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Oxidative Stress Enhances Mitochondrial DNA-dependent Type I Interferon Responses

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ABSTRACT

It is increasingly well-appreciated that mitochondrial DNA (mtDNA) can engage the cGAS-STING cytosolic DNA sensing machinery to trigger type I interferon (IFN-I) responses. However, the exact immunostimulatory features of mtDNA and whether other nucleic acid sensors participate in triggering IFN-I remain poorly defined. We have discovered that mitochondrial reactive oxygen species (mtROS) potentiate mtDNA-driven IFN-I responses by enhancing the immunostimulatory properties of this DNA toward the cGAS-STING pathway. Moreover, we report that Z-DNA binding protein (Zbp1) cooperates with cGAS to sustain IFN-I responses triggered by mtDNA instability. These results provide new insight into the molecular mechanisms of mtDNA recognition by the cytosolic DNA sensing machinery, with broad implications for understanding myriad human diseases where mtDNA release and sensing contributes to inflammation.

Figure 1. The mtDNA-mediated IFN-I Response is Dependent on Mitochondrial Reactive Oxygen Species.

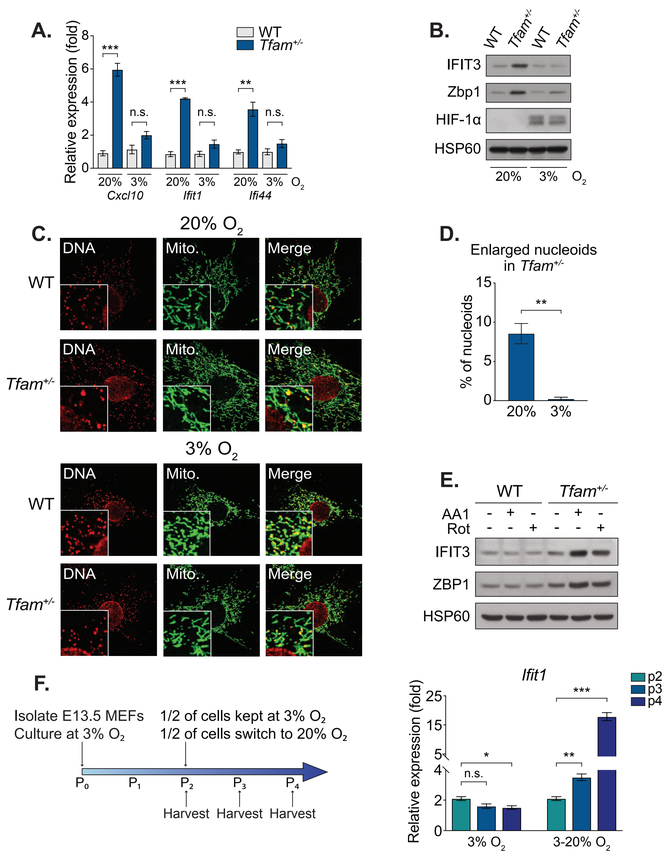


Figure 1. A-B. WT and *Tfam*$^{+/-}$ murine embryonic fibroblasts (MEFs) were cultured at 20% or 3% O_2. RNA was extracted for qRT-PCR analysis **(A)** or protein extracted for western blotting **(B)**. **C-D.** WT and *Tfam*$^{+/-}$ MEFs were cultured at 20% or 3% O_2. After 48h, cells were fixed, stained with anti-DNA (DNA), anti-Hsp60 (Mitochondria), and imaged **(C)**. Enlarged nucleoids from three independent images of each sample were quantified in **(D)**. **E.** WT and *Tfam*$^{+/-}$ MEFs were treated with 5 uM of Antimycin A1 (AA1) or 500 nM of Rotenone (Rot) for 8hrs at 20% O_2. Protein was extracted for western blotting. **F.** *Tfam*$^{+/-}$ MEFs were kept at 3% O_2 or switched from 3% to 20% O_2 for 3 passages (p2, p3, p4). RNA was extracted for qRT-PCR analysis.

Figure 2. Oxidation of mtDNA Potentiates the IFN-I Response.

