Molecular Cell, Volume *28*

Supplemental Data

Trm9-Catalyzed tRNA Modifications

Link Translation to the DNA Damage Response

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Table S2. Group 1 Codon Usage Statistics

Amino Acid	Codon	Group 1 Average	421 Random Average	Standard Deviation	Group Codon Z Score
Ala	GCA	0.09	0.30	0.007	-29.75
Ala	GCG	0.02	0.12	0.005	-20.79
Ala	GCC	0.29	0.22	0.006	10.89
Ala	GCU	0.61	0.36	0.008	32.41
Arg	AGG	0.04	0.21	0.006	-26.53
Arg	CGA	0.00	0.07	0.004	-16.35
Arg	CGG	0.00	0.04	0.003	-12.87
Arg	CGC	0.01	0.06	0.004	-12.45
Arg	CGU	0.19	0.14	0.006	7.84
Arg	AGA	0.76	0.48	0.009	30.98
Asn	AAU	0.25	0.57	0.009	-35.41
Asn	AAC	0.74	0.43	0.009	35.14
Asp	GAU	0.49	0.63	0.008	-16.79
Asp	GAC	0.51	0.37	0.008	16.79
Cys	UGC	0.13	0.37	0.014	-17.88
Cys	UGU	0.83	0.61	0.014	15.72
End	UGA	0.11	0.30	0.021	-8.72
End	UAG	0.16	0.23	0.019	-3.65
End	UAA	0.73	0.48	0.023	10.93
Gln	CAG	0.07	0.31	0.009	-27.68
Gln	CAA	0.93	0.68	0.009	27.73
Glu	GAG	0.11	0.31	0.007	-26.25
Glu	GAA	0.89	0.69	0.008	25.90
Gly	GGA	0.04	0.23	0.007	-27.08
Gly	GGG	0.02	0.13	0.005	-20.66
Gly	GGC	0.08	0.21	0.007	-19.69
Gly	GGU	0.86	0.43	0.010	43.39
His	CAU	0.41	0.62	0.011	-18.63
His	CAC	0.59	0.37	0.011	18.92
Ile	AUA	0.03	0.28	0.008	-32.15
Ile	AUU	0.48	0.45	0.007	5.08
Ile	AUC	0.48	0.27	0.007	29.67
Leu	CUG	0.03	0.11	0.004	-23.31
Leu	CUU	0.04	0.13	0.004	-21.28
Leu	CUC	0.01	0.06	0.003	-17.93
Leu	CUA	0.10	0.14	0.003	-12.01
Leu	UUA	0.22	0.26	0.005	-8.25
Leu	UUG	0.60	0.29	0.007	45.87
Lys	AAA	0.30	0.57	0.008	-34.18
Lys	AAG	0.70	0.43	0.008	34.18
Phe	UUU	0.29	0.57	0.009	-31.00
Phe	UUC	0.71	0.43	0.009	31.15
Pro	CCC	0.03	0.16	0.006	-21.29
Pro	CCG	0.01	0.12	0.006	-19.13
Pro	CCU	0.17	0.30	0.007	-17.69
Pro	CCA	0.78	0.41	0.009	39.18
Ser	UCA	0.09	0.20	0.005	-23.33
Ser	AGU	0.06	0.16	0.004	-22.56
Ser	UCG	0.02	0.10	0.003	-21.50
Ser	AGC	0.04	0.12	0.004	-18.26
Ser	UCC	0.32	0.17	0.005	30.54
Ser	UCU	0.47	0.26	0.006	35.55
Thr	ACA	0.09	0.30	0.007	-30.06
Thr	ACG	0.02	0.14	0.005	-23.31
Thr	ACU	0.49	0.34	0.007	21.87
Thr	ACC	0.40	0.22	0.007	26.97
Tyr	UAU	0.24	0.55	0.010	-29.89
Tyr	UAC	0.76	0.45	0.010	30.00
Val	GUA	0.04	0.22	0.006	-28.30
Val	GUG	0.06	0.20	0.006	-23.67
Val	GUU	0.53	0.37	0.007	22.33
Val	GUC	0.37	0.21	0.006	27.80

Boxes shaded yellow correspond to over-represented codons, while those shaded in purple correspond to under-represented codons.

