

Supplementary Information

Extracurricular Functions of tRNA Modifications in Microorganisms

Ashley M. Edwards, Maame A. Addo, Patricia C. Dos Santos*

Table S1. tRNA modifications with corresponding positions and varying types of stressors that affect each respective modification level

tRNA Modifications Affected by Nutritional and Environmental Stressors			
Position	Modification	Type of Stressor	References
34	mcm ⁵ s ² U	Nutrient limitation	[1-3]
34	mnm ⁵ s ² U	UVR, Oxidative stress, Cold stress	[4],[5-6],[7]
34	cmnm ⁵ s ² U	UVR, Antibiotic/Virulence	[4], [8-10]
37	ms ² i ⁶ A	Nutrient limitation, Oxidative stress, Hypoxia, UVR, Antibiotic/Virulence	[11-15], [6,16], [17], [4], [18-19]
34	m ⁵ C	Oxidative stress	[20-21]
32	Cm	Oxidative stress	[20-22]
32	Am	Oxidative stress	[22]
32	Um	Oxidative stress	[22]
32	s ² C	Oxidative stress	[6,23]
26	m ² ₂ G	Oxidative stress	[20-21]
34	oQ	Hypoxia	[17]
58	m ¹ A	Heat stress	[24-25]
54	s ² T	Heat stress, Oxidative stress	[24,26-29], [5]
46	m ⁷ G	Oxidative stress, Heat stress	[30,32], [31]
39 & 55	ψ	Heat stress, Cold stress	[33-34], [35]
34	ac ⁴ Cm	Heat stress	[26]
15	G ⁺	Heat stress	[25]
26	m ² ₂ Gm	Heat stress	[26]
26	m ² Gm	Heat stress	[28]
57	m ¹ I	Heat stress	[25]
14-21	D	Cold stress	[36-37]
37	ms ² io ⁶ A	Oxidative stress, Hypoxia	[16], [17]
34	xmo ⁵ U	Hypoxia	[38]
34	Q	Nutrient limitation, Antibiotic/Virulence	[39-40], [19]

37	m ¹ G	Antibiotic/Virulence	[41]
8	s ⁴ U	Oxidative stress, UVR	[5-6,42-46], [4,47-49]
34	s ² U	Oxidative stress	[50]

