Is mitochondrial function important for survival in cold temperatures?

Experimental Design

- Make Replica Plates of 307 Putative CS Mutants
- Screen on media requiring oxidative phosphorylation
- 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.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Control (YPD)</th>
<th>Experiment (YPEG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>26°C</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>30°C</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>37°C</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

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

- Identified 14 conditional mutants. These mutants utilize mitochondria in cold temperatures only.
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Gene Association Chart
- ARP1 and NIP100 strongly interact with each other.
- NIP100 and YKL118W have unknown relationship.

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: