"Post-transcriptional modification to the core of tRNAs modulates translational misreading errors"

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Supplementary material.

In this study we tested the effect of lack of each modification affecting any of the error-prone near-cognate tRNAs (blue columns in **Table S1**) or competing cognate tRNAs (green columns in **Table S1**) specific for each codon tested for misreading frequency. Each tRNA has a specific subset of possible modifications targeting the five structural regions of the tRNA: Acceptor Arm (A Arm), Dihydrouridine Arm (D Arm), Anticodon Arm (AC Arm), Extra Loop, or TΨC Arm (T Arm) shown labeled in the first column. **Table S1** presents the modification status (+ = modified) for each modification gene, modification and tRNA isoacceptor. Note that because deletion of the *TRM61* gene responsible for m^1A_{58} modification in the T loop is lethal we were unable to measure misreading error frequencies in the absence of this modification. The tRNAs are labeled by anticodon shown in 5'-3' order; e.g., $tRNA^{Glu}_{UUC}$ is the glutamate inserting isoacceptor with the anticodon 5'-UUC-3'. The predicted cognate codons for these tRNAs are also listed (e.g., 5'-GAA-3'/5'-GAG-3').

Tables S2 and S3 present the data used to create **Figures 3 and 4**. The values plus or minus SEM for enzyme assays are shown as the calculated value x 10^{-4} (e.g. for WT errors at UAG: 21 ± 3.5. The statistical significance of the values for the various mutants were calculated using the Student's T-test (two-tailed, homoscedastic) comparing all values for activity of each mutant strain to all values for the wild type.

The data from the quantification of results of tRNA Northerns is shown in **Table S4** for the indicated tRNAs purified from the wild type and all the relevant tRNA modification mutant strains and separated by denaturing polyacrylamide gel electrophoresis. The

volume of each band is indicated in units relative to the volume of the control 5S rRNA band visualized from the same RNA preparation. The bands were visualized using a C-DiGit® Blot Scanner (LI.COR, Lincoln, NE) and the signal intensities of various tRNA species were quantified using ImageJ software or by Image Studio (LI-COR) and normalized to the 5S RNA controls.

An *mnt1*Δ mutation, which blocks retrograde tRNA transport to the nucleus, was used to determine whether retrograde transport of any of the tRNAs could explain differences in misreading error frequencies. This data appears in **Figure 7** and **Table S5**. The tabular data are presented as in **Table S2**. The value of the wild type and each modification mutant strain are compared with the congenic *met10*Δ strain and in no case is the difference significant. This argues against a role for retrograde transport in the effects of the modification mutations.

Dot blot RNA blotting analysis of total RNA from each of the modification mutant strains used in the misreading studies is shown in **Figure S1**. Total RNA (20 µg) purified from cells grown in YPD medium to mid-exponential phase as described (Chatterjee et al. 2017) was spotted onto Hybond Nþ membrane (Amersham Biosciences) and crosslinked by using a Stratagene UV cross-linker (Model #2400). Probes designed to hybridize with each of the tRNAs relevant to this study and 5S rRNA were each successively hybridized to the membrane bound RNA and visualized as described (Wu, Huang and Hopper 2013). After each hybridization, the membrane was stripped of probe and rehybridized, with hybridization to the 5S rRNA probe. This experiment was qualitative, showing that the amount of each tRNA present appeared to be approximately equal for all the RNA samples. This analysis was supplemented by Northern blotting with a subset of the modification mutant strains, shown in **Figure 5** and **Table S4**.

To determine the level of specificity of the designed tRNA probes, we performed a dot blot analysis using DNA molecules corresponding to each full length tRNA molecule. **Figure S2** shows the result of that analysis. The image produced by visualizing the biotin-

labeled probes for each tRNA is shown on the right. Note that for each probe, labeling was predominant for a single spotted target DNA corresponding to the tRNA for which it was designed.