References

1. Laxman, S.; Sutter, B.M.; Wu, X.; Kumar, S.; Guo, X.; Trudgian, D.C.; Mirzaei, H.; Tu, B.P. Sulfur Amino Acids Regulate Translational Capacity and Metabolic Homeostasis through Modulation of tRNA Thiolation. *Cell* **2013**, *154*, 416–429, doi:10.1016/j.cell.2013.06.043.
2. Gupta, R.; Laxman, S. tRNA wobble-uridine modifications as amino acid sensors and regulators of cellular metabolic state. *Curr Genet* **2019**, doi:10.1007/s00294-019-01045-y.
3. Gupta, R.; Walvekar, A.S.; Liang, S.; Rashida, Z.; Shah, P.; Laxman, S. A tRNA modification balances carbon and nitrogen metabolism by regulating phosphate homeostasis. *Elife* **2019**, *8*, doi:10.7554/eLife.44795.
4. Sun, C.; Jora, M.; Solivio, B.; Limbach, P.A.; Addepalli, B. The Effects of Ultraviolet Radiation on Nucleoside Modifications in RNA. *ACS Chemical Biology* **2018**, *13*, 567–572, doi:10.1021/acscchembio.7b00898.
5. Watanabe, K. Reactions of 2-thioribothymidine and 4-thiouridine with hydrogen peroxide in transfer ribonucleic acids from *Thermus thermophilus* and *Escherichia coli* as studied by circular dichroism. *Biochemistry* **1980**, *19*, 5542–5549, doi:10.1021/bi00565a013.
6. Rao, Y.S.P.; Cherayil, J.D. Studies on chemical modification of thionucleosides in the transfer ribonucleic acid of *Escherichia coli*. *Biochem J* **1974**, *143*, 285–294.
7. Singh, A.K.; Shivaji, S. A cold-active heat-labile t-RNA modification GTPase from a psychrophilic bacterium *Pseudomonas syringae* (Lz4W). *Research in Microbiology* **2010**, *161*, 46–50, doi:10.1016/j.resmic.2009.11.002.
8. Shippy, D.C.; Eakley, N.M.; Lauhon, C.T.; Bochsler, P.N.; Fadl, A.A. Virulence characteristics of *Salmonella* following deletion of genes encoding the tRNA modification enzymes *GidA* and *MnmE*. *Microb. Pathog.* **2013**, *57*, 1–9, doi:10.1016/j.micpath.2013.01.004.
9. Cho, K.H.; Caparon, M.G. tRNA modification by *GidA/MnmE* is necessary for *Streptococcus pyogenes* virulence: a new strategy to make live attenuated strains. *Infect. Immun.* **2008**, *76*, 3176–3186, doi:10.1128/IAI.01721-07.
10. Shippy, D.C.; Fadl, A.A. Immunological characterization of a *gidA* mutant strain of *Salmonella* for potential use in a live-attenuated vaccine. *BMC Microbiology* **2012**, *12*, 286, doi:10.1186/1471-2180-12-286.
11. Rhaese, H.-J.; Grade, R.; Dichtelmüller, H. Studies on the Control of Development. *European Journal of Biochemistry* **1976**, *64*, 205–213, doi:10.1111/j.1432-1033.1976.tb10289.x.
12. Menichi, B.; Heyman, T. Thiomethylation of Tyrosine Transfer Ribonucleic Acid Is Associated with Initiation of Sporulation in *Bacillus subtilis*: Effect of Phosphate Concentration. *J. BACTERIOL.* **4**.
13. Buck, M.; Griffiths, E. Iron mediated methylthiolation of tRNA as a regulator of operon expression in *Escherichia coli*. *Nucleic Acids Res.* **1982**, *10*, 2609–2624, doi:10.1093/nar/10.8.2609.
14. Vecerek, B.; Moll, I.; Bläsi, U. Control of *Fur* synthesis by the non-coding RNA *RyhB* and iron-responsive decoding. *EMBO J.* **2007**, *26*, 965–975, doi:10.1038/sj.emboj.7601553.
15. Pollo-Oliveira, L.; de Crécy-Lagard, V. Can protein expression be regulated by modulation of tRNA modifications profiles? *Biochemistry* **2019**, *58*, 355–362, doi:10.1021/acs.biochem.8b01035.
16. Skovran, E.; Lauhon, C.T.; Downs, D.M. Lack of *YggX* Results in Chronic Oxidative Stress and Uncovers Subtle Defects in Fe-S Cluster Metabolism in *Salmonella enterica*. *Journal of Bacteriology* **2004**, *186*, 7626–7634, doi:10.1128/JB.186.22.7626-7634.2004.

