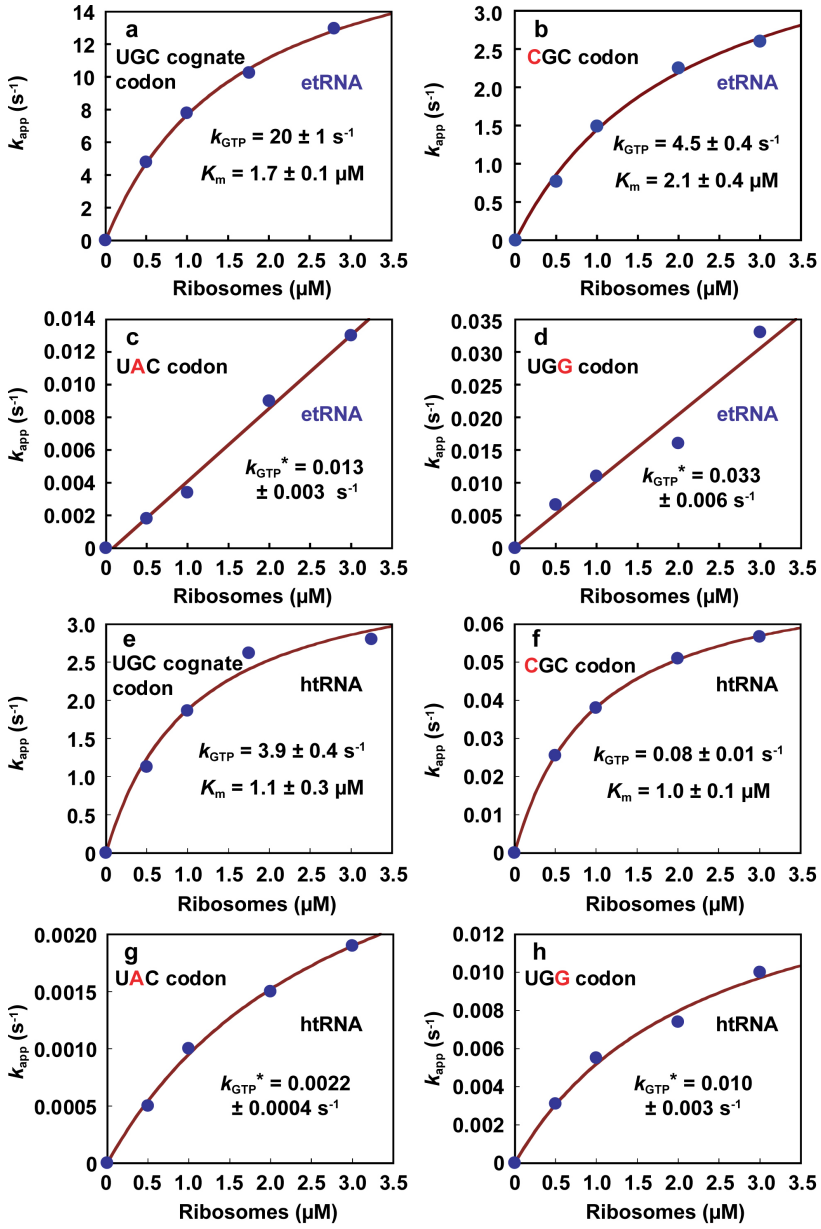
Supplementary Figure S1. Indirect readout of etRNA^{Cys} by eCysRS. (a) The crystal structure of eCysRS-etRNA^{Cys} complex, showing the enzyme core domains: 1, the connective polypeptide (CP); 2, Rossmann fold; 3, the stem-contact (SC) fold; 4, the helical bundle domain; and 5, the anticodon-binding (AB) domain. The C-terminus of eCysRS is localized between the helical bundle and AB domains, shown by the bent arrow, which in hCysRS is extended with the CTE. (b) Recognition of G15-G48 by eCysRS through hydrogen-bonding interactions between N351 and the 5' phosphate of G15 and between E354 and 2'-OH of A14. A water molecule that coordinates the network of hydrogen bonds is shown as a red sphere. (c) Recognition of A37 by eCysRS through hydrogen-bonding interactions between the 2'- and 3'-OH of A37 with L449 and R459, respectively. The enzyme residues involved in both types of indirect readout interactions are not conserved between eCysRS and hCysRS. The figures were drawn based on the structure of the eCysRS-tRNA complex[18] (PDB code 1U0B) and were made using PyMoL.

