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Retrograde signaling targets under conditions of mitochondria failure

Abstract

RNaseZ is a highly conserved gene, whose mutations were linked to prostate cancer and infantile hypertrophic cardiomyopathy in humans[1,2]. In order to understand how mutations in this gene can lead to such serious conditions, the function of the protein encoded by this gene is studied using Drosophila flies as a model. When this protein is removed from mitochondria, the function of respiratory chain fails. It was demonstrated that in such cells the amount of mitochondria and mitochondrial DNA is drastically increased[6]. The goal of this study is to identify the targets of retrograde signaling activating mitochondria biosynthesis. The expression of genes impacting mtDNA replication was analyzed. I found that the *tamas* and *mtSSB* genes are upregulated in the mutant cells lacking RNaseZ from mitochondria.

Introduction

Eucaryotic cells have two different sets of tRNAs which are encoded by nuclear and mitochondrial DNA. In mitochondria, tRNAs serve as punctuation marks separating subunits of mitochondrial respiratory chain which are transcribed in a long polycistronic transcript[3]. Both populations of tRNAs go through maturation process in order to become fully functional. One of the enzymes essential for this process is RNaseZ which is a tRNA endonuclease RNaseZ cuts at the 3' end[4], which in mitochondrial transcript separates tRNA from mRNA[5] (figure 1).

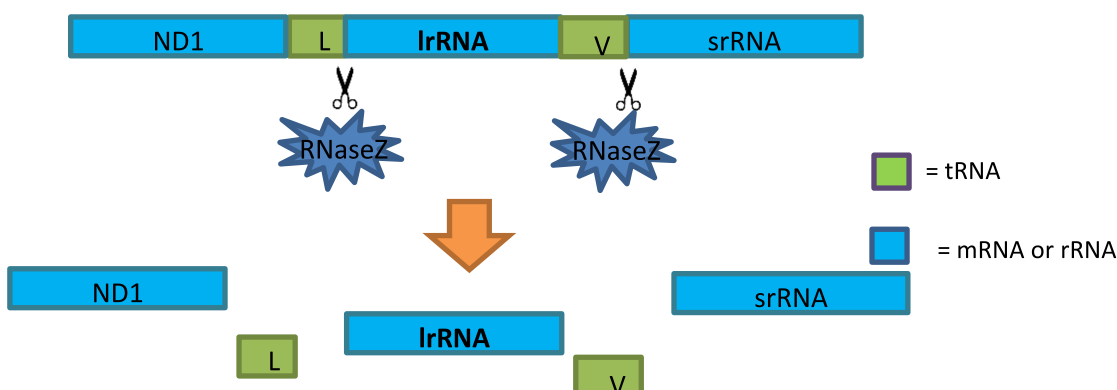


Figure 1. Model for role of RNaseZ in mitochondrial transcript processing. RNaseZ cuts 3' end of each tRNA thus separating them from mRNAs and rRNAs

Mutations of RNaseZ coding gene can lead to genetic diseases; to understand the mode of action of this protein it is studied in the Drosophila model. Removal of RNaseZ from mitochondria leads to complete mitochondrial failure due to the absence of mature subunits of respiratory chain. Interestingly in such cells the mass of mitochondria and amount of mtDNA is increased indicating possibly compensatory mechanism[6] (figure 2).