Table S1: Core modifications of tRNA in this study

			Error-prone near-cognate tRNAs			Competing cognate tRNAs					
Structural Region	Gene	Modification*	$tRNA^{Glu}_{UUC}$	$tRNA^{Lys}_{UUU}$	$tRNA^{Lys}_{CUU}$	$tRNA^{Arg}_{CCU}$	$tRNA^{Arg}_{UCU}$	$tRNA^{Asn}_{GUU}$	$tRNA^{Asp}_{GUC}$	$tRNA^{Gly}_{UCC}$	$tRNA^{Gly}_{CCC}$
			GAA/ GAG	AAA/ AAG	AAG	AGG	AGA	GAU/ GAC	GAU/ GAC	GGA/ GGG	GGG
A Arm	*TRM10*	m^1G_9			+	+	+				+
	TRM13	Cm_4									+
	TRM11	m^2G_{10}		+	+	+	+	+			+
D Arm	*PUS7*	Ψ_{13}	+						+	+	
	DUS1	D_{16}/D_{17}		+	+	+	+	+	+	+	+
	DUS2	D_{20}		+	+			+	+	+	+
	DUS4	D_{20a}	+					+			
	TRM1	$m^2_2G_{26}$		+	+	+	+	+			
AC Arm	*PUS1*	Ψ_1/Ψ_{27}	+	+	+	+	+				
	PUS3	Ψ_{39}		+	+			+			
Extra Loop	*TRM8*	m^7G_{46}		+							
	DUS3	D_{47}		+				+			
	TRM4	m^5C_{48}/ m^5C_{49}	+	+				+	+		+
T Arm	*PUS4*	Ψ_{55}	+	+	+	+	+	+	+	+	+
	TRM2	m^5U_{54}	+	+	+	+	+	+	+	+	+
	TRM61	m^1A_{58}			+		+	+			

* D, dihydrouridine; Y, pseudouridine; m^2_2G, *N2,N2*-dimethylguanosine; m^5U, 5-methyluridine; m^5C, 5-methylcytidine; m^7G, 7-methylguanosine; m^1G, 1-methylguanosine; m^2G, 2-methylguanosine; Cm, 2-O-methylcytidine; m^1A, 1-methyladenosine

Table S2: Effect of lack of modification enzymes of misreading frequencies by $tRNA^{Lys}$

Mutation	Lys	Ter UAG	Arg AGG	Asn AAU	Asn AAC
		Activity of *P. pyralis* luciferase relative to wild type enzyme (x 10^{-4})			
WT		21 ± 3.5	8.4 ± 0.86	1.6 ± 0.27	1.8 ± 0.29
*dus1*Δ	+	11 ± 1.0 *	+ 14 ± 1.2 **	+ 3.3 ± 0.85*	3.1 ± 0.16*
*dus2*Δ	+	4.7 ± 1.5***	+ 11 ± 0.31 *	+ 1.5 ± 0.32	2.0 ± 0.44
*dus3*Δ	+	7.6 ± 1.2**	- 9.6 ± 1.4	+ 2.5 ± 0.55	2.4 ± 0.48
*dus4*Δ	-	22 ± 2.3	- 8.9 ± 0.95	+ 3.0 ± 0.55*	3.2 ± 0.13***
*pus1*Δ	+	6.6 ± 1.0***	+ 10 ± 1.5	- 3.1 ± 0.61*	3.8 ± 0.47***
*pus3*Δ	+	16 ± 0.57	- 15 ± 1.0 ***	+ 4.9 ± 1.3 **	3.7 ± 0.80*
*pus4*Δ	+	12 ± 0.96	+ 17 ± 0.99 ***	+ 3.4 ± 0.49**	2.7 ± 0.48
*trm1*Δ	+	15 ± 1.9	+ 13 ± 2.4 *	+ 2.5 ± 0.76	2.7 ± 0.77
*trm2*Δ	+	15 ± 4.2	+ 11 ± 2.1	+ 3.8 ± 0.47***	4.1 ± 0.45***
*trm4*Δ	+	7.6 ± 1.2**	- 12 ± 0.79 **	+ 2.1 ± 0.22	2.7 ± 0.35
*trm8*Δ	+	6.3 ± 0.98 ***	- 9.4 ± 1.0	- 2.6 ± 0.43	2.4 ± 0.41
*trm8*Δ *trm4*Δ	+/+	21 ± 3.1	-/- 6.5 ± 0.86	-/- 1.3 ± 0.19	1.3 ± 0.11
*trm 10*Δ	+	9.2 ± 0.93 **	+ 10 ± 0.54	- 1.5 ± 0.12	1.5 ± 0.15
*trm11*Δ	+	9.2 ± 0.65 **	+ 12 ± 0.42 **	+ 1.9 ± 0.19	1.7 ± 0.16

P values: (*, P < 0.05; **, P < 0.01; ***, P < 0.001)

Table S3: Effect of lack of modification enzymes of misreading frequencies by $tRNA^{Glu}$