17. Buck, M.; Ames, B.N. A modified nucleotide in tRNA as a possible regulator of aerobiosis: synthesis of cis-2-methyl-thioribosylzeatin in the tRNA of Salmonella. *Cell* **1984**, *36*, 523–531, doi:10.1016/0092-8674(84)90245-9.
18. Durand, J.M.; Björk, G.R.; Kuwae, A.; Yoshikawa, M.; Sasakawa, C. The modified nucleoside 2-methylthio-N6-isopentenyladenosine in tRNA of Shigella flexneri is required for expression of virulence genes. *J. Bacteriol.* **1997**, *179*, 5777–5782, doi:10.1128/jb.179.18.5777-5782.1997.
19. Durand, J.M.B.; Dagberg, B.; Uhlin, B.E.; Björk, G.R. Transfer RNA modification, temperature and DNA superhelicity have a common target in the regulatory network of the virulence of Shigella flexneri: the expression of the virF gene. *Molecular Microbiology* **2000**, *35*, 924–935, doi:10.1046/j.1365-2958.2000.01767.x.
20. Chan, C.T.Y.; Pang, Y.L.J.; Deng, W.; Babu, I.R.; Dyavaiah, M.; Begley, T.J.; Dedon, P.C. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* **2012**, *3*, 937, doi:10.1038/ncomms1938.
21. Chan, C.T.Y.; Dyavaiah, M.; DeMott, M.S.; Taghizadeh, K.; Dedon, P.C.; Begley, T.J. A Quantitative Systems Approach Reveals Dynamic Control of tRNA Modifications during Cellular Stress. *PLoS Genetics* **2010**, *6*, e1001247, doi:10.1371/journal.pgen.1001247.
22. Jaroensuk, J.; Atichartpongkul, S.; Chionh, Y.H.; Wong, Y.H.; Liew, C.W.; McBee, M.E.; Thongdee, N.; Prestwich, E.G.; DeMott, M.S.; Mongkolsuk, S.; et al. Methylation at position 32 of tRNA catalyzed by TrmJ alters oxidative stress response in Pseudomonas aeruginosa. *Nucleic Acids Res* **2016**, *44*, 10834–10848, doi:10.1093/nar/gkw870.
23. Romsang, A.; Duang-nkern, J.; Khemsom, K.; Wongsaroj, L.; Saninjuk, K.; Fuangthong, M.; Vattanaviboon, P.; Mongkolsuk, S. Pseudomonas aeruginosa ttcA encoding tRNA-thiolating protein requires an iron-sulfur cluster to participate in hydrogen peroxide-mediated stress protection and pathogenicity. *Scientific Reports* **2018**, *8*, doi:10.1038/s41598-018-30368-y.
24. Shigi, N.; Suzuki, T.; Terada, T.; Shirouzu, M.; Yokoyama, S.; Watanabe, K. Temperature-dependent biosynthesis of 2-thioribothymidine of Thermus thermophilus tRNA. *J. Biol. Chem.* **2006**, *281*, 2104–2113, doi:10.1074/jbc.M510771200.
25. Orita, I.; Futatsuishi, R.; Adachi, K.; Ohira, T.; Kaneko, A.; Minowa, K.; Suzuki, M.; Tamura, T.; Nakamura, S.; Imanaka, T.; et al. Random mutagenesis of a hyperthermophilic archaeon identified tRNA modifications associated with cellular hyperthermotolerance. *Nucleic Acids Res* **2019**, *47*, 1964–1976, doi:10.1093/nar/gky1313.
26. Kowalak, J.A.; Dalluge, J.J.; McCloskey, J.A.; Stetter, K.O. The Role of Posttranscriptional Modification in Stabilization of Transfer RNA from Hyperthermophiles. *Biochemistry* **1994**, *33*, 7869–7876, doi:10.1021/bi00191a014.
27. Kumagai, I.; Watanabe, K.; Oshima, T. Thermally induced biosynthesis of 2'-O-methylguanosine in tRNA from an extreme thermophile, Thermus thermophilus HB27. *Proceedings of the National Academy of Sciences* **1980**, *77*, 1922–1926, doi:10.1073/pnas.77.4.1922.
28. McCloskey, J.A.; Graham, D.E.; Zhou, S.; Crain, P.F.; Ibba, M.; Konisky, J.; Söll, D.; Olsen, G.J. Post-transcriptional modification in archaeal tRNAs: identities and phylogenetic relations of nucleotides from mesophilic and hyperthermophilic Methanococcales. *Nucleic Acids Res* **2001**, *29*, 4699–4706.
29. Horie, N.; Hara-Yokoyama, M.; Yokoyama, S.; Watanabe, K.; Kuchino, Y.; Nishimura, S.; Miyazawa, T. Two tRNA^{Ile} species from an extreme thermophile, Thermus thermophilus

