



Stressin' and slicin': stress-induced tRNA fragmentation codon-adapts translation to repress cell growth

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Transfer RNAs (tRNAs) are central adaptors that decode genetic information during translation and have been long considered static cellular components. However, whether dynamic changes in tRNAs and tRNA-derived fragments actively contribute to gene regulation remains debated. In this issue, Huh *et al* (2020) highlight tyrosine tRNA_{GUA} fragmentation at the nexus of an evolutionarily conserved adaptive codon-based stress response that fine-tunes translation to restrain growth in human cells.

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See also: D Huh *et al* (2020)

Protein synthesis is a fundamental process that relies on tRNAs for decoding the genetic information in mRNAs. Their central role as universal adaptor molecules in translation contributed to the prevailing notion that tRNAs are static cellular components with limited or no regulatory function. This view is changing due to increasing evidence that tRNA abundance and modifications are rewired to direct cellular translomes during physiological and stress conditions in different types of normal and malignant cells (Gingold *et al*, 2014; Goodarzi *et al*, 2016; Chou *et al*, 2017). A renewed interest in tRNA biology was further sparked by the discovery of evolutionarily conserved stable tRNA-derived fragments (tRFs) with pleiotropic roles in gene expression control across different cell types and upon stress in organisms from all kingdoms of life (Oberbauer & Schaefer, 2018). Findings that only a small portion of tRNA is cleaved suggested that the

functional effects of tRFs on gene expression are mostly uncoupled from the translational impairments caused by the depletion of precursor or mature tRNAs (Thompson *et al*, 2008). However, an outstanding question is to what extent tRNA fragmentation coordinates the tRNA and tRF cellular pools at the codon level in order to molecularly adapt the stress response.

In this issue of *The EMBO journal*, Huh *et al* (2020) set out to address this question in human epithelial cells undergoing oxidative stress, which was known to induce robust tRF accumulation in multiple cell systems (Thompson *et al*, 2008). The authors employed a highly sensitive probe-based sequencing approach to uncover reductions within a limited subset of tRNAs following stress. Depletion of these tRNAs overlapped, at least in part, with the generation of isodecoder-specific fragments, highlighting tyrosine tRNA_{GUA} (tRNA^{Tyr}_{GUA}) fragmentation as one of the most striking oxidative-stress-induced event. In an attempt to delineate the consequences of tRNA^{Tyr}_{GUA} depletion, authors performed elegant loss-of-function studies and revealed specific translation impairments in distinct tyrosine codon-enriched mRNAs encoding metabolic and growth-promoting factors. These defects were consistent with the cytostatic effects observed following oxidative stress and tRNA^{Tyr}_{GUA} downregulation. Findings that ribosome stalling was increased on tyrosine codons upon depletion of tRNA^{Tyr}_{GUA} strongly support that the availability of this cognate tRNA needs to meet the demand imposed by the mRNA codon usage during stress. This is concordant with previous

reports illustrating a direct correspondence between tRNA abundance and codon usage that selectively impacts ribosome elongation rates and gene expression in response to stress and various biological cues (Gingold *et al*, 2014; Goodarzi *et al*, 2016).

Emerging evidence further suggests that reprogramming of tRNA modifications may provide an additional layer of translation control that impacts codon optimality in cells exposed to different environmental signals (Chan *et al*, 2012; Chou *et al*, 2017). Previous work by the Dedon laboratory demonstrated that increased levels of Trm4 methyltransferase-induced 5-methylcytosine (m5C) modification at the wobble position of tRNA^{Leu}_{CAA} promoted selective translation of UUG codon-enriched mRNAs involved in survival upon oxidative stress in yeast (Chan *et al*, 2012). Additional research indicates that the expression of tRNA modifying enzymes may be regulated and influence tRNA fragmentation across different types of normal and malignant cells and following stress (Blanco *et al*, 2016; Guzzi *et al*, 2018). These results raise a key question: do epitranscriptomic modifications impact processing and function of tRNA^{Tyr}_{GUA} and other stress-sensitive isoacceptor tRNAs identified by Huh and colleagues (2020)? This may provide further mechanistic explanations for differences in selectivity of tRNA modulation observed between mammalian cells and unicellular organisms such as yeast and bacteria, which display profound rearrangements of their tRNA pools upon exposure to oxidative stress (Torrent *et al*, 2018).