Mutation	Glu	Asp				Gly			
		GAU		GAC		GGA		GGG	
		Activity of *E. coli* β-galactosidase relative to wild type enzyme (x 10^{-4})							
WT			1.4 ± 0.17		1.1 ± 0.07		1.8 ± 0.29		0.27 ± 0.03
*dus1*Δ	-	+	1.7 ± 0.15		1.9 ± 0.02	**	+ 2.1 ± 0.78	+	0.59 ± 0.07 ***
*dus2*Δ	-	-	1.1 ± 0.22		0.85 ± 0.34		+ 1.2 ± 0.20	+	0.14 ± 0.03 **
*dus4*Δ	+	-	2.1 ± 0.12	**	1.9 ± 0.07	***	+ 1.6 ± 0.18	-	0.39 ± 0.02 **
*pus1*Δ	+	-	2.2 ± 0.15	**	1.5 ± 0.09	**	- 3.5 ± 0.31 **	-	0.56 ± 0.07 **
*pus4*Δ	+	+	0.55 ± 0.10	**	0.66 ± 0.10	**	+ 0.47 ± 0.07 ***	+	0.14 ± 0.02 **
*pus7*Δ	+	+	0.71 ± 0.11	**	0.80 ± 0.07	**	+ 1.1± 0.11 *	-	0.12 ± 0.01 ***
*trm2*Δ	+	+	3.7 ± 0.40	**	3.6 ± 0.49	***	+ 1.8 ± 0.31	+	1.2 ± 0.10 ***
*trm4*Δ	+	+	1.2 ± 0.22		1.5 ± 0.19		+ 2.1 ± 0.66	+	0.38 ± 0.07
trm10	-	-	1.3 ± 0.16		1.1 ± 0.20		- 2.5 ± 0.07	+	0.23 ± 0.03
*trm11*Δ	-	-	1.8 ± 0.15		1.3 ± 0.19		- 2.4 ± 0.08	+	0.41 ± 0.11
*trm13*Δ	-	-	1.6 ± 0.14		1.1 ± 0.16		- 2.2± 0.10	+	0.26 ± 0.05

P values: (*, P < 0.05; **, P < 0.01; ***, P < 0.001)

Table S4: Steady state levels of tRNAs in modification backgrounds

tRNA	Scanned Northern value ± SEM (corrected for 5S rRNA standard)					
	WT	*pus1*Δ	*trm2*Δ	*trm4*Δ	*trm9*Δ	*trm4*Δ *trm8*Δ
Lys (UUU)	1.07 ± 0.08	0.94 ± 0.11	1.00 ± 0.08	0.94 ± 0.03	0.93 ± 0.13	0.99 ± 0.03
Lys (CUU)	1.10 ± 0.08	1.06 ± 0.13	1.03 ± 0.08	1.05 ± 0.02	0.95 ± 0.12	0.99 ± 0.03
Arg (UCU)	1.05 ± 0.04	0.98 ± 0.06	0.93 ± 0.02	0.93 ± 0.04	0.87 ± 0.05	1.00 ± 0.04
Arg (CCU)	1.13 ± 0.04	1.14 ± 0.02	0.98 ± 0.05	1.07 ± 0.06	0.87 ± 0.07	1.02 ± 0.02
Asn (GUU)	1.04 ± 0.08	0.99 ± 0.04	0.97 ± 0.08	1.01 ± 0.02	0.85 ± 0.04	0.97 ± 0.03
Glu (UUC)	0.97 ± 0.05	0.98 ± 0.05	0.99 ± 0.05	1.00 ± 0.04	0.97 ± 0.04	1.08 ± 0.03
Gly (UCC)	1.11 ± 0.10	1.09 ± 0.04	0.97 ± 0.02	0.98 ± 0.04	0.90 ± 0.03	1.11 ± 0.05
Gly (CCC)	1.09 ± 0.09	1.03 ± 0.07	1.00 ± 0.04	1.10 ± 0.10	0.85 ± 0.07	1.01 ± 0.06
Asp (GUC)	1.12 ± 0.02	1.10 ± 0.10	1.00 ± 0.02	1.10 ± 0.06	0.91 ± 0.02	1.01 ± 0.05

Table S5: Effect of *mtr10*

Mutation	GAU			GAC			GGA			GGG		
	MTR10	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P
WT	1.36 ± 0.16	1.32 ± 0.04	0.80	1.09 ± 0.06	1.16 ± 0.08	0.49	1.83 ± 0.29	1.60 ± 0.28	0.56	0.27 ± 0.02	0.30 ± 0.02	0.33
pus1Δ	2.14 ± 0.15	1.73 ± 0.18	0.09	1.52 ± 0.09	1.39 ± 0.17	0.53	3.45 ± 0.31	3.06 ± 0.58	0.59	0.56 ± 0.06	0.50 ± 0.05	0.58
trm2Δ	3.70 ± 0.39	4.81 ± 0.20	0.06	3.63 ± 0.49	3.11 ± 0.16	0.35	1.84 ± 0.30	1.17 ± 0.05	0.13	1.18 ± 0.09	1.35 ± 0.17	0.36
trm4Δ	1.22 ± 0.21	1.29 ± 0.05	0.84	1.50 ± 0.19	1.00 ± 0.17	0.06	2.10 ± 0.65	1.89 ± 0.36	0.78	0.37 ± 0.07	0.35 ± 0.00	0.75