Table S3. Functional Categories Over-Represented in Group 1 Proteins

Biological Theme	Process	p-value
Protein Production	Ribosome Biogenesis	1.0E-14
	Protein Synthesis	3.4E-14
	Aminoacyl-tRNA-Synthetases	5.3E-12
	Amino Acid Biosynthesis	6.6E-10
	Translation	2.3E-09
	Amino Acid Metabolism	8.3E-06
Energy and Metabolism	Metabolism	2.5E-14
	Glycolysis and Gluconeogenesis	1.0E-13
	Nucleotide Metabolism	1.5E-06
	Energy	1.6E-06
	Carbohydrate Metabolism	1.8E-06
	Homeostasis of Protons	8.2E-04
	Phosphate Metabolism	9.2E-04
Damage and Stress Response	Stress Response	3.6E-06
	Cell Rescue, Defense and Virulence	1.5E-05
	Purine Ribonucleotide Metabolism	4.5E-05
	Cell Wall	5.1E-05
	Deoxyribonucleotide Metabolism	2.9E-03
	Transport	3.8E-03

Table S4. Codon Usage Numbers for Some Group 1 Genes

Gene Name	Arg^{AGA}	Arg^{AGG}	Glu^{GAA}	Glu^{GAG}
YEF3	41	0	91	1
RNR1	18	1	44	14
RNR2	13	0	34	11
RNR3	15	2	44	14
RNR4	7	0	31	2
PAB1	14	0	42	7
Expected Ratio	2.3 to 1		2.3 to 1	

Table S5. Strains and Oligonucleotides

Strain	Genotype
BY4741 (Open Biosystems)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
Yml014w (Open Biosystems)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trm9Δ G418*
CenPK2-1C (Kahlor and Clark, 2003)	*MAT ura3 his3 leu2 trp1*
HKY102 (Kahlor and Clark, 2003))	*CEN.PK2-1c trm9::TRP1*
BY4743 (Open Biosystems)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 / MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
20559 (Open Biosystems)	Heterozygous Diploid Knockout for *trm9Δ* in *BY4743*
30559 (Open Biosystems)	Homozygous Diploid Knockout for *trm9Δ* in *BY4743*
ATCC 201388 (TAP tag parent strain, Ghaemmaghami S, et al., 2003)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
Oligonucleotides	**Sequence**
10X AGA codon up primer	ATGATGATGGTACCT AGA AGA AGA AGA AGA AGA AGAAGA AGA AGA CCTAAGGATCAGCTTGGAGTTGA
10X AGG codon up primer	ATGATGATGGTACCT AGG AGG AGG AGG AGG AGG AGG AGG AGG AGG CCTAAGGATCAGCTTGGAGTTGA
10X GAA codon up primer	ATGATGATGGTACCTGAAGAAGAAGAAGAAGAAGAAGAAGAAGAACCTAAGGATCAGC TTGGAGTTGA
5X AGG up primer	ATGATGATGGTACCT AGG AGG AGG AGG AGG CCTAAGGATCAGCTTGGAGTTGA
Reporter down primer (lacZ bot)	GCTGGATATCTGCAGAATTCG
pYES Trm9 up primer	ATT CCG CTC GAG TCA TCT CTT CTG GGC CAC CA
pYES Trm9 down primer	CGC AGG ATC CAG ATG GAG ATA AAC CAA GCG GCT
pRS416 Trm9 up primer	CGGGATCCCGTTGTGTTTGCTGTTGTAAATG
pRS416 Trm9 down primer	ATCAGTCGAGCTCGGAAAGGGGTTACCATCATAAC
RNR1 up primer	CGCAGG TAC CTA TGT ACG TTT ATA AAA GAG ACG GTC
RNR1 down primer	TGC AGC TCG AGA ACC CGA ACA CAT TTC ACA AG
RNR3 up primer	CAGTGAAATCGTATGTACGTTATTAAA AGA GAC GGC
RNR3 down primer	GAC ATC TCG AGA ACC GGA ACA TGA CTC ACA A

