The optimization of a genetic screen to identify genes involved in paternal mitochondrial DNA elimination in *C. elegans*

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Abstract

Caenorhabditis elegans were used to study the genes responsible for paternal mitochondrial DNA (mtDNA) degradation. A genetic screen was conducted using hermaphrodites containing a nuclear mutation that causes larval arrest on restrictive conditions, crossed with males containing a mitochondrial mutation which rescues that larval arrest. Parents of the males were exposed to a mutagen, with the goal of inducing paternal mtDNA transmission, and thus providing rescue of the larval arrest. After optimizing this genetic screen we are poised to identify genes involved in paternal mtDNA degradation.

Introduction

Mitochondria are energy-producing organelles which contain their own genomes called mitochondrial DNA (mtDNA). Each cell contains many mtDNA copies, which in most species are maternally inherited. The presence of multiple mtDNA haplotypes in one cell is defined as heteroplasmy (**Figure 1**). In *C. elegans*, heteroplasmy is correlated with increased embryonic lethality, suggesting a cost to heteroplasmy. Additionally, studies in *C. elegans* have identified factors involved in paternal mtDNA elimination in embryos, including LGG-1, which is involved in ubiquitination and autophagy of sperm mitochondria-related organelles, and CPS-6 (endonuclease G), which degrades paternal mtDNA upon fertilization (**Figure 2**).

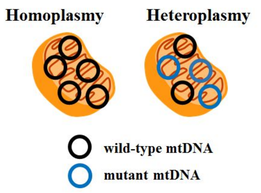

Figure 1. Homoplasmy (left) in which all copies of mtDNA have the same haplotype and heteroplasmy (right) in which copies of mtDNA exhibit different haplotypes are depicted.

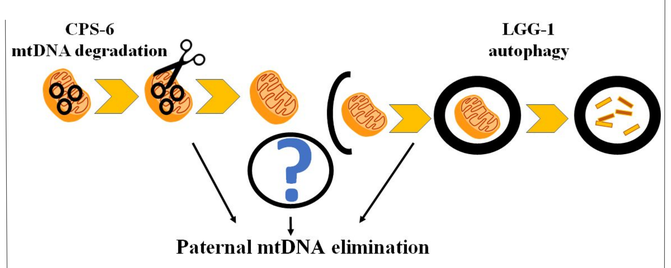

Figure 2. Previous studies have demonstrated the roles of LGG-1 and CPS-6 in paternal mtDNA elimination; we sought to identify other factors.

The goal of this experiment was to use a forward genetic screen to identify genes in the *C. elegans* genome involved in paternal mtDNA elimination (**Figure 2**). We utilized mutations in the *isp-1* and *ctb-1* genes with *vhp-1* RNAi to track mtDNA transmission. *isp-1(qm150)* (nuclear) slows growth, *vhp-1* (nuclear) RNAi knocks down a stress response pathway and causes larval arrest when combined with *isp-1(qm150)*. *ctb-1(qm189)* (mitochondrial) partially restores wild-type activity of *isp-1(qm150)* to rescue larval arrest and cause persistence through the L4 stage. Males with a random induced nuclear mutation and *ctb-1(qm189)* were crossed with non-mutagenized hermaphrodites wild-type for *ctb-1;* the presence of persisting animals suggested paternal transmission of the mitochondrial DNA mutation *ctb-1(qm189)* caused by the newly introduced mutation (**Table 1**). Understanding paternal mtDNA elimination and transmission will provide us with tools to study heteroplasmy more effectively in the laboratory and will pave the way for future research and therapies for mitochondrial diseases.

	MRP83 *isp-1* *(qm150)*	MRP84 *isp-1(qm150);* *ctb-1(qm189)*
vhp-1 RNAi	L3 arrest (no rescue, arrestors)	L4+ (rescue, persistors)
Empty Plasmid	L4+	L4+

Table 1. Conditions under which worms will arrest at L3 or develop into L4s.

