

Spotlight

Cytosine methylation
flags mitochondrial RNA
for degradationEmeline Recazens ¹, and
Alexis A. Jourdain ^{1,*}

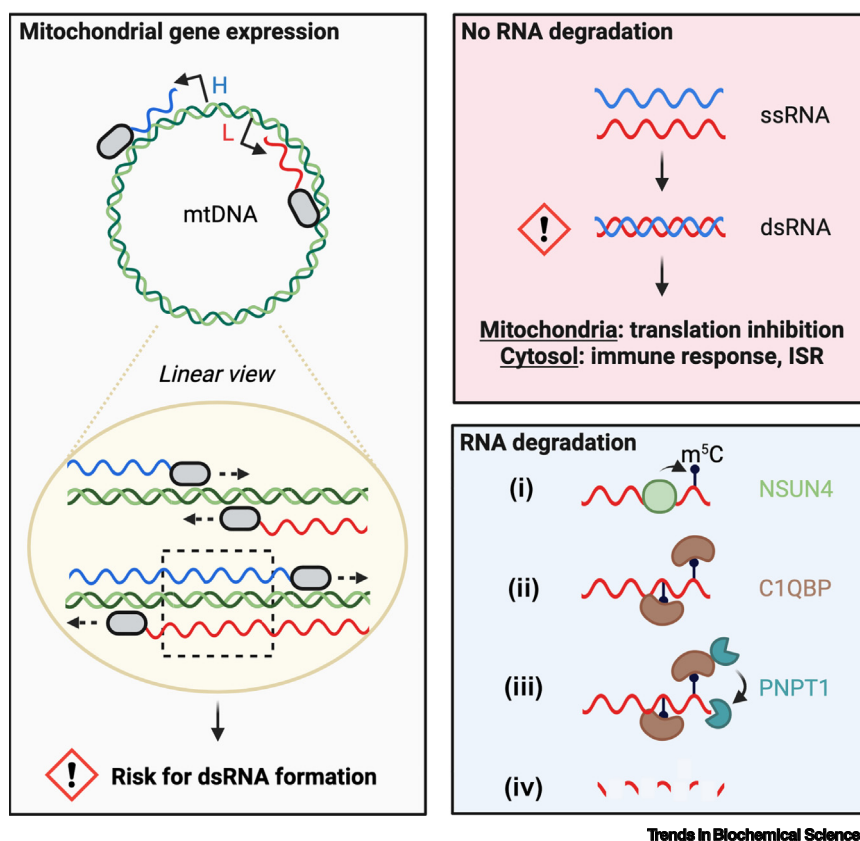
Mitochondrial double-stranded RNA (dsRNA) can form spontaneously in mitochondria, blocking mitochondrial gene expression and triggering an immune response. A recent study by Kim, Tan, *et al.* identified a safeguard mechanism in which NOP2/Sun RNA methyltransferase 4 (NSUN4)-mediated RNA methylation (m⁵C) recruits the RNA degradation machinery to prevent dsRNA formation.

Mitochondria have emerged as significant signaling organelles that can release nucleic acids into the cytosol and trigger an immune response. Cytosolic mitochondrial DNA (mtDNA) is known to activate cGAS/STING and the NLRP3 inflammasome, causing interferon signaling, inflammation, and cell death [1,2]. More recently, mtRNA was also highlighted as a potent immunogenic molecule since the bidirectional mode of transcription of the circular mtDNA genome generates long transcripts with a high propensity to form dsRNA (Figure 1) [3]. Similar to viral genomes, mitochondria-derived dsRNA is recognized as foreign in the cytosol and can initiate an immune response.

To prevent dsRNA formation, mitochondrial noncoding transcripts, primarily encoded by the light strand of the mtDNA molecule, must be constantly degraded. In human mitochondria, this function is catalyzed by the mitochondrial degradosome, a protein complex composed of the RNA helicase SUV3 (SUPV3L1) and the 3'-5' exonuclease polyribonucleotide nucleotidyltransferase 1

(PNPT1) [4]. Depletion of either subunit perturbs mtRNA turnover and results in dsRNA accumulation, which affects mitochondrial gene expression and leads to mtDNA instability [5,6]. Similar to mtDNA release, leakage of mitochondrial dsRNA into the cytosol can be detected by cytosolic sensors such as MDA5 and RIG-I, as well as the dsRNA-activated protein kinase R (PKR). In cellular models and in patients with mutations in *PNPT1* [3], this triggers

proinflammatory and interferon signaling, as well as the integrated stress response (Figure 1). Several modifications exist in mtRNA, including methylation and pseudouridylation, but their role in RNA degradation, dsRNA formation, and innate immunity remains unknown. Mitochondrial dsRNA accumulation has also been implicated in various inflammatory diseases, including autoimmune Sjögren's syndrome, osteoarthritis, and Huntington's disease. Multiple models