- HB8: effect of 2-thiolation of ribothymidine on the thermostability of tRNA. *Biochemistry* **1985**, *24*, 5711–5715, doi:10.1021/bi00342a004.
30. Thongdee, N.; Jaroensuk, J.; Atichartpongkul, S.; Chittrakanwong, J.; Chooyoung, K.; Srimahaeak, T.; Chaiyen, P.; Vattanaviboon, P.; Mongkolsuk, S.; Fuangthong, M. TrmB, a tRNA m7G46 methyltransferase, plays a role in hydrogen peroxide resistance and positively modulates the translation of katA and katB mRNAs in *Pseudomonas aeruginosa*. *Nucleic Acids Res* **2019**, *47*, 9271–9281, doi:10.1093/nar/gkz702.
 31. Takano, Y.; Takayanagi, N.; Hori, H.; Ikeuchi, Y.; Suzuki, T.; Kimura, A.; Okuno, T. A gene involved in modifying transfer RNA is required for fungal pathogenicity and stress tolerance of *Colletotrichum lagenarium*. *Molecular Microbiology* **2006**, *60*, 81–92, doi:10.1111/j.1365-2958.2006.05080.x.
 32. Reichle, V.F.; Weber, V.; Kellner, S. NAIL-MS in *E. coli* Determines the Source and Fate of Methylation in tRNA. *ChemBioChem* **2018**, *19*, 2575–2583, doi:10.1002/cbic.201800525.
 33. Durant, P.C.; Davis, D.R. Stabilization of the anticodon stem-loop of tRNA^{Lys,3} by an A+-C base-pair and by pseudouridine11 Edited by I. Tinoco. *Journal of Molecular Biology* **1999**, *285*, 115–131, doi:10.1006/jmbi.1998.2297.
 34. Booth, I.R.; O’Byrne, C.P.; Stansfield, I.; Kinghorn, S.M. Physiological analysis of the role of truB in *Escherichia coli*: a role for tRNA modification in extreme temperature resistance. *Microbiology* **2002**, *148*, 3511–3520, doi:10.1099/00221287-148-11-3511.
 35. Ishida, K.; Kunibayashi, T.; Tomikawa, C.; Ochi, A.; Kanai, T.; Hirata, A.; Iwashita, C.; Hori, H. Pseudouridine at position 55 in tRNA controls the contents of other modified nucleotides for low-temperature adaptation in the extreme-thermophilic eubacterium *Thermus thermophilus*. *Nucleic Acids Res* **2011**, *39*, 2304–2318, doi:10.1093/nar/gkq1180.
 36. Dalluge, J.J.; Hashizume, T.; Sopchik, A.E.; McCloskey, J.A.; Davis, D.R. Conformational flexibility in RNA: the role of dihydrouridine. *Nucleic Acids Res* **1996**, *24*, 1073–1079.
 37. Dalluge, J.J.; Hamamoto, T.; Horikoshi, K.; Morita, R.Y.; Stetter, K.O.; McCloskey, J.A. Posttranscriptional modification of tRNA in psychrophilic bacteria. *J Bacteriol* **1997**, *179*, 1918–1923.
 38. Chionh, Y.H.; McBee, M.; Babu, I.R.; Hia, F.; Lin, W.; Zhao, W.; Cao, J.; Dziergowska, A.; Malkiewicz, A.; Begley, T.J.; et al. tRNA-mediated codon-biased translation in mycobacterial hypoxic persistence. *Nature Communications* **2016**, *7*, 13302, doi:10.1038/ncomms13302.
 39. Müller, M.; Hartmann, M.; Schuster, I.; Bender, S.; Thüring, K.L.; Helm, M.; Katze, J.R.; Nellen, W.; Lyko, F.; Ehrenhofer-Murray, A.E. Dynamic modulation of Dnmt2-dependent tRNA methylation by the micronutrient queuine. *Nucleic Acids Res.* **2015**, *43*, 10952–10962, doi:10.1093/nar/gkv980.
 40. Müller, M.; Legrand, C.; Tuorto, F.; Kelly, V.P.; Atlasi, Y.; Lyko, F.; Ehrenhofer-Murray, A.E. Queuine links translational control in eukaryotes to a micronutrient from bacteria. *Nucleic Acids Res* **2019**, *47*, 3711–3727, doi:10.1093/nar/gkz063.
 41. Masuda, I.; Matsubara, R.; Christian, T.; Rojas, E.R.; Yadavalli, S.S.; Zhang, L.; Goulian, M.; Foster, L.J.; Huang, K.C.; Hou, Y.-M. tRNA Methylation Is a Global Determinant of Bacterial Multi-drug Resistance. *Cell Systems* **2019**, *8*, 302-314.e8, doi:10.1016/j.cels.2019.03.008.
 42. Nair, D.P.; Podgórski, M.; Chatani, S.; Gong, T.; Xi, W.; Fenoli, C.R.; Bowman, C.N. The Thiol-Michael Addition Click Reaction: A Powerful and Widely Used Tool in Materials Chemistry. *Chem. Mater.* **2014**, *26*, 724–744, doi:10.1021/cm402180t.