Supplementary Figure S2 (Hou)



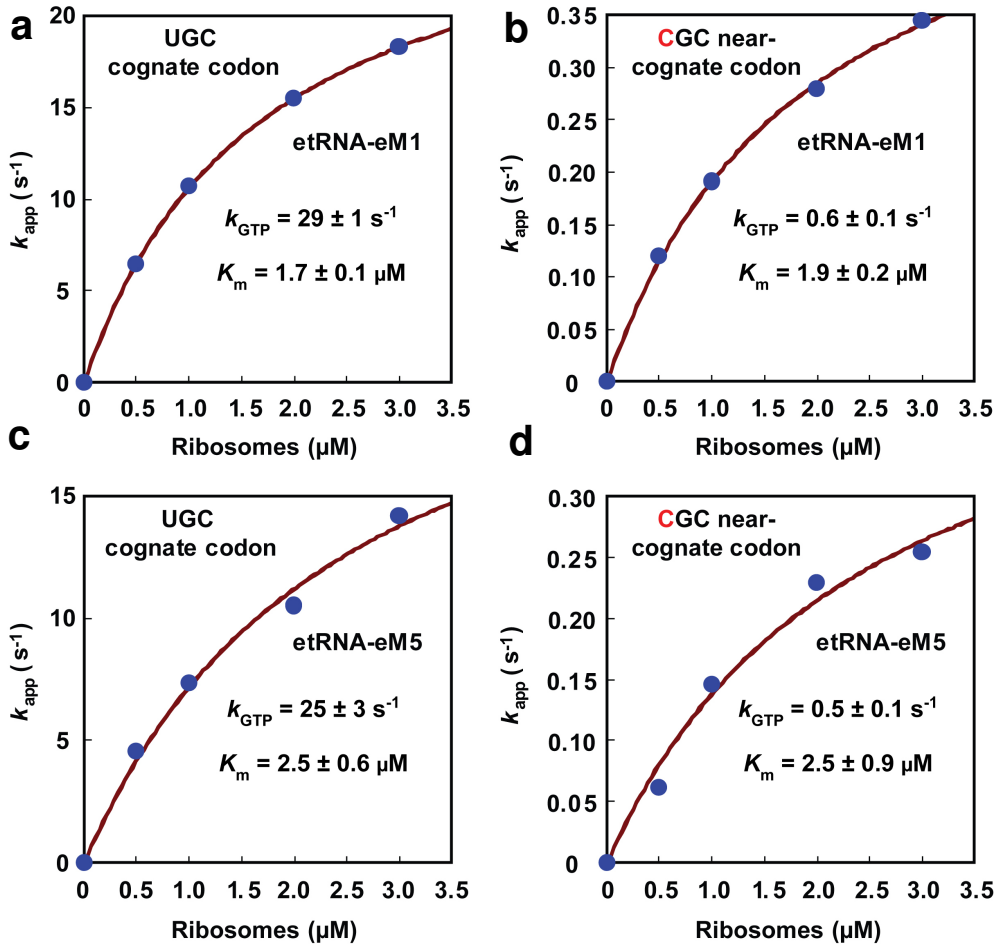
Supplementary Figure S2. Sequences of tRNA. Nucleotide sequence of htRNA^{Cys} and etRNA^{Cys} and their mutants. Nucleotide substitutions are shown in red by arrows.