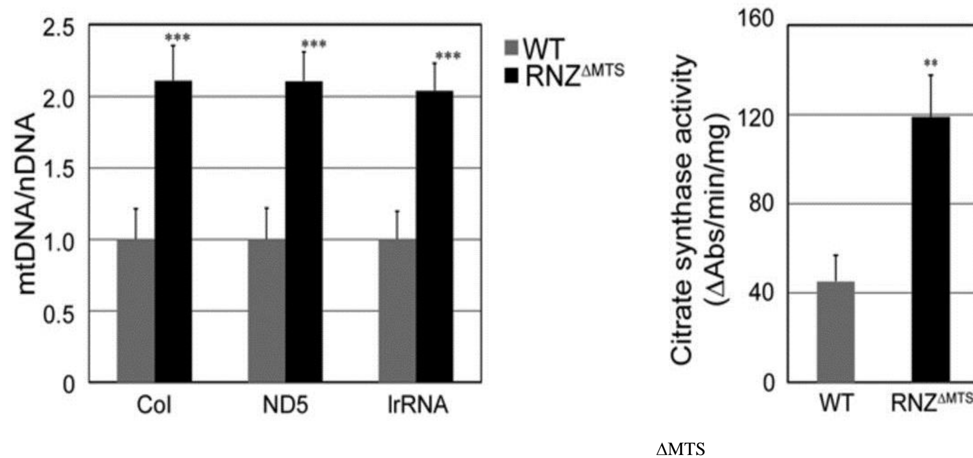


Figure 2. (a) qPCR analysis of mtDNA in WT and $Z^{\Delta MTS}$ wing discs, mtDNA values are normalized to nuclear DNA (b) citrate synthase activity, the marker for mitochondrial mass

In order to understand how the cell recognizes mitochondrial failure and proceeds to increase their amount the first step is to study which targets in nucleus are upregulated in conditions of mitochondria failure. In this study the expression of the genes which encode the mtDNA replication machinery proteins such as mitochondrial DNA polymerase γ (*tamas*), single-stranded DNA binding protein (*mtSSB*) and mitochondrial DNA helicase (*mtHel*) will be analyzed

Methods and materials

Drosophila Melanogaster flies were kindly provided by Dr. Dubrovsky's lab. Genetic construct $Z^{\Delta MTS}$ was created to exclude RNaseZ from mitochondria. Flies were raised to late 3rd instar larvae stage which for wild type is 5 days AED (After Egg Deposition) and for $Z^{\Delta MTS}$

mutant larvae 7 days AED due to delay in development. Imaginal discs were dissected out of mutant and wild type larvae of appropriate age and stored in 100 EtOH at -80°C until RNA extraction.

RNA was extracted using a QIAGEN RNeasy Plus Mini Kit following the manufacturer protocol.

Primers for PCR reaction were designed as listed below (Table 1).

RT-PCR was done with 3 different pairs of primers for each gene using the QIAGEN® One-Step RT-PCR Kit (QIAGEN®, Hilden, Germany). The conditions for thermocycler were as following: 1st hold at 50°C for 30m, 2nd hold at 95°C for 15m, 32 cycles of 94°C 30s, 58°C 30s and 72°C 30s and final extension hold for 5m.

The RT-PCR products were separated and visualized in the 2% agarose gel. The images of gel were analyzed with ImageJ for relative density of each band. RT-PCR products were purified using QIAquick® PCR Purification Kit and the purified DNA products were sequenced by Genewiz Inc.(South Plainfield, NJ, USA). The sequencing results were analyzed by BLAST(Basic Local Alignment Search Tool)(BLAST, NCBI).

tamas		length	%G/C	Tm	Sequence 3'-5'	Product length
Pair 1	Forward	24	50	60	TTCGCAGCGATTCAGAAGGAG	260
	Reverse	22	45	57	GAAGAAGCAGCAATCATCCAGT	
Pair 2	Forward	21	52	59	TGTCCCAGAAGCAACAACGTC	417
	Reverse	21	52	58	TCATCCATGTGGGTTCCATCG	
Pair 3	Forward	20	55	59	ATGACGAACTGGAGGACAGG	270
	Reverse	22	50	61	TCTGTACCACCCAATTGATGCG	
mtSSB		length	%G/C	Tm	Sequence 3'-5'	Product length
Pair 1	Forward	22	50	59	CATGCTGAATCCTCTGTTGACC	258
	Reverse	21	47	59	TTGAACACCACTACACGATGC	
Pair 2	Forward	18	50	54	TGAATCCTCTGTTGACCG	307
	Reverse	18	55.5	57	ATGGTTCGCTGTCCCTTC	
Pair 3	Forward	22	50	59	GCATGCTGAATCCTCTGTTGAC	198
	Reverse	21	52	61	AGTTGGTGTGTGTAGCAACCG	
mtHel		length	%G/C	Tm	Sequence 3'-5'	Product length

