Cold Adaptation & Mitochondrial Function in *S. cerevisiae*

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Is mitochondrial function important for survival in cold temperatures?

Experimental Design

Make Replica Plates of 307 Putative CS Mutants

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Screen on media requiring oxidative phosphorylation

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30°C | 37°C | 26°C | 10°C

Replica Plating: Velvet Stamp Technique

- Replica plates were produced by velvet stamping.
- Stamped by order of assigned temperature: 10°C, 26°C, 30°C, and 37°C.
- It is important to start stamping by lowest temperature first to ensure plates assigned to least favorable conditions contain maximum number of cells.

Temp.	Control (YPD)	Experiment (YPEG)
10°C	1	2
26°C	1	2
30°C	0	2
37°C	0	2

Data Acquisition

Each temperature treatment was photographed for two successive days once sufficient growth was observed.

- Day 1: Plates growing at 37°C plates were photographed
- Day 2: Both YPD and YPEG plates growing at 26°C and 30°C were photographed
- Day 13: Both YPD and YPEG plates grown at 10°C were photographed

Data Analysis

- The presence of growth on YPEG denoted that the deletion did not impede cellular respiration or other factors of cold adaptation.
- The absence of growth on YPEG denoted the deleted gene coded for a vital component in the mitochondria that also impacted cold sensitivity.

Expected Results

- All mutants should have reduced or no growth at 10°C on YPD
- Mitochondrial mutants should have more drastic phenotype on YPEG than on YPD at 10°C

Results

6000 Genes

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307 Putative CS Mutants

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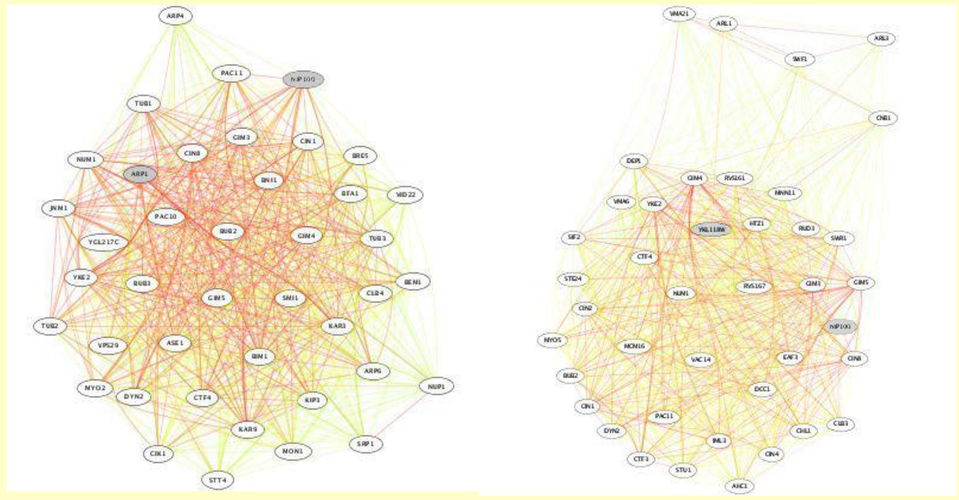
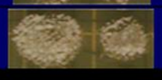
269 not CS | 24 CS on both YPD & YPEG | 14 CS only on YPEG

Identified 14 conditional mutants. These mutants utilize mitochondria in cold temperatures only



Gene Association Chart

ARP1 and NIP100 strongly interact with each other.
NIP100 and YKL118W have unknown relationship.

Gene	Control (YPD)	Experiment (YPEG)	Known Function
Nuclear Function			
SAC3			Pore-associated protein, forms complex with Thp1p, which is involved in transcription & mRNA export from nucleus
NUP188			Nuclear pore complex (NPC) subunit, involved in structural organization and permeability of envelope
MAC1			Transcription factor, induces genes required for reduction & utilization of Fe and Cu[1]
SNU66			Nuclear RNA splicing
IES6			Protein that associates with INO80 chromatin remodeling complex under low-salt conditions
ARP1			Arp1-actin short filament related to dynactin complex via linking cytoplasmic dynein to its cargo through p150(glued)[2]; nuclear import
NIP100			mitotic spindle positioning protein in dynactin complex involved in partitioning mitotic spindle between mother & daughter cells[1]; nuclear import
Vacuolar Function			
PEP3			Protein involved in vacuolar protein sorting[1]
VPH2			Vacuolar ATPase assembly protein that acts in ER[1]
Cytosolic Function			
SER1			3-phosphoserine aminotransferase, catalyzes formation of phosphoserine from 3-phosphohydroxypyruvate, needed for Ser & Gly biosynthesis
RMD8			Cytosolic protein required for sporulation
Mitochondrial Function			
YME1			Degrades improperly folded proteins in intermembrane space[1]
Unknown Function			
BUD28			Unlikely to encode protein
YKL118W			Overlaps with both NIP100 and VPH2[1]

Conclusion

Of the fourteen genes found to be cold sensitive on YPEG conditions, only one is directly related to mitochondrial function. The results suggest:

- the reduction of growth in YME1 is due to the dysfunctional mitochondria
- overall, reduced cell growth in the mutants was a result of errors in cell transportation or protein processing and maintenance
- genes other than YME1 may code for proteins that contribute to processes indirectly related to mitochondrial processes

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References:

[1]Dimmer KS, Fritz S, Fuchs F, Messerschmitt M, Weinbach N, Neupert W, Westermann B. Genetic basis of mitochondrial function and morphology in Saccharomyces cerevisiae. *Mol Biol Cell.* 2002;13:847–53. doi: 10.1091/mbc.01-12-0588.

[2]Hirokawa N. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science.* 1998;279:519–526. doi: 10.1126/science.279.5350.519.