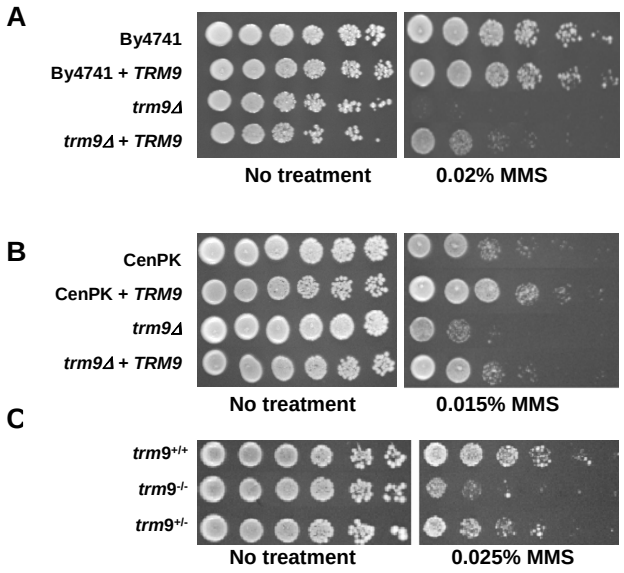

Figure S1. **Haploid and Diploid *trm9Δ* Cells are Sensitive to MMS**

(A) The MMS-sensitivity of *trm9Δ* cells in the haploid By4741 background was complemented by over-expression of *TRM9* from pYES2.0-TRM9. Cultures were grown overnight in SD-URA and then serially diluted onto YP-galactose plates. **(B)** The MMS-sensitivity of *trm9* insertion mutants in the haploid CenPK2 background was complemented by the expression of *TRM9*, under the control of its own promoter, from pRS416-TRM9. Cultures were grown overnight in SD-TRP and then serially diluted onto YPD plates. **(C)** Heterozygous and homozygous deficient *trm9Δ* cells from the By4743 diploid background were assayed for MMS sensitivity. Overnight cultures were grown in YPD and then serially diluted onto YPD plates. All plates were imaged 3-5 days after spotting.

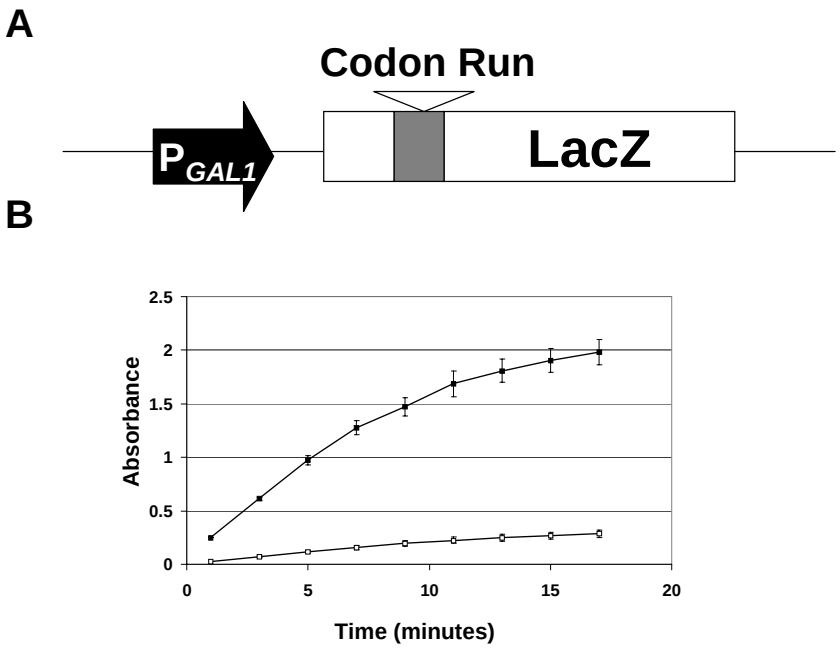

Figure S2. Trm9 Enhances the Activity of Codon-Specific Reporters

(A) Codon runs (none, 10X AGA, 10X AGG, and 5X AGG) were cloned in frame with *lacZ*, in pYESNT/LacZ, and the reporter was transcriptionally induced using a strong galactose inducible *GAL1* promoter. **(B)** Time course of β-galactosidase activity for the 10X GAA in wild-type (closed squares) and *trm9Δ* (open squares) cells. Absorbance at 410 nm. The 10X GAA reporter was expressed form pYES-10XGAA-lacZ.