Supplementary Figure S3 (Hou)



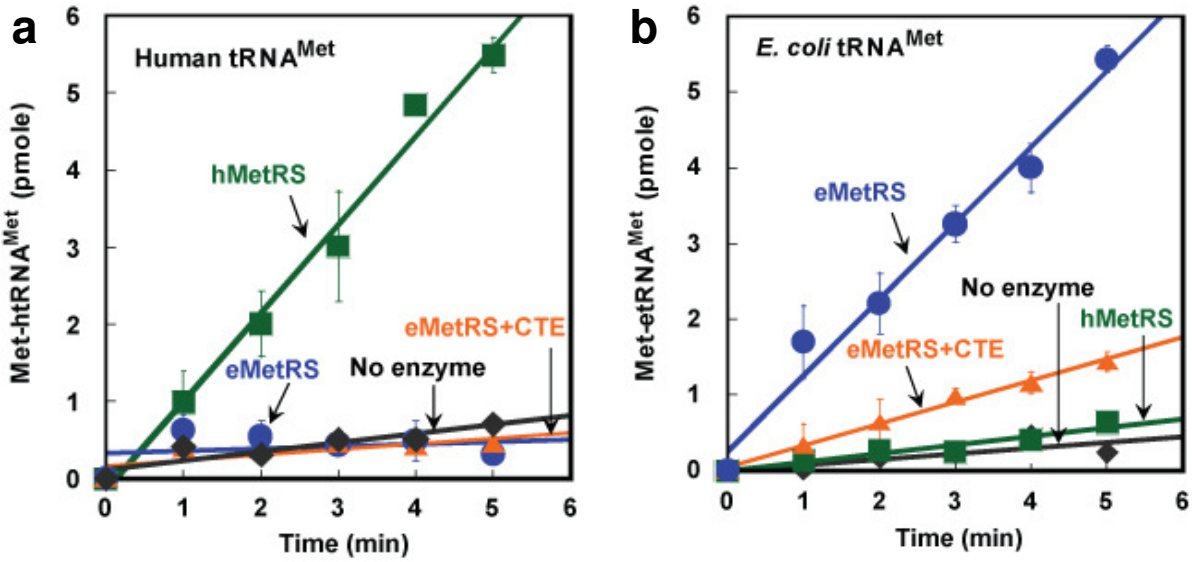
Supplementary Figure S3. GTPase activity for codon recognition. (a) Recognition of the cognate UGC codon by etRNA^{Cys}; (b) recognition of the 1st-position near-cognate CGC codon by etRNA^{Cys}; (c) recognition of the 2nd-position near-cognate UAC codon by etRNA^{Cys}; and (d) recognition of the 3rd-position near-cognate UGG codon by etRNA^{Cys}, where the site-specific substitutions in near-cognate codons are in red. (e) Recognition of the cognate UGC codon by htRNA^{Cys}; (f) recognition of the first-position near-cognate CGC codon by htRNA^{Cys}; (g) recognition of the 2nd-position near-cognate UAC codon by htRNA^{Cys}; and (h) recognition of the 3rd-position near-cognate UGG codon by htRNA^{Cys}, where the site-specific substitutions in near-cognate codons are in red. The error margins of each plot were determined from curve fitting and were 20% or less of the respective experimental value.

Supplementary Figure S4 (Hou)



Supplementary Figure S4. GTPase activity of mutants of etRNA^{Cys}. (a) Recognition of the cognate UGC codon by etRNA^{Cys}-eM1; (b) recognition of the near-cognate CGC codon by etRNA^{Cys}-eM1; (c) recognition of the cognate UGC codon by etRNA^{Cys}-eM5; (d) decognition of the near-cognate CGC codon by etRNA^{Cys}-eM5. GTPase activity was plotted as a function of ribosome concentration for codon recognition. The error margins of each plot were determined from curve fitting and were within 20% or less of the respective experimental value.