Methods

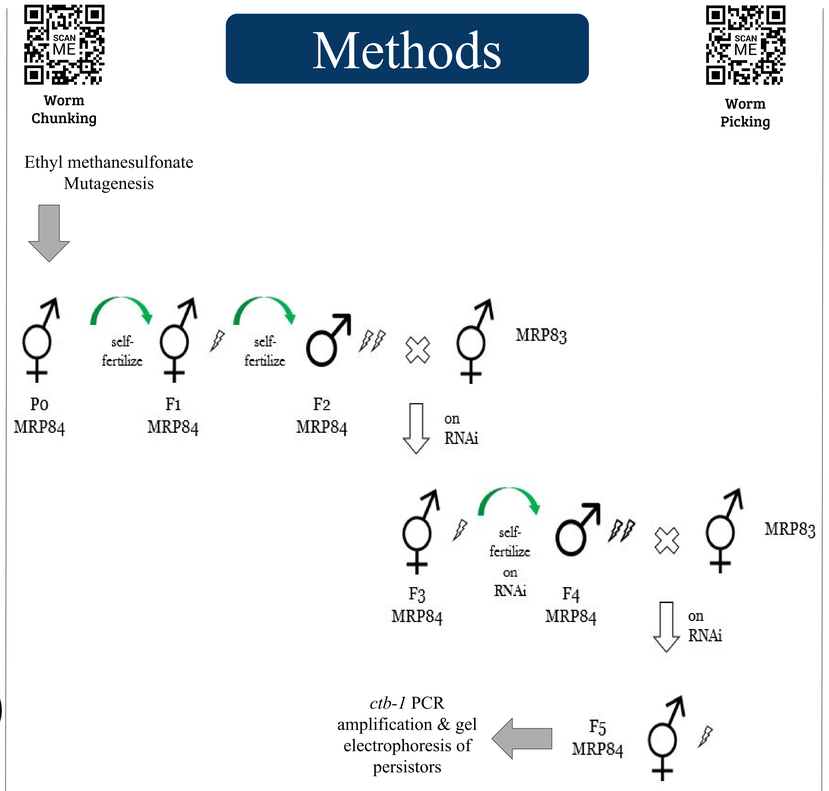

Figure 3 Schematic of crosses performed. The lightning bolts represent a mutation induced by EMS (one bolt represents heterozygosity for the mutation, two bolts respresent homozygosity). MRP83 animals contain the mutant *isp-1(qm150)* allele. MRP84 animals contain the mutant *isp-1(qm150);ctb-1(qm189)* alleles.

Results

Two trials of the crosses were carried out. For trial one, a total of 360 haploid genomes were screened, and 23 persisted past the L3 developmental stage. In trial two, a total of 522 hapolid genomes were screened, and 28 offspring persisted past the L3 stage. PCR was performed on DNA isolated from MRP83, MRP84, arresting, and persisting worms, but the results were inconclusive (**Figure 3)**.

Trial	# of haploid genomes	# of persistors
1	360	23
2	522	28

Table 2. Number of haploid genomes screened and number of persisting offspring from each trial.

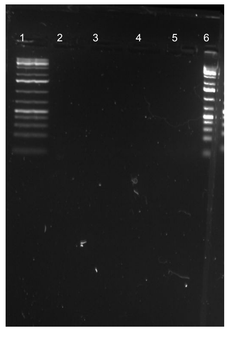

Figure 3. Gel electrophoresis of MRP83 control PCR. Wells 1 and 6 contain reference ladders. Wells 2-5 contain the failed *ctb-1* PCR as indicated by lack of bands.

Discussion

The high rate of persistors in trial 1 likely resulted from carry-over MRP84 animals or failed *vhp-1* knockdown. Thus, in subsequent trials, extra efforts were taken to reduce the number of small animals and embryos on target plates. Positive and negative controls were also established to ensure the efficacy of the RNAi. The lower rate of persistors in trial 2 suggests true paternal mtDNA transmission, and thus warrants further exploration. Given more time, we would have optimized our PCR and subsequently used Sanger sequencing on a portion of the *ctb-1* gene to identify whether our persistors were "true" or "false" rescues. A sample chromatogram showing a portion of the *ctb-1* gene shown below suggests heteroplasmy (**Figure 4**), as evidenced by the two peaks at base position 308. If these data were real, taken together, they could suggest that persisting animals in subsequent trials displayed interrupted degradation of paternal mtDNA. We successfully optimized a screen to be used by future students to identify genes involved in paternal mtDNA degradation.

Figure 4. Theoretical chromatogram of a heteroplasmic worm, shown by two peaks (green and black) at base position 308.

Future Directions

In the future, steps should be taken to improve the efficacy of the experimental methods. Additionally, future screens could include mutations or knockouts of *lgg-1* and *cps-6*, two known paternal mtDNA elimination factors, to increase the likelihood of achieving paternal mtDNA transmission in *C. elegans*.

Acknowledgements

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