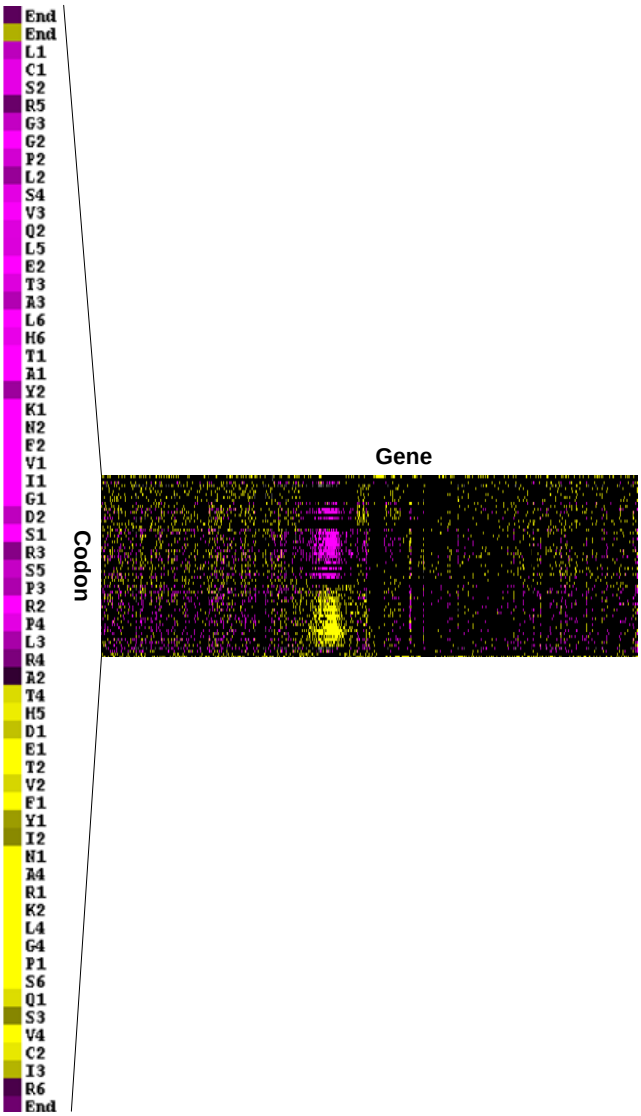

Figure S3. Heat Map of Significant GSCU Z-Scores

Hierarchical clustering and heat map analysis of all GSCU data for 5,783 *S. cerevisiae* genes. Z-scores greater then 1.5 ($p < 0.067$) are displayed in yellow, Z-scores <-1.5 are displayed in purple, and Z-scores between -1.5 and 1.5 are displayed as black. Note figure S3 has been rotated 90º clockwise to facilitate viewing of the codon axis and corresponds to figure 2A. A zoomed view of the codon key specific to the *YEF3* gene. A1, GCA (Ala), A2, GCC (Ala); A3, GCG (Ala); A4, GCT (Ala); R1, AGA (Arg); R2, AGG (Arg); R3, CGA (Arg); R4, CGC (Arg); R5, CGG (Arg); R6, CGT (Arg); N1, AAC (Asn); N2, AAT (Asn); D1, GAC (Asp); D2, GAT (Asp); C1, TGC (Cys); C2, TGT (Cys); Q1, CAA (Gln); Q2, CAG

(Gln); E1, GAA (Glu); E2, GAG (Glu); G1, GGA (Gly); G2, GGC (Gly); G3, GGG (Gly); G4, GGT (Gly); H5, CAC (His); H6, CAT (His); I1, ATA (Ile); I2, ATC (Ile); I3, ATT (Ile); L1, CTA (Leu); L2, TTA (Leu); L3, CTC (Leu); L4, TTG (Leu); L5, CTG (Leu); L6, CTT (Leu); K1, AAA (Lys); K2, AAG (Lys); F1, TTC (Phe); F2, TTT (Phe); P1, CCA (Pro); P2, CCC (Pro); P3, CCG (Pro); P4, CCT (Pro); S1, TCA (Ser); S2, AGC (Ser); S3, TCC (Ser); S4, TCG (Ser); S5, AGT (Ser); S6, TCT (Ser); T1, ACA (Thr); T2, ACC (Thr); T3, ACG (Thr); T4, ACT (Thr); Y1, TAC (Tyr); Y2, TAT (Tyr); V1, GTA (Val); V2, GTC (Val); V3, GTG (Val); V4, GTT (Val)