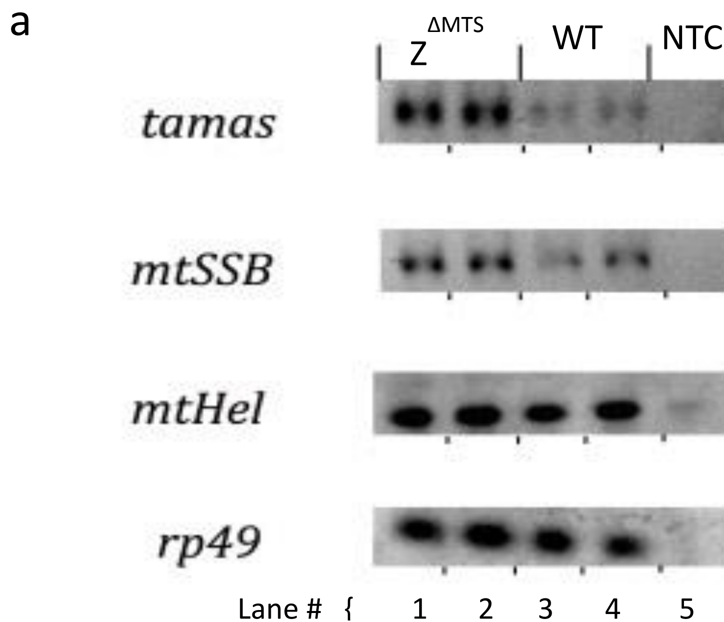
Pair 1	Forward	24	50	59	GGAGGACCAACTAAATGCCATAGG	358
	Reverse	20	55	59	ACTGGCATCGTAGTGCAACC	
Pair 2	Forward	20	55	59	TGCTGATTCACGGTGCGATG	134
	Reverse	23	48	59	TGGTAGAGTCTTCAGTTCGTAGG	
Pair 3	Forward	24	45	59	CCTTGCCACACAGAACATTGAAAC	313
	Reverse	20	50	58.5	TGCTCCGAATGTTGTGATGG	

Table 1. Primer design. Three pairs of primers were designed for each gene. Each primer pair was designed to on different exons with at least one intron in between. The product length is an expected mRNA length.

Results:

The expression of mtDNA polymerase and mtSSB but not mtDNA helicase genes are upregulated in the mitotically replicating tissues of $Z^{\Delta MTS}$ mutant larvae in comparison to wild type larvae of the same stage (figure 3a). Analysis of relative intensity of bands of genes in mutant flies compared to the wild type, allowed to calculate that the expression of *mtSSB* is almost doubled and the expression of *tamas* is upregulated four-fold in the $Z^{\Delta MTS}$ mutant larvae compared to the wild type (figure 3b)

