Mechanism of Miltefosine Resistance in *Saccharomyces cerevisiae* Mutants Sac1 and Stt4

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Background

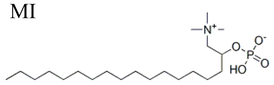
- *Saccharomyces cerevisiae* (baker's yeast) is a eukaryotic organism with genes orthologous to humans and thus an effective model organism to study how eukaryotic plasma membranes interact with phospholipids.
- Sac1 was identified from the MAT-alpha knockout strain collection, while Stt4 was identified from the Essential knockout gene collection.
- In these strain collections one gene replaced with a drug resistance marker allowed us to correlate gene disruption with a growth phenotype under a particular condition.
- Doxycycline (Dox) is a tetracycline antibiotic. Here we use Dox as a way to reduce expression of Stt4[1].
- Miltefosine (Mil) is an alkylphospholipid and analog to Phosphatidylcholine (PC) that induces apoptosis in some cells by collecting on intracellular membranes[2].
- TopFluor Lyso-PC (TF-PC) is a fluorescent phospholipid analog that can be used to visualize lyso-lipid trafficking[3].

Research Questions

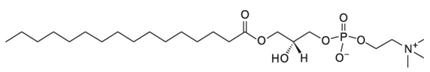
- Where is Miltefosine depositing within the cell?
- How is Miltefosine getting to specific intracellular membranes?

Miltefosine and TopFluor LysoPC Structures

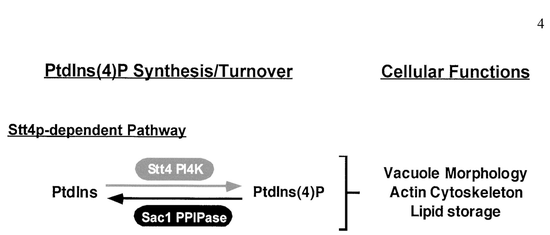
MI



TF-PC



PI4P Synthesis/ Turnover



Dilution Screen Mil Resistant *S. cerevisiae* Strains Sac1 and Stt4

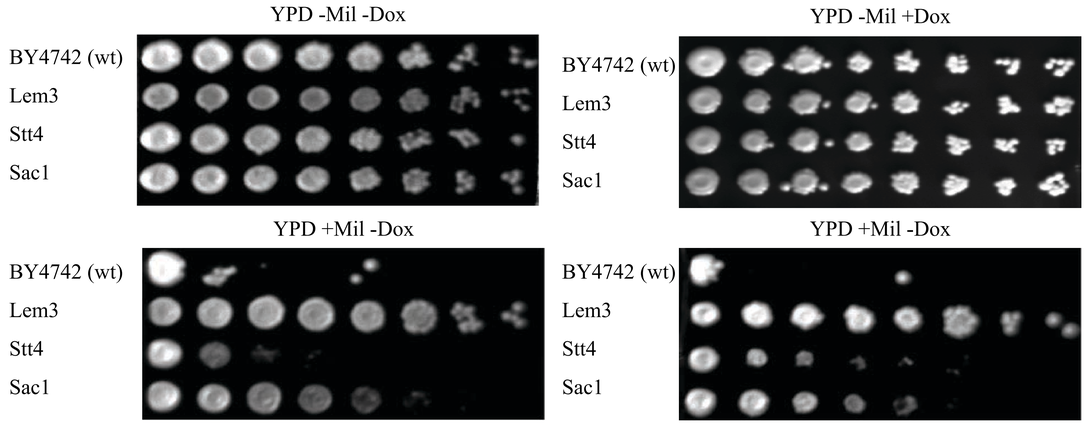


Figure 1. Plates were grow at 30°C for four days. Pictures were taken on the fourth day using a UVP Epi Chemi II Darkroom Camera with LabWorks as the developing software.

Fluorescence Microscopy of Identified Mil Resistant Strains Sac1 and Stt4

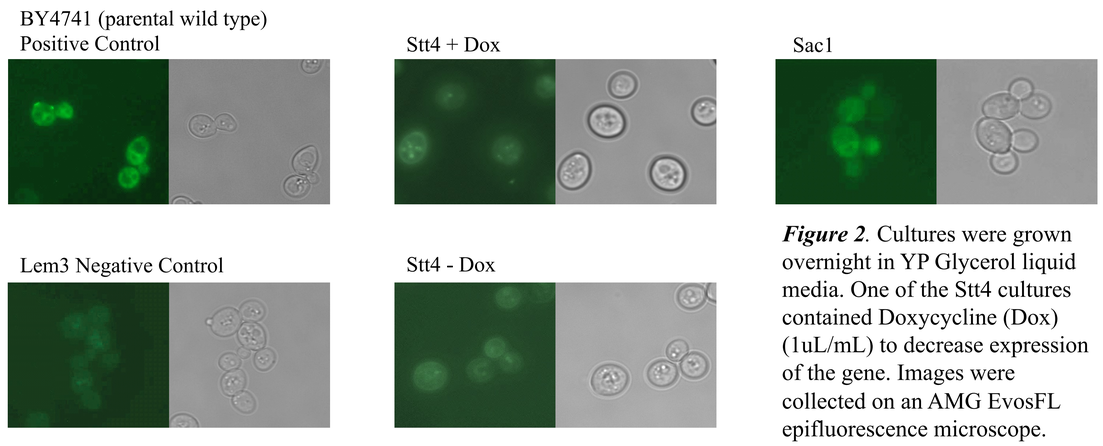
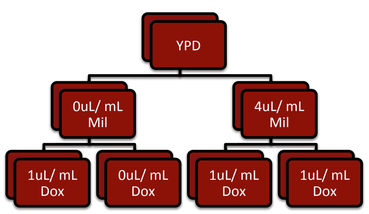


Figure 2. Cultures were grown overnight in YP Glycerol liquid media. One of the Stt4 cultures contained Doxycycline (Dox) (1uL/mL) to decrease expression of the gene. Images were collected on an AMG EvosFL epifluorescence microscope.

Methodology

Miltefosine Dilution Screen Media Conditions



Fluorescent Microscopy

- YP Glycerol liquid cultures grown for 3 days
- PBS Tergitol wash x2
- SC Glycerol resuspension
- Top- Fluor lysoPC (TF-PC) incubation for 15 min
- PBS wash of unincorporated label
- Distilled water resuspension

Key Findings

- Deletion in Sac1 or under expression in the essential gene Stt4 causes Mil resistance
- TF-PC found in vesicles in Stt4 + Dox
- TopFluor lyso-PC imaging reveals that deletion in Sac1 and under expression of Stt4 alter localization of lyso-lipids once inside the cell.
- Sac1 and Stt4 mutants tend to have a larger concentration of TF-PC around the vacuole and the plasma membrane respectively.
- These results indicate different gene disruptions confer Mil resistance by different molecular mechanisms.

Implications

- Confirmed that a disruption in these genes leads to Mil resistance
- Key implications in improvement and development of more effective chemotherapy drugs

Future Directions

- Transformations for both Sac1 and Stt4 mutants will be conducted.
- Bioinformatic approaches will be used to generate hypotheses about the function and localization of each protein identified in the screen.
- TF-PC microscopy of these strains will be conducted under + or – Mil conditions to see if TF-PC transport is affected.
- Imaging will be conducted on cultures grown with different sources of carbon to see the effect of mitochondria on lyso-lipid transport.

Acknowledgements

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Works Cited

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