One of most prominent mechanism of tRF generation relies on angiogenin (ANG),

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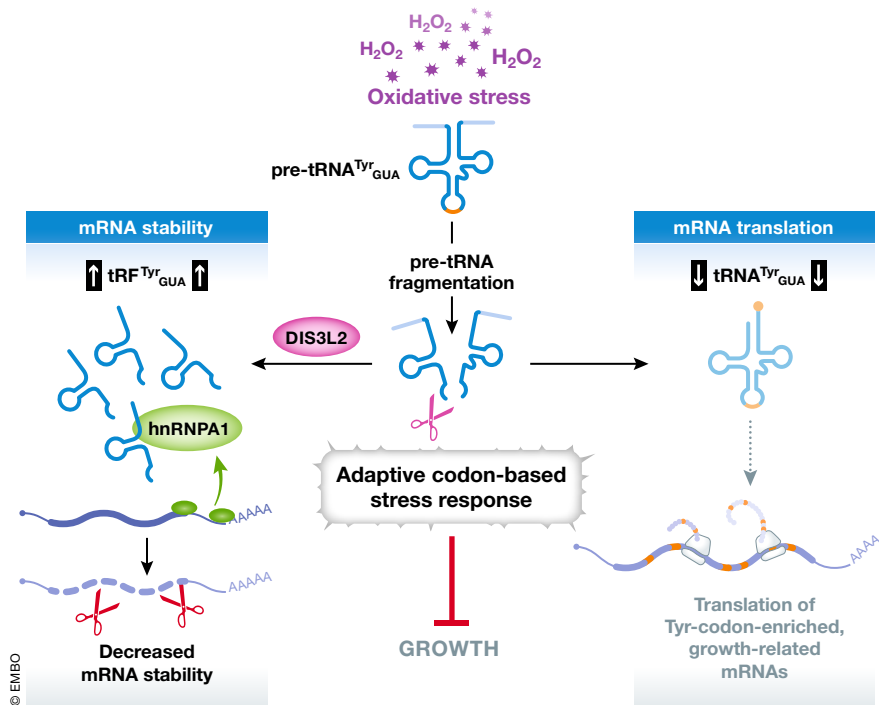


Figure 1. Stress-induced tyrosine tRNA fragmentation controls cell growth.

Schematic model depicts the adaptive codon-based stress response governed by tyrosine tRNA^{Tyr_{GUA}} fragmentation in human cells. Through an unknown mechanism, oxidative stress induces rapid depletion of pre-tRNA^{Tyr_{GUA}} and accumulation of tRNA fragments (tRF^{Tyr_{GUA}}) in an exoribonuclease DIS3L2-dependent manner. Left, tRF^{Tyr_{GUA}} competes for hnRNPA1 binding with specific growth-associated mRNA subsets promoting their destabilization. Right, pre-tRNA^{Tyr_{GUA}} fragmentation subsequently depletes mature tRNA^{Tyr_{GUA}}, thereby hampering the translation of growth-related mRNAs (e.g., USP3, EPCAM, and SCD) that are enriched in tyrosine codons. The synergistic effects on the transcriptome and translome induced by the accumulation of tRF^{Tyr_{GUA}} and depletion of tRNA^{Tyr_{GUA}} drive an evolutionarily conserved program that limits cell growth upon oxidative stress.

a member of the RNase A superfamily, which is activated under stress to cleave multiple mature cytoplasmic tRNAs into halves (Ivanov *et al*, 2011). Focusing on the accumulation of tRF^{Tyr_{GUA}}, Huh *et al* (2020) report a new processing pathway that involves DIS3L2, a Wilms tumor-associated exoribonuclease. Interestingly, tRF^{Tyr_{GUA}} induction occurs rapidly following oxidative stress and is concomitant with a drastic reduction of pre-tRNA^{Tyr_{GUA}} levels without changes in the mature tRNA pool. That DIS3L2 depletion does not fully restore pre-tRNA levels upon oxidative stress points to an intermediate precursor tRF^{Tyr_{GUA}} form that is processed. Evidence that this DIS3L2-mediated processing event is evolutionarily conserved in *C. elegans* suggests that it may have widespread physiological implications for organismal growth. Thus, a future challenge is to determine how DIS3L2-mediated tRF processing is regulated to generate mature tRF^{Tyr_{GUA}} and possibly other tRFs.