Supplementary Figure S5 (Hou)



Supplementary Figure S5. The CTE-fusion to eMetRS. (a) Time courses of aminoacylation of the transcript of human $tRNA^{Met}$ (0.6 μM) by eMetRS, hMetRS, and the fusion protein eMetRS+CTE, where the CTE of human CysRS is fused to the C-terminus of eMetRS. The enzyme concentration was 2 nM each. The results show that eMetRS and eMetRS+CTE are unable to cross-acylate the human tRNA, indicating the existence of a cross-species barrier to aminoacylation. (b) Time courses of aminoacylation of the transcript of *E. coli* $tRNA^{Met}$ (0.6 μM) by eMetRS, hMetRS, and the fusion protein eMetRS+CTE, showing a high activity of eMetRS but interference of this activity by the fusion with the CTE. The enzyme concentration was 1 nM each. In (a), the aminoacylation assay was in a buffer containing 150 mM KCl, 10 mM $MgCl_2$, 4 mM DTT, 2 mM ATP, 50 mM HEPES, 20 uM ^{35}S-methionine for eMetRS and eMetRS+CTE fusion protein, whereas in (b), the buffer for human MetRS was the same as above except that the concentration of KCl was reduced to 20 mM. The eMetRS clone was constructed by inserting the *metS* gene into pET22-b, while the human MetRS clone (PET/MHS) was provided by Professor Marc Mirande[47]. Each point in the graphs was the average of three experiments and error bars represented standard deviations.

Supplementary Table S1. Aminoacylation of tRNA

A. Steady-state aminoacylation of tRNA					
tRNA	Enzyme	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} M^{-1})	Relative activity to human homologous pair
$etRNA^{Cys}$	eCysRS	1.0 ± 0.2	1.0 ± 0.1	(1.0 ± 0.2) × 10^6	
$htRNA^{Cys}$		4.5 ± 0.7	0.011 ± 0.003	(2.4 ± 0.8) × 10^3	**1/583**
$etRNA^{Cys}$	hCysRS	1.0 ± 0.2	0.62 ± 0.04	(6 ± 1) × 10^5	
$htRNA^{Cys}$		0.9 ± 0.1	1.3 ± 0.1	(1.4 ± 0.2) × 10^6	**1**
$etRNA^{Cys}$	eCysRS + CTE	0.28 ± 0.04	0.44 ± 0.02	(1.6 ± 0.2) × 10^6	
$htRNA^{Cys}$		1.4 ± 0.1	0.07 ± 0.01	(5.0 ± 0.8) × 10^4	**1/28**
B. Single turnover kinetics of aminoacylation					
tRNA	Enzyme	K_d (μM)	k_{chem} (s^{-1})	k_{chem}/K_d (s^{-1} M^{-1})	Relative activity to human homologous pair
$etRNA^{Cys}$	eCysRS	3.2 ± 0.3	15.2 ± 0.5	(4.8 ± 0.5) × 10^6	
$htRNA^{Cys}$		6 ± 1	0.034 ± 0.002	(6 ± 1) × 10^3	**1/733**
$etRNA^{Cys}$	hCysRS	3.2 ± 0.5	20 ± 1	(6 ± 1) × 10^6	
$htRNA^{Cys}$		3.2 ± 0.5	14 ± 1	(4.4 ± 0.8) × 10^6	**1**
$etRNA^{Cys}$	eCysRS + CTE	1.4 ± 0.1	9.5 ± 0.4	(6.8 ± 0.6) × 10^6	
$htRNA^{Cys}$		1.5 ± 0.2	0.7 ± 0.02	(4.7 ± 0.6) × 10^5	**1/9.4**

Aminoacylation of $etRNA^{Cys}$ and $htRNA^{Cys}$ by eCysRS, hCysRS, and the fusion protein eCysRS+CTE, showing the existence of a cross-species barrier to aminoacylation of $htRNA^{Cys}$ by eCysRS in both steady state and single turnover assays. Each data point is the average of three experiments. Fold difference in activity (k_{cat}/K_m or k_{chem}/K_d) is shown in bold face. Error bars represent standard deviations.