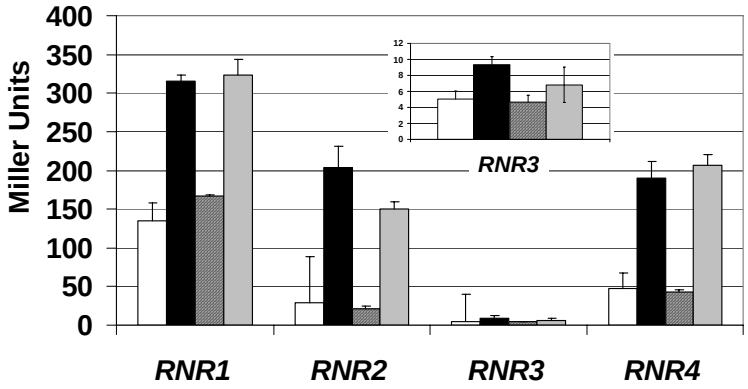

Figure S4. ***RNR* transcript Levels in Wild-type and *trm9Δ* Cells**

lacZ promoter fusion assays were used to quantitate the transcription of *RNR1*, *RNR2*, *RNR3*, and *RNR4* in wild-type and *trm9Δ* cells before and after MMS treatment. β-galactosidase activity was determined in untreated wild-type (white bars), MMS-treated wild-type (black bars), untreated *trm9Δ* (striped bars), and MMS-treated *trm9Δ* cells (gray bars). The *RNR* transcription reporters (YCp(33)*RNR1Z*, YCp(33)*RNR2Z*, YCp(33)*RNR3Z*, and YCp(33)*RNR4Z*) were transformed into wild-type (By4741) and *trm9Δ*, cells and assayed as described (Klinkenberg et al., 2006).

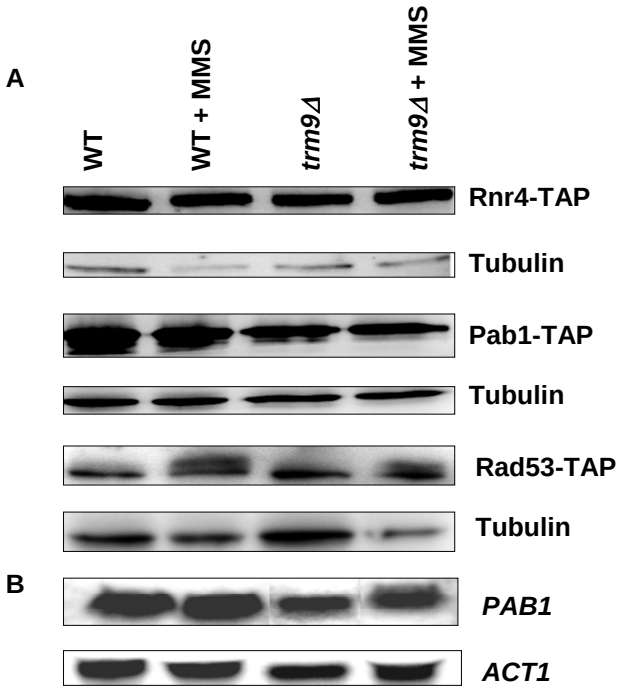

Figure S5. Rnr4 and Pab1 Levels in Wild-type and *trm9Δ* Cells

(A) Western blot analysis of endogenous Rnr4-TAP, Pab1-TAP, Rad53-TAP, and β-tubulin in wild-type and *trm9Δ* cells, before and after MMS-treatment. **(B)** Northern blot results for both cell types, before and after MMS-treatment, for the Trm9-influenced transcript *PAB1* and the loading control *ACT1*. Experiments were performed as described in Figure 2.