43. Herzog, V.A.; Reichholf, B.; Neumann, T.; Rescheneder, P.; Bhat, P.; Burkard, T.R.; Wlotzka, W.; von Haeseler, A.; Zuber, J.; Ameres, S.L. Thiol-linked alkylation of RNA to assess expression dynamics. *Nature Methods* **2017**, *14*, 1198–1204, doi:10.1038/nmeth.4435.
44. Schofield, J.A.; Duffy, E.E.; Kiefer, L.; Sullivan, M.C.; Simon, M.D. TimeLapse-seq: adding a temporal dimension to RNA sequencing through nucleoside recoding. *Nature Methods* **2018**, *15*, 221–225, doi:10.1038/nmeth.4582.
45. Yano, M.; Hayatsu, H. Permanganate oxidation of 4-thiouracil derivatives: Isolation and properties of 1-substituted 2-pyrimidone 4-sulfonates. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* **1970**, *199*, 303–315, doi:10.1016/0005-2787(70)90073-0.
46. Ziff, E.B.; Fresco, J.R. Chemical transformation of 4-thiouracil nucleosides to uracil and cytosine counterparts. *J. Am. Chem. Soc.* **1968**, *90*, 7338–7342, doi:10.1021/ja01028a027.
47. Kramer, G.F.; Baker, J.C.; Ames, B.N. Near-UV stress in *Salmonella typhimurium*: 4-thiouridine in tRNA, ppGpp, and ApppGpp as components of an adaptive response. *Journal of Bacteriology* **1988**, *170*, 2344–2351, doi:10.1128/JB.170.5.2344-2351.1988.
48. Thiam, K.; Favre, A. Role of the stringent response in the expression and mechanism of near-ultraviolet induced growth delay. *European Journal of Biochemistry* **1984**, *145*, 137–142, doi:10.1111/j.1432-1033.1984.tb08532.x.
49. Favre, A.; Hajnsdorf, E.; Thiam, K.; Caldeira de Araujo, A. Mutagenesis and growth delay induced in *Escherichia coli* by near-ultraviolet radiations. *Biochimie* **1985**, *67*, 335–342, doi:10.1016/S0300-9084(85)80076-6.
50. Sochacka, E.; Bartos, P.; Kraszewska, K.; Nawrot, B. Desulfuration of 2-thiouridine with hydrogen peroxide in the physiological pH range 6.6–7.6 is pH-dependent and results in two distinct products. *Bioorganic & Medicinal Chemistry Letters* **2013**, *23*, 5803–5805, doi:10.1016/j.bmcl.2013.08.114.