Supplementary Table S2. Steady-state aminoacylation of htRNA^{Cys} and etRNA^{Cys}

	tRNA	Enzyme	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} M^{-1})	Relative activity to WT htRNA
htRNA	WT	eCysRS	4.5±0.7	$(1.1±0.3)\times10^{-2}$	$(2.4±0.8)\times10^{3}$	1
	U73A				(0.12±0.02)[b]	1/(**20,000 ± 7,000**)
	G34C		(64±13)[a]	$(7.5±1.5)\times10^{-5}$	1.2±0.3	1/(**2,000 ± 800**)
	C48G		12.8±1.2	$(2.7±0.2)\times10^{-3}$	$(2.1±0.3)\times10^{2}$	1/(**11 ± 4**)
	G37A (hM1)				(1.6±0.1)[b]	1/(**1,500 ± 500**)
	hM2				$(1.6±0.2)\times10^{6}$	
etRNA	WT		1.0±0.2	1.0±0.1	$(1.0±0.2)\times10^{6}$	
	A37G (eM1)				$((1.2±0.1)\times10^{3})$[b]	
htRNA	WT	hCysRS	0.9±0.1	1.3±0.1	$(1.4±0.2)\times10^{6}$	1
	U73A		38.2±7.6	$(3.3±0.5)\times10^{-4}$	8.6±2.2	1/ (**160,000± 40,000**)
	G34C		(172±25)[a]	$(8.7±0.5)\times10^{-3}$	51±8	1/(**27,000± 5,000**)
	C48G		13.0±3.0	0.14±0.02	$(1.1±0.3)\times10^{4}$	1/(**127±36**)
	G37A (hM1)				(5.9±0.7)[b]	1/(**240,000± 30,000**)
	hM2				$(0.4±0.1)\times10^{6}$	
etRNA	WT		1.0±0.2	0.62±0.04	$(6±1)\times10^{5}$	
	A37G (eM1)				$((1.1±0.1)\times10^{3})$[b]	
htRNA	WT	eCysRS +CTE	1.4±0.1	0.07±0.01	$(5.0±0.8)\times10^{4}$	1
	U73A				(0.69±0.10)[b]	1/(**72,000± 16,000**)
	G34C		19.2±1.0	$(3.3±0.2)\times10^{-4}$	17±1	1/(**2,900 ± 500**)
	C48G		10.5±1.2	$(6.2±0.8)\times10^{-3}$	$(5.9±1.0)\times10^{2}$	1/(**85 ± 20**)
	G37A (hM1)				(0.39±0.07)[b]	1/(**130,000 ± 30,000**)
	hM2				$(0.5±0.1)\times10^{6}$	
etRNA	WT		0.28±0.04	0.44±0.02	$(1.6±0.2)\times10^{6}$	
	A37G (eM1)				$((2.8±0.4)\times10^{3})$[b]	

a. Estimated values from curve fitting; b. Estimated from the initial rate of aminoacylation under sub-K_m concentration of the substrate. Each data point is the average of 3 experiments. Error bars represent standard deviations.

Supplementary Table S3. The specificity factor k_{cat}/K_m ($M^{-1}s^{-1}$) of aminoacylation.

	15-48	37	hCysRS	eCysRS+CTE	eCysRS	Ref
htRNA-wt	G-C	G	$(2.6 \pm 0.3) \times 10^6$	$(5.0 \pm 0.8) \times 10^4$	$(2.4 \pm 0.8) \times 10^3$	Tab S1
htRNA-hM1	G-C	A	5.9 ± 0.7	0.4 ± 0.1	1.6 ± 0.1	Tab S2
htRNA-hM2	G-G + others[a]	A	$(0.4 \pm 0.1) \times 10^6$	$(0.5 \pm 0.1) \times 10^6$	$(1.6 \pm 0.2) \times 10^6$	Tab S2
etRNA-wt	G-G	A	$(0.6 \pm 0.1) \times 10^6$	$(1.6 \pm 0.2) \times 10^6$	$(1.0 \pm 0.2) \times 10^6$	Tab S2
etRNA-eM1	G-G	G	$(1.1 \pm 0.1) \times 10^3$	$(2.8 \pm 0.4) \times 10^3$	$(1.2 \pm 0.1) \times 10^3$	Tab S2