Mutation	AAU			AAC			AGG			UAG		
	MTR10	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P	*MTR10*	*mtr10Δ*	P
WT	1.61 ± 0.26	2.11 ± 0.24	0.21	1.81 ± 0.29	2.40 ± 0.30	0.24	8.42 ± 0.85	11.0 ± 0.71	0.63	21.3 ± 3.45	24.8 ± 1.74	0.50
pus1Δ	3.06 ± 0.60	4.10 ± 0.41	0.28	3.77 ± 0.46	4.50 ± 0.45	0.21	10.2 ± 1.46	8.70 ± 2.05	0.51	6.56 ± 1.01	5.91 ± 0.36	0.64
trm2Δ	3.75 ± 0.47	4.35 ± 0.41	0.26	4.13 ± 0.44	4.80 ± 0.35	0.37	11.1 ± 2.04	16.6 ± 1.18	0.05	14.5 ± 4.16	9.37 ± 0.61	0.32
trm4Δ	2.10 ± 0.22	2.89 ± 0.24	0.50	2.68 ± 0.34	3.12 ± 0.23	0.69	12.3 ± 0.78	16.2 ± 3.13	0.09	12.5 ± 4.39	7.56 ± 0.47	0.47
trm8Δ	2.54 ± 0.42	2.81 ± 0.05	0.66	2.35 ± 0.41	2.64 ± 0.08	0.66	9.37 ± 1.03	10.6 ± 0.17	0.52	7.87 ± 0.98	6.96 ± 0.42	0.69
trm4Δ/trm8Δ	1.29 ± 0.19	1.46 ± 0.19	0.21	1.27 ± 0.11	1.53 ± 0.17	0.59	6.53 ± 0.86	6.92 ± 0.21	0.77	20.9 ± 3.14	16.9 ± 3.01	0.43

Table S6. S. cerevisiae strains used in this work

Strain	Strain Number	*Genotype*	Source
dus1Δ	PF999	*dus1::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
dus2Δ	PF1002	*dus2::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
dus3Δ	PF1005	*dus3::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
dus4Δ	PF1186	*dus4::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
pus1Δ	PF991	*pus1::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
pus1Δ	PF1247	*pus1::HphMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
pus1Δ	PF1261	*pus1::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
pus1Δ mtr10Δ	PF1251	*pus1::HphMX mtr10::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
pus4Δ	PF993	*pus4::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm11Δ	PF1185	*trm11::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm2Δ	PF996	*trm2::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm2Δ	PF1245	*trm2::HphMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm2Δ	PF1260	*trm2::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm2Δ mtr10Δ	PF1248	*trm2::HphMX mtr10::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm4Δ	PF887	*trm4::NatMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	Dr. Eric Phizicky
trm4Δ	PF1262	*trm4::NatMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm4Δ mtr10Δ	PF1243	*trm4::NatMX mtr10::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm4Δ trm8Δ	PF1249	*trm4::HphMX trm8::NatMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm4Δ trm8Δ	PF1264	*trm4::KanMX trm8::NatMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm4Δ trm8Δ mtr10Δ	PF1252	*trm4::HphMX trm8::NatMX mtr10::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm8Δ	PF890	*trm8::NatMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	Dr. Eric Phizicky
trm8Δ	PF1263	*trm8::NanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm8Δ met22Δ	PF891	*trm8::NatMX met22::HphMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	Dr. Eric Phizicky
trm8Δ mtr10Δ	PF1244	*trm8::NatMX mtr10::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	This study
trm8Δ trm4Δ	PF876	*trm8::NatMX trm4::KanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	Dr. M. Conner
BY4742	PF884	*WT his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MATα*	Dr. Eric Phizicky

Figure S1: Southern blotting to tRNA cDNA copies

		Probes						
		Arg CCU	Arg UCU	Lys UUU	Lys CUU	Gly CCC	Gly UCC	Gly UUC
DNA Oligos (3 nM)	Arg CCT	0	3293	0	94	0	0	206
	Arg TCT	2217	0	0	0	0	0	0
	Lys TTT	0	0	6522	876	0	172	409
	Lys CTT	319	0	3130	3807	757	0	805
	Gly CCC	0	0	519	0	184	0	6446
	Gly TCC	0	0	0	0	2506	2697	376
	Glu TTC	0	0	0	0	6958	228	1781