There is a growing realization that tRFs may provide additional means to direct genetic information upon exposure to stress, during development and in disease (Oberbauer & Schaefer, 2018). Although still little is known about the function of tRFs, exciting studies suggest that specific fragments perturb mRNA stability and repress translation through critical interactions with RNA binding proteins (RBPs) and translation initiation factors (Ivanov *et al*, 2011; Goodarzi *et al*, 2015; Guzzi *et al*, 2018). Huh *et al* (2020) expand the tRF-protein interactome by uncovering selective binding between tRF^{Tyr_{GUA}} and two RBPs with multiple roles in RNA regulation, namely hnRNPA1 and La/SSB, in response to oxidative stress. Intriguingly, tRF^{Tyr_{GUA}} may directly compete for hnRNPA1 binding with specific growth-associated mRNA subsets, promoting their destabilization under stress conditions. This effect consolidates the translational repression of growth-promoting transcripts

imposed by the subsequent depletion of mature tRNA^{Tyr_{GUA}}. As such, these findings highlight divergent control programs that converge on pre-tRNA^{Tyr_{GUA}} to adapt the cellular transcriptome and translome during oxidative stress (Fig 1).

In conclusion, the recent study by Huh *et al* (2020) supports evidence that multifaceted gene regulatory mechanisms intrinsic to the genetic code are integral to the cellular stress response. While new exciting horizons are opening within this emerging research field, future work is needed to delineate the signaling cascades that coordinate tRNA fragmentation and reprogram protein synthesis during critical cell state transitions in development and tumorigenesis.

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References

- Blanco S, Bandiera R, Popis M, Hussain S, Lombard P, Aleksic J, Sajini A, Tanna H, Cortes-Garrido R, Gkatzka N *et al* (2016) Stem cell function and stress response are controlled by protein synthesis. *Nature* 534: 335–340
- Chan CTY, Pang YL, Deng WJ, Babu IR, Dyavaiah M, Begley TJ, Dedon PC (2012) Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* 3: 937
- Chou HJ, Donnard E, Gustafsson HT, Garber M, Rando OJ (2017) Transcriptome-wide analysis of roles for tRNA modifications in translational regulation. *Mol Cell* 68: 978–992
- Gingold H, Tehler D, Christoffersen NR, Nielsen MM, Asmar F, Kooistra SM, Christophersen NS, Christensen LL, Borre M, Sorensen KD *et al* (2014) A dual program for translation regulation in cellular proliferation and differentiation. *Cell* 158: 1281–1292
- Goodarzi H, Liu X, Nguyen HC, Zhang S, Fish L, Tavazoie SF (2015) Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. *Cell* 161: 790–802
- Goodarzi H, Nguyen HCB, Zhang S, Dill BD, Molina H, Tavazoie SF (2016) Modulated expression of specific tRNAs drives gene expression and cancer progression. *Cell* 165: 1416–1427

- Guzzi N, Ciesla M, Ngoc PCT, Lang S, Arora S, Dimitriou M, Pimkova K, Sommarin MNE, Munita R, Lubas M *et al* (2018) Pseudouridylation of tRNA-derived fragments steers translational control in stem cells. *Cell* 173: 1204–1216
- Huh D, Passarelli MC, Gao J, Dusmatova SN, Goin C, Fish L, Pinzaru AM, Molina H, McMillan EA, Asgharian H *et al* (2020) A stress-induced Tyrosine tRNA depletion response mediates codon-based translational repression and growth suppression. *EMBO J* 39: e106696
- Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P (2011) Angiogenin-induced tRNA fragments inhibit translation initiation. *Mol Cell* 43: 613–623
- Oberbauer V, Schaefer MR (2018) tRNA-derived small RNAs: biogenesis, modification, function and potential impact on human disease development. *Genes-Basel* 9: 607
- Thompson DM, Lu C, Green PJ, Parker R (2008) tRNA cleavage is a conserved response to oxidative stress in eukaryotes. *RNA* 14: 2095–2103
- Torrent M, Chalancon G, de Groot NS, Wuster A, Babu MM (2018) Cells alter their tRNA abundance to selectively regulate protein synthesis during stress conditions. *Sci Signal* 11: eaat6409