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Identifying Genes Associated with Elevated Rates of Mitochondrial DNA Instability in a Yeast Model System

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Identifying Alleles Associated with Elevated Rates of Mitochondrial DNA Instability in *Saccharomyces cerevisiae*

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Abstract

Problem: Mitonuclear interactions have been shown to influence the maintenance of mtDNAs, which directly affects the efficacy of mitochondrial function. mtDNA loss is involved in many diseases, including some cancers. Despite their importance to organismal health, identifying mitonuclear interactions proves very challenging, and few successful examples exist.

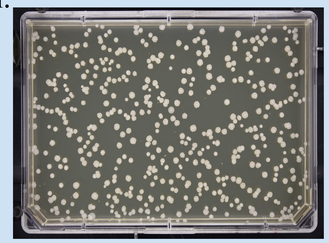
Hypothesis: Naturally occurring genetic variation in yeast populations will alter the inheritance rate of stable mtDNAs.

Approach:

- Use *Saccharomyces cerevisiae* as a genetic model to identify mitonuclear interactions via petite assays in strains from a previously created mapping population.
- Employ GWAS to detect mitonuclear interactions that contribute to mtDNA instability.
- Validate GWAS information through expression analysis of significant genes.

Mitonuclear Interactions Contribute to mtDNA Instability

1.



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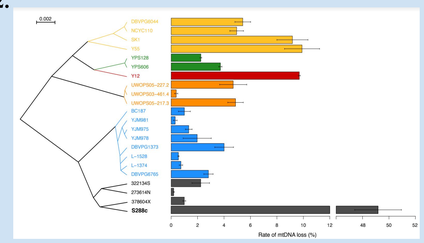

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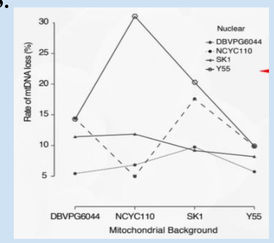
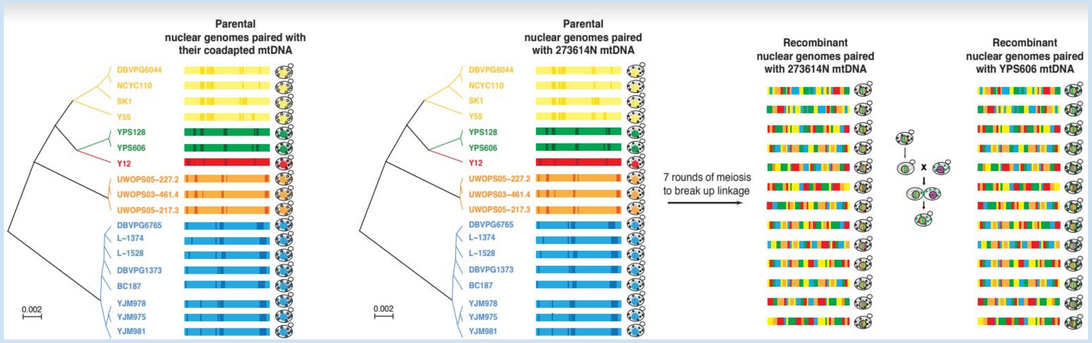


Figure 1: Frequency of petite colony formation was used as a proxy for mtDNA loss.

Figure 2: mtDNA stability differs across yeast isolates

Figure 3: mtDNA stability influenced by mitonuclear interactions

Mitonuclear Mapping Population

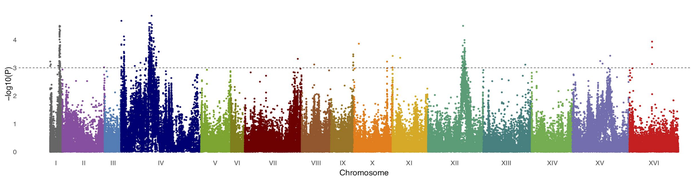


- About 200 recombinant strains containing 2 different mtDNAs were sequenced.
- 180,122 SNPs were identified in the Recombinant collection, allowing for GWAS approaches to map mitonuclear alleles.

Association Test for Mitonuclear Alleles

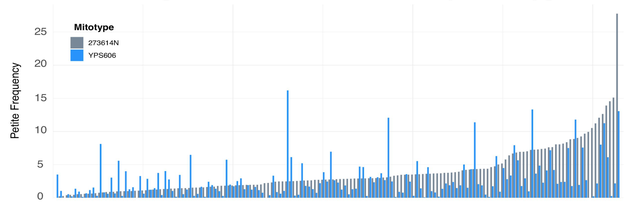
Petite frequencies were determined for each strain in the Mitonuclear Mapping Population

MitoNuclear Alleles



- **298 SNPs were linked with significant changes in petite frequency between mitotypes.**
- **The SNPs identified were located within 75 different genes.**
- **GWAS Model: Petite Frequency ~ SNP + mtDNA +SNP * mtDNA**

Petite Frequencies of Each Strain by Mitotype



- **Epistatic interactions between nuclear and mitochondrial DNA leads to discrepancies in petite frequency.**
- **These interactions vary by mitotype.**

Significant Genes Tended to Function in Protein Quality Control and Cell-Cycle Regulation

Gene	Function	Petite Frequency
DOA4	Required to recycle ubiquitin from proteasome-bound ubiquitinated intermediates.	29.3 %
VMS1	Peptidyl-tRNA hydrolase that is required for releasing stalled peptides from ribosomes.	25.0 %
ZUO1	Ribosome-associated chaperone involved in ribosome biogenesis. Localizes to the mitochondria.	100 %
WHI4	RNA-binding protein that regulates cell size requirement throughout cell division.	37.7 %
YAT1	Outer mitochondrial carnitine acetyltransferase; transports activated acyl groups into the matrix.	15.5 %
PHO13	A conserved phosphatase acting as a metabolite repair enzyme.	20.9 %
COQ11	Subunit of Coenzyme Q biosynthetic complexes.	29.5 %
CTS1	Endochitinase required for cell separation after mitosis.	3.3 %
AIM7	Stimulates actin filament debranching and inhibits actin nucleation.	27.9 %
PAU7	Function unknown. Active during alcoholic fermentation. Inhibited by oxygen.	17.7 %

Conclusions

- Mitotype, and the associated epistatic interactions, had a significant impact on petite frequency. This is a strong indicator that mitonuclear interactions have been detected.
- Alleles that function in protein quality control and cell cycle regulation were the most significant. We suspect this relates to how stable mtDNA is transmitted to daughter cells.

Future Studies

- Verify that the identified genes contribute to mtDNA stability through gene KO and allele swapping.
- Monitor and compare gene expression in strains that perform differently between mitotypes to elucidate the pathways that these mitonuclear interactions act upon.

Acknowledgments

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