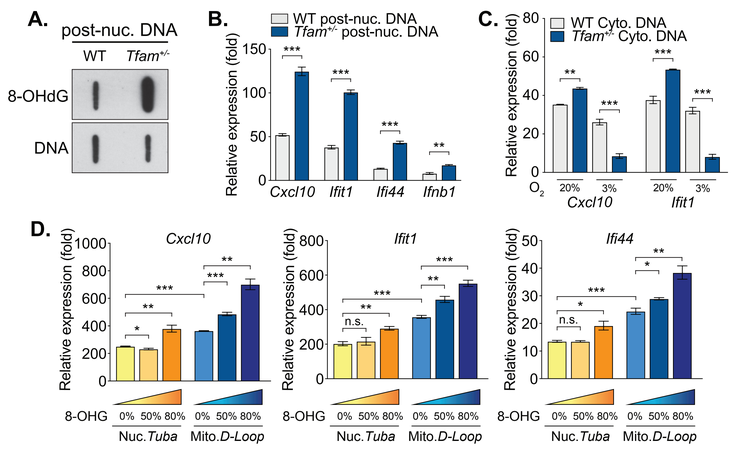


Figure 2. A. Dot blotting of post-nuclear DNA isolated from WT and *Tfam*$^{+/-}$ MEFs. **B.** Cytosolic DNA was extracted from WT and *Tfam*$^{+/-}$ MEFs, and then transfected into WT MEFs for 6hrs. RNA was extracted for qRT-PCR analysis. C. Cytosolic DNA was extracted from WT and *Tfam*$^{+/-}$ MEFs cultured in 20% or 3% O_2, and then transfected into WT MEFs for 6hrs. RNA was extracted for qRT-PCR analysis. **D.** 77bp template of nuclear Tuba (Nuc. *Tuba*) or mitochondrial D-Loop (Mito. *D-Loop*) sequence was used for PCR using different amout of 8-OHG (0%, 50% or 80%). The PCR products were then transfected into WT MEFs. RNA was extracted for qRT-PCR analysis.

Figure 3. Zbp1 Contributes to mtDNA-driven IFN-I Responses.

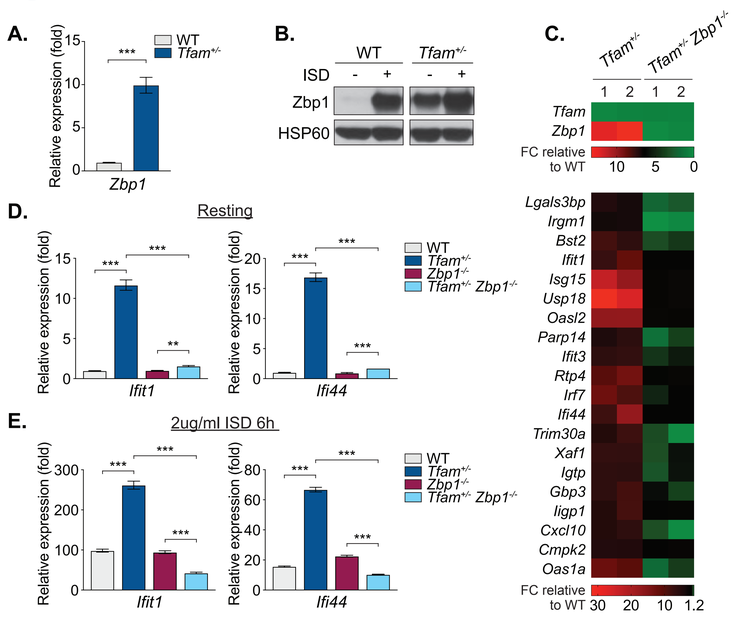


Figure 3. A-B. RNA was extracted from WT and *Tfam*$^{+/-}$ MEFs for qRT-PCR analysis **(A)** or protein was extracted from WT and Tfam$^{+/-}$ MEFs for western blotting **(B)**. **C.** RNA was extracted from WT and *Tfam*$^{+/-}$ MEFs, and sbjected to RNA sequencing. **D.** RNA was extracted from resting WT, *Tfam*$^{+/-}$, *Zbp1*$^{-/-}$ and *Tfam*$^{+/-}$*Zbp1*$^{-/-}$ MEFs for qRT-PCR analysis. **E.** RNA was extracted from interferon stimulatory DNA (ISD) treated WT, *Tfam*$^{+/-}$, *Zbp1*$^{-/-}$ and *Tfam*$^{+/-}$*Zbp1*$^{-/-}$ MEFs for qRT-PCR analysis.