[a]The core mutations in the hM2 mutant of $htRNA^{Cys}$ contains the G15-G48 base pair and others[8], including A13-A22-A46, U21, and deletion of U47. Abbreviations: wt: wild-type; Tab: Table. The data showed that hCysRS discriminates the G37A mutation by nearly 10^6-fold (see values of htRNA-wt *versus* htRNA-hM1 in red boxes), whereas eCysRS discriminates the A37G mutation by only 10^3-fold (see values of etRNA-wt *versus* etRNA-eM1 in red boxes).

Supplementary Table S4. Single nucleotide substitution on tRNA selectivity on the ribosome.

Lab	tRNA	Cognate codon*			Near-cognate codon*			Selectivity	Selectivity relative to wt tRNA
		K_m (μM)	k_{GTP} (s^{-1})	k_{GTP}/K_m ($\mu M^{-1}s^{-1}$)	K_m (μM)	k_{GTP} (s^{-1})	k_{GTP}/K_m ($\mu M^{-1}s^{-1}$)		
Hou (this work)	Wt-etRNA^{Cys} (A37)	1.7	20	12	2.1	4.5	2.1	5.7	1
	eM1etRNA^{Cys} (G37)	1.7	29	17	1.9	0.6	0.3	57	10
Uhlenbeck[32]	Wt tRNA^{Ala} (A32-U38)	4.5	31	6.9	4.6	2.4	0.52	13	1
	Mut tRNA^{Ala} (U32-A38)	2.9	27	9.3	4.9	26	5.3	1.8	1/7.2
Green[34]	Wt tRNA^{Trp} (U11-G24)	5.6	80	14	3.0	5.6	1.9	7.6	1
	Mut tRNA^{Trp} (U11-A24)	8.5	81	9.6	9.8	45	4.6	2.1	1/3.6

The values K_m's were estimated from published hyperbolic fits of k_{app} *versus* ribsome concentration, whereas k_{GTP}'s are the reported values. In this work, the cognate and near-cognate codons are UGC and CGC, respectively. In the Uhlenbeck work, the cognate and near-cognate codons are GCC and GCA, respectively. In the Green work, the cognate and near-cognate codons are UGG and UGA, respectively.
Selectivity = $[k_{GTP}/K_m]_{(Cognate)}/[k_{GTP}/K_m]_{(Near\text{-}cognate)}$.

Supplementary Table S5. Anticodon loop sequences of etRNA and htRNA.

tRNA	Anticodon 5' NNN 3'		*E. coli*		Human			
	E. coli	Human	A37	G37	A37	G37	U37	C37
Ala	GGC		2					
	UGC	UGC	3		12			
		AGC			32			
		CGC			5			
Arg	ACG	ACG	4			8		
	CCG	CCG		1		4		
		UCG				7		
	CCU	CCU	1		8	2		
	UCU	UCU	1		6			
Asn	GUU	GUU	4		39			
		AUU			2			
Asp	GUC	GUC	3		20			
Cys	GCA	GCA	1			31		
Gln	CUG	CUG	2		24	3		
	UUG	UUG	2		14	16		
Glu	UUC	UUC	4		20	1		
		CUC			24	1		
Gly	GCC	GCC	4		17			
	CCC	CCC	1		12			
	UCC	UCC	1		12			
His	GUG	GUG	1			12		
Ile		AAU			16	1		
	GAU	GAU	3		3			
		UAU			5			
Leu		AAG			2	11	1	
	CAG	CAG		4	1	9		
	UAG	UAG		1		5		
	CAA	CAA	1		5	2		
		CUA			1			
	UAA	UAA	1		4	7		1
	GAG			1				
Lys		CUU			21			1
	UUU	UUU	6		21			1
Met	CAU	CAU	8		25			
Phe	GAA	GAA	2		2	16		
Pro		AGG			1	13		
	GGG	GGG		1		1		
	CGG	CGG		1		4		
	UGG	UGG		1	2	9		
			A37	G37	A37	G37	U37	C37
tRNA	Anticodon 5' NNN 3'		*E. coli*		Human			
Sec	UCA	UCA	1		3	1		
Ser		AGA			12	2		