Trends in Biochemical Sciences

Figure 1. Mitochondria RNA degradation prevents double-stranded RNA formation. Left: mitochondrial DNA (mtDNA) is a circular double-stranded DNA whose mode of expression generates long complementary strands of RNA, with a high propensity to form double-stranded RNA (dsRNA). Grey superellipse: mitochondrial RNA polymerase. Top right: in the absence of RNA degradation, long complementary strands of RNA may base-pair to form dsRNA, leading to translation inhibition in mitochondria and immune and integrated stress responses in the cytosol. Bottom right: to prevent dsRNA formation, mitochondrial noncoding RNA, mostly encoded by the light strand, must be constitutively degraded by the exonuclease polyribonucleotide nucleotidyltransferase 1 (PNPT1). Kim, Tan *et al.* [8] provide a novel mechanism to explain how noncoding RNA is degraded in mitochondria, based on (i) 5-methylcytosine (m⁵C) methylation of noncoding RNA by NOP2/Sun RNA methyltransferase 4 (NSUN4), (ii) recruitment of C1QBP to the m⁵C sites, and (iii) recruitment of PNPT1 for (iv) RNA degradation. Abbreviations H, heavy strand of the mtDNA; ISR, integrated stress response; L, light strand of the mtDNA.

have been proposed to explain the release of mtRNA to the cytosol, including BAX/BAK pores and the opening of the mitochondrial permeability transition pore (MPTP) [2,7]. Detection, degradation, and thus elimination of mitochondrial dsRNA appears to be crucial to prevent a chronic immune response. Until recently, however, a key aspect of mtRNA turnover remained unknown, namely, how the mitochondrial degradosome is able to discriminate between coding and non-coding RNA.

In a recent publication, Kim, Tan, *et al.* revealed that mitochondrial NSUN4 can methylate mtRNAs, targeting them for degradation [8]. The authors depleted the expression of 89 putative RNA-binding proteins of the mitochondrial matrix and performed strand-specific RT-qPCR, probing various regions of the mitochondrial transcriptome, including noncoding RNA from the light strand. This approach highlighted the importance of the methyltransferase NSUN4, an enzyme known to methylate a cytosine nucleotide on the mitochondrial small ribosomal subunit, creating 5-methylcytosine (m⁵C), and whose ablation in skeletal muscle or cardiomyocytes was shown to result in mitochondrial dysfunction, cardiomyopathy, and shortened lifespan in mice [9]. Kim, Tan, *et al.* have now reported that in *NSUN4*-depleted cells, noncoding RNA accumulates in mitochondria and generates increased levels of dsRNA. This appears to depend mainly on the catalytic activity of NSUN4 and is independent of defects in mitochondrial ribosome assembly and PNPT1, prompting the authors to directly measure m⁵C methylation. Using bisulfite sequencing (Bis-seq), Kim, Tan, *et al.* generated an ‘epitranscriptome’ map of m⁵C sites on mtRNA, and by comparing their data with *NSUN4*-depleted cells they highlighted *NSUN4*-dependent m⁵C methylation sites, many of which were reduced on noncoding transcripts in cells depleted from this

methyltransferase, while several methylation sites were independent from NSUN4. Thus, these data provide a strong link between methylation and degradation of RNA in mitochondria and highlight the importance of this pathway in regulating dsRNA formation. However these observations also raise further questions, such as how the noncoding RNAs are recognized by NSUN4, since the authors’ search for conserved motifs or secondary structures around the methylation sites remained inconclusive due to the lack of diversity in methylated mtRNAs, and how does RNA methylation recruit the mitochondrial degradosome.

