mt-LAF3 is a pseudouridine synthase ortholog required for mitochondrial rRNA and mRNA gene expression in *Trypanosoma brucei*

Suzanne M McDermott[1,2,*], Vy Pham[1], Isaac Lewis[1], Maxwell Tracy[1], and Kenneth Stuart[1,2,*]

[1]Seattle Children's Research Institute, Seattle, WA, USA, and [2]Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA.
*To whom correspondence should be addressed.
Email: suzanne.mcdermott@seattlechildrens.org, ken.stuart@seattlechildrens.org

Abstract

Trypanosoma brucei and related kinetoplastid parasites possess unique RNA processing pathways, including in their mitochondria, that regulate metabolism and development. Altering RNA composition or conformation through nucleotide modifications is one such pathway, and modifications including pseudouridine regulate RNA fate and function in many organisms. We surveyed pseudouridine synthase (PUS) orthologs in trypanosomatids, with a particular interest in mitochondrial enzymes due to their potential importance for mitochondrial function and metabolism. *T. brucei* mt-LAF3 is an ortholog of human and yeast mitochondrial PUS enzymes, and a mitoribosome assembly factor, but structural studies differ in their conclusion as to whether it has PUS catalytic activity. Here, we generated *T. brucei* cells that are conditionally null for mt-LAF3 and showed that mt-LAF3 loss is lethal and disrupts mitochondrial membrane potential (ΔΨm). Addition of a mutant gamma-ATP synthase allele to the conditionally null cells permitted ΔΨm maintenance and cell survival, allowing us to assess primary effects on mitochondrial RNAs. As expected, these studies showed that loss of mt-LAF3 dramatically decreases levels of mitochondrial 12S and 9S rRNAs. Notably, we also observed decreases in mitochondrial mRNA levels, including differential effects on edited vs. pre-edited mRNAs, indicating that mt-LAF3 is required for mitochondrial rRNA and mRNA processing, including of edited transcripts. To assess the importance of PUS catalytic activity in mt-LAF3 we mutated a conserved aspartate that is necessary for catalysis in other PUS enzymes and showed it is not essential for cell growth, or maintenance of ΔΨm and mitochondrial RNA levels. Together, these results indicate that mt-LAF3 is required for normal expression of mitochondrial mRNAs in addition to rRNAs, but that PUS catalytic activity is not required for these functions. Instead, our work, combined with previous structural studies, suggests that *T. brucei* mt-LAF3 acts as a mitochondrial RNA-stabilizing scaffold.

Keywords

Trypanosome, mitochondria, pseudouridine, RNA modification, RNA editing