05

Disruption in vacuolar pH perturbs mitochondrial copper homeostasis

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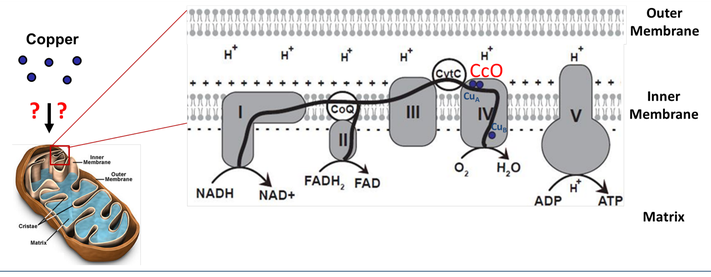
Abstract

Copper is an essential cofactor for cytochrome *c* oxidase (CcO), the terminal enzyme of the mitochondrial respiratory chain. Loss-of-function mutations in genes required for copper transport to CcO results in fatal human disorders. Despite the fundamental importance of copper in cellular and organismal health, intracellular copper trafficking and distribution are not well understood. To identify the genes involved in copper trafficking to mitochondria, we performed a genome-wide copper-sensitized screen using yeast deletion mutants. This screen identified many genes required for vacuolar biogenesis as putative regulators of mitochondrial copper homeostasis. The yeast vacuole is the major storage site for metals including copper, but exactly how vacuolar function impacts mitochondrial metal homeostasis is not well understood. Investigations of the "hits" identified in our screen revealed that yeast mutants defective in maintaining vacuolar acidity exhibit reduced mitochondrial copper with a concomitant decrease in CcO activity. Respiratory growth and CcO activity could be rescued by either restoring vacuolar pH or by supplementing growth media with copper. Consistent with this genetic data, pharmacologically inhibiting vacuolar proton pump leads to a decrease in mitochondrial copper and CcO activity. Collectively, our work identifies a number of novel genetic regulators of mitochondrial copper homeostasis and links vacuolar pH to mitochondrial energy metabolism via copper transport.

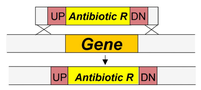
Mitochondria store copper for metallating cytochrome *c* oxidase



Genome wide screen identifies genes involved in vacuole biogenesis as regulators of mitochondrial copper homeostasis

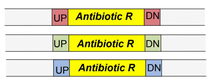
A

1. Yeast deletion collection ~5,000 different gene KO's

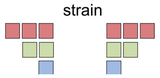


2. Grow deletion collection to an optical density of 5.0

3. Harvest cells and isolate genomic DNA



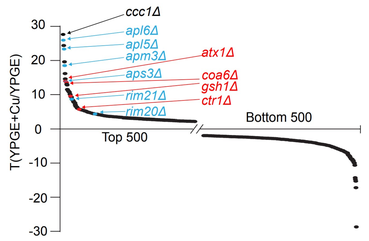
4. PCR amplify tags for each strain



5. Sequence DNA Barcodes

6. Statistical Analysis

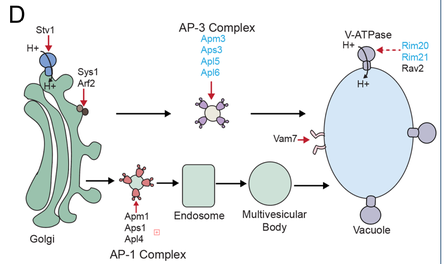
B



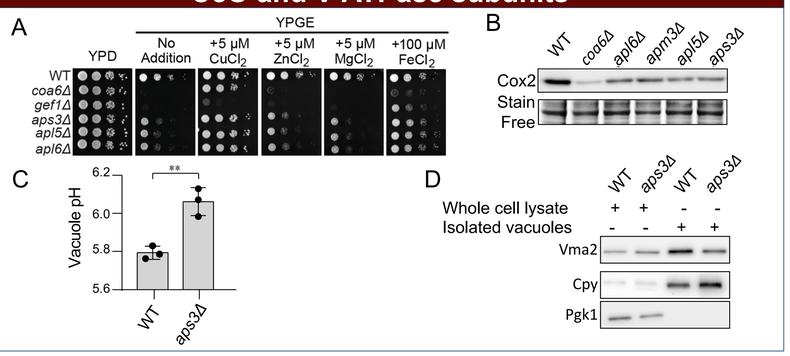
C

Process	P-Value	Q-Value
Golgi to vacuole transport	1.54E-6	7.95E-3
Post-Golgi vesicle-mediated transport targeting to vacuole	4.53E-6	1.17E-2
Transition metal ion homeostasis	1.76E-5	3.02E-2
Chemical homeostasis	1.77E-5	2.29E-2
Cation homeostasis	3.18E-5	3.29E-2