To address the latter question, the authors reanalyzed pull-down experiment data performed using m⁵C-methylated RNA as bait. They identified C1QBP as a binding partner for m⁵C and were able to demonstrate that C1QBP binding to mtRNA is greatly attenuated in *NSUN4*-depleted cells. C1QBP was first identified as a complement-binding protein and, to date, multiple functions have been attributed to the protein, possibly due to its high affinity for various factors. However, human genetic studies reported that biallelic *C1QBP* mutations result specifically in mitochondrial defects characterized by impaired mitochondrial translation [10], reminiscent of *Nsun4* deletion in mice. Kim, Tan, *et al.* now showed that *C1QBP*-deficient cells exhibit higher levels of mtRNA and dsRNA due to reduced degradation rates, confirming the role of m⁵C in mtRNA turnover. These observations also suggest that patients with *C1QBP* mutations may potentially present with higher levels of mitochondrial dsRNA and interferonopathy. Finally, the authors added another piece to the puzzle by performing an additional pull-down experiment, which allowed them to identify an interaction between C1QBP and PNPT1: Bis-seq in either *C1QBP*- or *PNPT1*-deficient cells

revealed an accumulation of *NSUN4*-dependent methylation sites, confirming the sequential actions of all three factors in dsRNA degradation, namely: (i) methylation of light strand non-coding mtRNA by NSUN4, (ii) C1QBP binding to m⁵C, and (iii) recruitment of PNPT1, thus (iv) promoting RNA degradation (Figure 1).

A curious aspect of this work is that whereas depletion of *NSUN4*, *C1QBP*, and *PNPT1* all lead to dsRNA accumulation, only *C1QBP* and *PNPT1* depletion cause immune signaling, suggesting an absence of dsRNA release in *NSUN4*-depleted cells. This observation, confirmed by cellular subfractionation, suggests that m⁵C methylation might not only label RNA for degradation but may also be a signal for efflux to the cytosol. In the future, it will be important to investigate this finding in the context of the proposed mechanism of mtRNA release to the cytosol.

The work by Kim, Tan, *et al.* provides new insight into the mechanism of RNA degradation in mitochondria, with strong implications for mitochondrial gene expression and innate immunity. Future research should focus on elucidating how NSUN4 recognizes specific RNA targets for methylation, for example, using *in vitro* assays, and how this modification influences RNA efflux to the cytosol. Investigating the implications of the interaction between C1QBP and PNPT1 should also provide further insight into the regulation of mitochondrial gene expression and the pathology of these disease-associated genes, possibly offering new therapeutic strategies for mitochondrial and immune disorders.

Acknowledgments

The authors would like to thank the members of the Jourdain lab. This work was supported by an EMBO postdoctoral fellowship (ALTF 944-2022 to E.R.) and a grant from the Swiss National Science Foundation (310030_200796 to A.A.J.).

Declaration of interests

No interests are declared.

¹Department of Immunobiology, University of Lausanne, 1066 Epalinges, Switzerland

*Correspondence:

alexis.jourdain@unil.ch (A.A. Jourdain).

✉: [@jourdainlab](https://twitter.com/jourdainlab)

<https://doi.org/10.1016/j.tibs.2024.08.001>

© 2024 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

References

1. West, A.P. *et al.* (2015) Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* 520, 553–557
2. Xian, H. *et al.* (2022) Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling. *Immunity* 55, 1370–1385 e1378
3. Dhir, A. *et al.* (2018) Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature* 560, 238–242
4. Szczesny, R.J. *et al.* (2010) Human mitochondrial RNA turnover caught in flagranti: involvement of hSuv3p helicase in RNA surveillance. *Nucleic Acids Res.* 38, 279–298
5. Pajak, A. *et al.* (2019) Defects of mitochondrial RNA turnover lead to the accumulation of double-stranded RNA *in vivo*. *PLoS Genet.* 15, e1008240
6. Silva, S. *et al.* (2018) Human mitochondrial degradosome prevents harmful mitochondrial R loops and mitochondrial genome instability. *Proc. Natl. Acad. Sci. USA* 115, 11024–11029
7. Krieger, M.R. *et al.* (2024) Trafficking of mitochondrial double-stranded RNA from mitochondria to the cytosol. *Life Sci. Alliance* 7, e202302396
8. Kim, S. *et al.* (2024) RNA 5-methylcytosine marks mitochondrial double-stranded RNAs for degradation and cytosolic release. *Mol. Cell* 84, 2935–2948.e7
9. Metodiev, M.D. *et al.* (2014) NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly. *PLoS Genet.* 10, e1004110
10. Feichtinger, R.G. *et al.* (2017) Biallelic C1QBP mutations cause severe neonatal-, childhood-, or later-onset cardiomyopathy associated with combined respiratory-chain deficiencies. *Am. J. Hum. Genet.* 101, 525–538