a



b

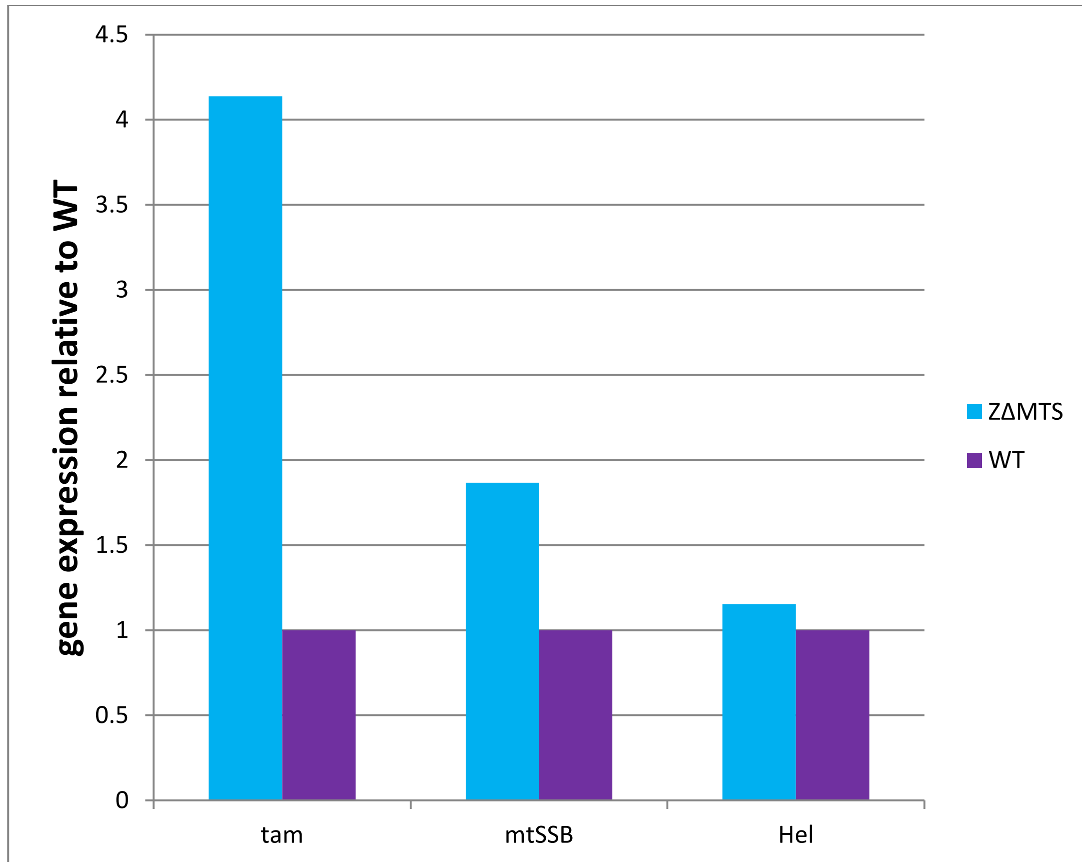


Figure 3. $Z^{\Delta MTS}$ = mutant phenotype, WT = wild type, NTC = no-template control. (a) RT-PCR results for the studied gene products. *rp49* is a housekeeping gene. (b) Results of analysis of relative band intensity.

Discussion

It has been demonstrated in *D. melanogaster* that mitochondria fail to function when RNaseZ is modified not to go inside of mitochondria[7]. Because of the mitochondria failure the cells are arrested in cell cycle and tissue growth is significantly slowed down in the mutant flies. However there is no evidence that such cells go through cell death, rather a compensatory mechanism is turned on and production of mitochondria is increased resulting in increased mitochondrial mass and amount of mtDNA per cell[7] (figure 1). To understand how mitochondria signals to cell about its damage the retrograde signaling nuclear targets were studied to see which

genes are expressed at higher level compared to the wild type flies. According to the results shown, the expression of mtDNA polymerase and single-stranded DNA binding protein is upregulated in RNZ$^{\Delta MTS}$ mutant larvae which provides an explanation to the fact that amount of mtDNA is increased. The figure 4 demonstrates a proposed model of retrograde signaling under the conditions of mitochondria failure.

There is no observable difference in expression of mtDNA helicase and a possible explanation is that the mtHEL molecule might have a longer half-life or higher processivity than the mtDNA polymerase.

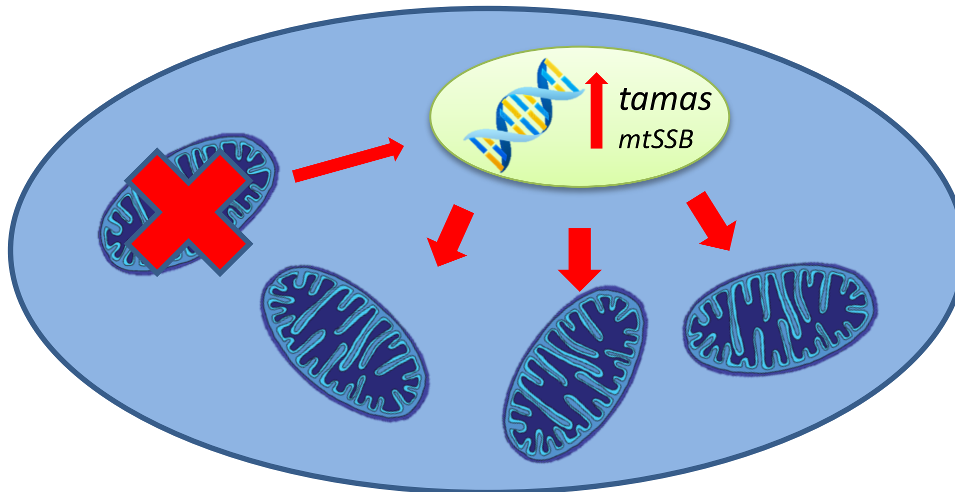


Figure 4. Model for the mechanism, which enables the increase of mitochondrial mass and amount of mtDNA under conditions of mitochondria failure. When mitochondrial function fails due to lack of RNaseZ, *tamas* and *mtSSB* genes are upregulated thus enabling increased mitochondrial DNA replication

References

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