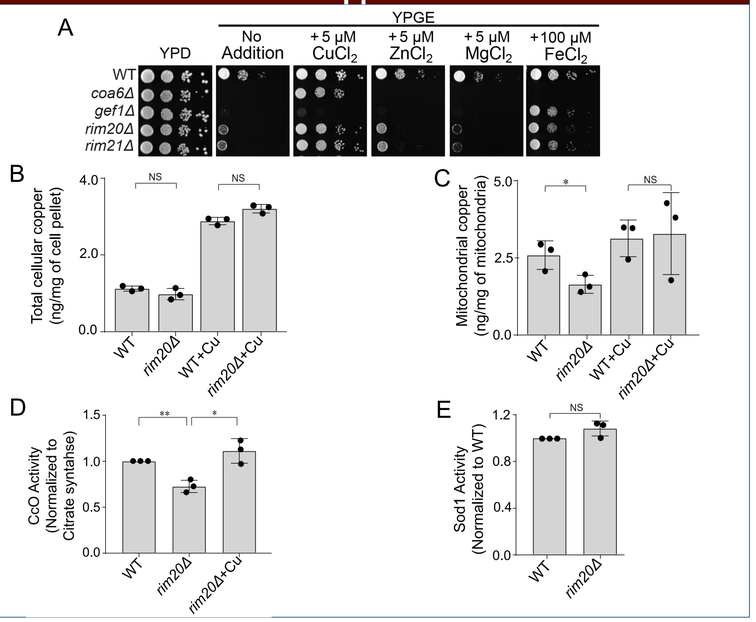
D



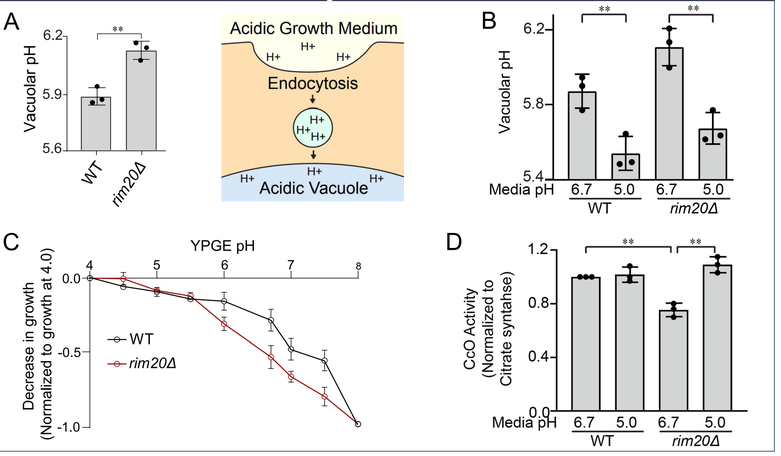
AP-3 mutants exhibit reduced abundance of CcO and V-ATPase subunits



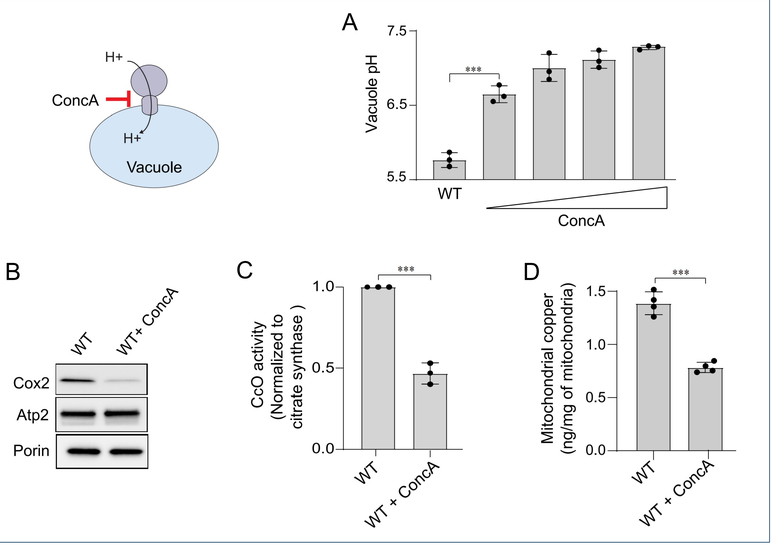
Genetic defects in Rim101 pathway perturbs mitochondrial copper homeostasis



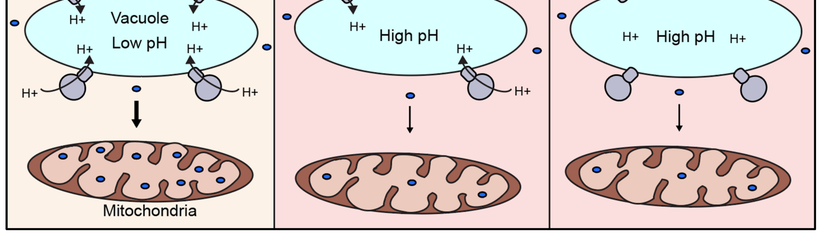
Respiratory defect in *rim20Δ* is rescued by restoring the acidity of vacuole



Pharmacological inhibition of the V-ATPase results in decreased mitochondrial copper



Proposed model



Conclusions

1. Yeast mutants defective in maintaining vacuolar pH display reduced respiratory growth, which is restored by copper supplementation.
2. AP-3 is required for the proper localization of the V-ATPase to the vacuolar membrane.
3. AP-3 mutants have less acidic vacuoles and exhibit reduced respiratory growth.
4. Rim20 is required for maintaining copper transport to the mitochondria.
5. Mitochondrial copper homeostasis is dependent on vacuolar pH.

Acknowledgements

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