	CGA	CGA	1		5			
	UGA	UGA	1		10			
		ACU			1			
	GCU	GCU	1		8			
	GGA		2					
Thr		AGU			11			
	CGU	CGU	2		6			
	UGU	UGU	1		8			
	GGU		2					
Trp	CCA	CCA	1			8		1
Tyr	GUA	GUA	3		1	19		
		AUA				1		
Val	GAC		2					
	UAC	UAC	5		6			
		AAC			15			
		CAC			23			
sum			75	10	465	194	1	4
% use			88.24	11.76	70.03	29.22	0.15	0.6
			A37	G37	A37	G37	U37	C37
			E. coli		Human			
Numbers represent copy numbers of tRNAs based on genomic tRNA database (http://lowelab.ucsc.edu/GtRNAdb).								

Sequence analysis was based on the genomic tRNA database (http://lowelab.ucsc.edu/GtRNAdb). The sum refers to the total number of tRNA genes in *E. coli* or in humans that contain A37, G37, C37, or U37. For example, the total number of tRNA genes in *E. coli* with A37 is 75, while the total number of tRNA genes with G37 is 10. Thus, the frequency of A37 among the total number of tRNA genes in *E. coli* is 88.24 % (= (75)/(75 + 10)).

Supplementary Table S6. Anticodon loop sequences of yeast and human mitochondrial tRNAs.

tRNA	Anticodon 5' NNN 3'		Yeast mitochondria		Human mitochondria	
	yeast	Human	A37	G37	A37	G37
Ala	UGC	UGC	2		1	1
Arg	ACG		1			
		UCG			1	1
	UCU		1			
Asn	GUU	GUU	1		2	
Asp	GUC	GUC	1		1	
Cys	GCA	GCA	1 (A)		1 (A)	
Gln	UUG	UUG	1			2
Glu	UUC	UUC	1		1	
Gly	UCC	UCC	1		1	
His	GUG	GUG		1	1	
Ile	GAU	GAU	1		1	
Leu		UAG				1
	UAA	UAA		1	1	
Lys	UUU	UUU	1		1	
Met	CAU	CAU	1	1	2	
Phe	GAA	GAA		1	1	
Pro	UGG	UGG		1		1
Ser	UGA	UGA		1	1	
	GCU	GCU	1		1	
Thr	UAG			1		
	UGU	UGU	1		1	
Trp	UCA	UCA	1		1	
Tyr	GUA	GUA	2		1	
Val	UAC	UAC	2		1	
sum			20	7	21	6
Usage			20/27	7/27	21/27	6/27
% use			74.07	25.93	77.78	22.22
			A37	G37	A37	G37
			Yeast mitochondria		Human mitochondria	
Values represent the number of each tRNA gene. Sequence analysis was based on the transfer RNA database (http://trnadb.bioinf.uni-leipzig.de)						

The sum refers to the total number of tRNA genes in yeast mitochondria or in human mitochondria that contain A37 or G37. Note that mitochondria do not encode C37 or U37 in tRNA genes. Based on the analysis, for example, the total number of tRNA genes in yeast mitochondria with A37 is 20, while that with G37 is 7. Thus, the frequency of A37 among the total number of tRNA genes in yeast mitochondria is 74.07 % (= (20)/(20 + 7)).

Supplementary Reference

47. Kaminska, M., Shalak, V. & Mirande, M. The appended C-domain of human methionyl-tRNA synthetase has a tRNA-sequestering function. *Biochemistry* **40**, 14309-16 (2001).