Figure 4. Zbp1 interacts with cGAS to sustain mtDNA-driven IFN-I Responses.

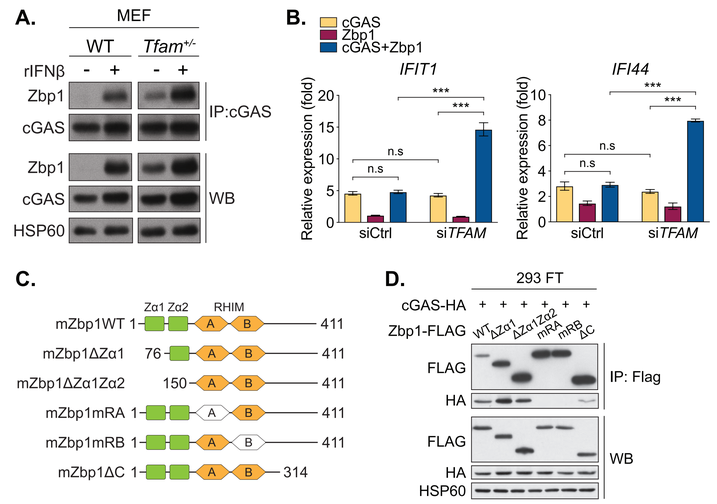
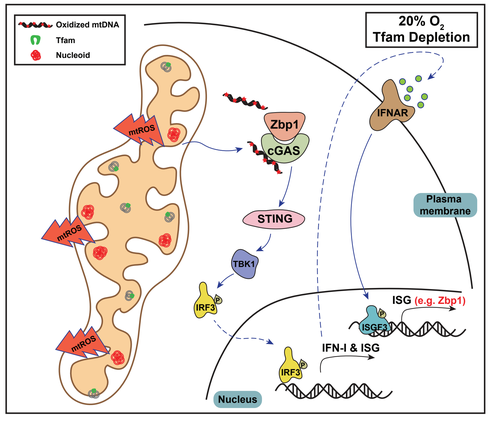


Figure 4. A. WT and *Tfam*$^{+/-}$ MEFs were treated with recombinant interferon β (rIFNβ). Cells were then lysed and immunoprecipitated using cGAS antibody. Protein was extracted for western blotting. **B.** 293FT cells that are stably expressing STING were transfected with siRNA (siCtrl or si*TFAM*), then transfected with cGAS, Zbp1 or cGAS+Zbp1 plasmids. RNA was extracted for qRT-PCR analysis. **C.** Schematic representation of the murine ZBP1 mutants. **D.** 293FT cells were transfected with indicated plasmids for 24hrs. Cells were then lysed and immunoprecipitated using Flag antibody. Protein was extracted for western blotting.

CONCLUSIONS



Loss of the mtDNA packaging protein Tfam drives release of fragmented mtDNA species into the cytosol to trigger type I interferon responses via the cGAS-STING innate immune pathway. Here, we show that, in addition to mtDNA liberation, the oxidation status of mtDNA enhances its immunostimulatory capacity. Morover, we find that specific mtDNA sequences, particulatory those derived from the D-Loop mtDNA regulatory region, are more stimulatory toward cGAS-STING when they enter the cytosol.

Zbp1, the first identified cytosolic DNA sensor, is highly expressed during mtDNA-driven IFN-I responses. Our work suggested that Zbp1 works with cGAS to sustain these responses. Mechanistically, Zbp1 interacts with cGAS through its RHIM domains to potentiate higher interferon stimulated gene (ISG) expression.

In summary, our findings advance understanding of the mtDNA-cGAS-STING-IFN-I signaling axis, which is increasingly implicated in the development and/or progression of many human diseases. Our work and additional studies to characterize the molecular mechanisms of cytosolic mtDNA recognition may contribute to the development of future therapies.

ACKNOWLEDGEMENTS

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