Effects of Oxidative Stress on Genomic Stability in *Candida albicans*

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Large genome changes in *Candida albicans*

- *C. albicans* is a prevalent fungal pathogen.
- *C. albicans* has a highly plastic genome, and a wide variety of large genome changes occur.
- Large genome changes include polyploidy, copy number variation (CNV) and loss of heterozygosity (LOH).
- Genome instability is important for adaptation to new environments or stressors, such as oxidative stress.

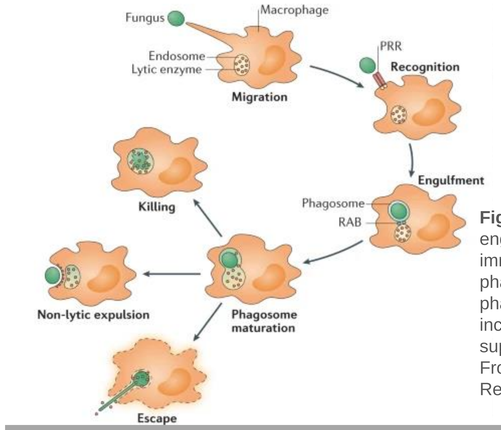


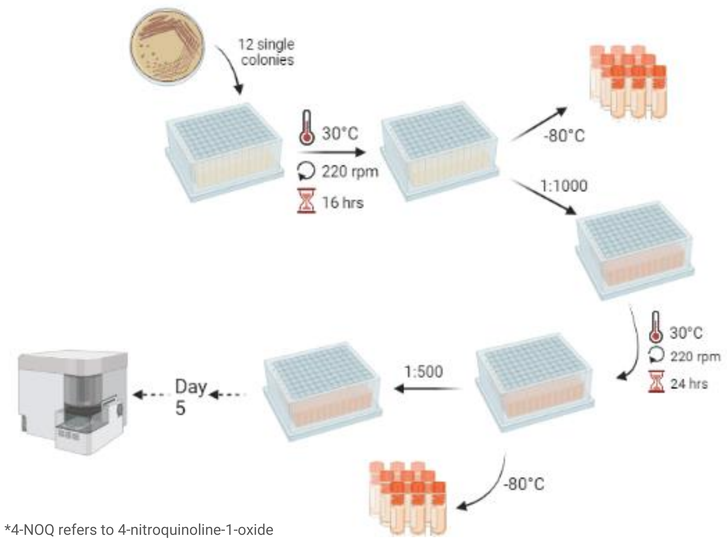
Fig. 1: Fungal pathogens are engulfed by cells of the innate immune system during phagocytosis. The subsequent phagosome is flooded with ROS including hydrogen peroxide and superoxide.
From Erwig & Gow, 2016 Nature Reviews Microbiology

Hypothesis

Exposure to oxidative stressors will result in increased rates of large genome changes and this genomic instability will provide a pathway to adaptation to oxidative stress and antifungal drug resistance.

Evolution of *C. albicans* in oxidative stress to measure genome stability

- *In vitro* evolution experiments with oxidative stress provide isolates that can be further analyzed using flow cytometry to determine genome stability.
- Treatment conditions include YPAD (control); 5 mM H_2O_2; 10 mM H_2O_2; 0.125 µg/mL 4-NOQ; 0.25 µg/mL 4-NOQ.



*4-NOQ refers to 4-nitroquinoline-1-oxide

Flow cytometry system for detection of genome stability

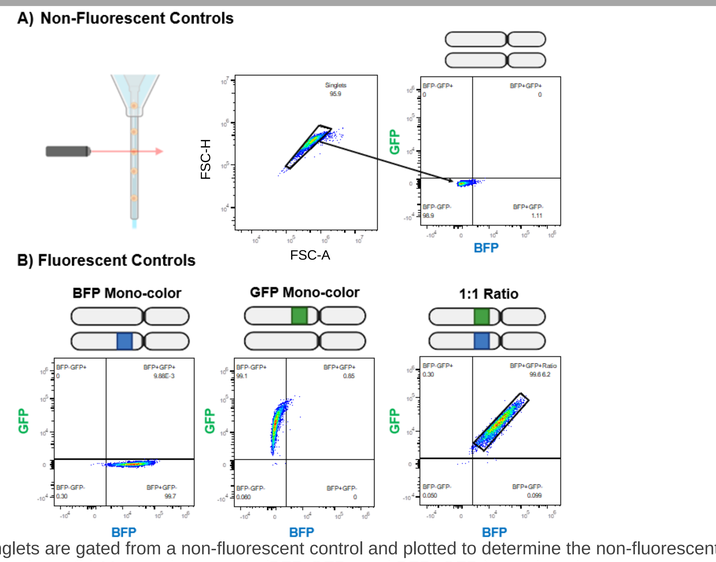


Fig. 2: (a) Singlets are gated from a non-fluorescent control and plotted to determine the non-fluorescent (BFP-GFP-) quadrant; **(b)** Mono-color controls (BFP-GFP+ and BFP+GFP-) are plotted to ensure correct positioning of quadrants. Heterozygous progenitor strains are also plotted and gated for 1:1 ratio cells.

Heterogeneity of populations identified by rapid screening of single colonies

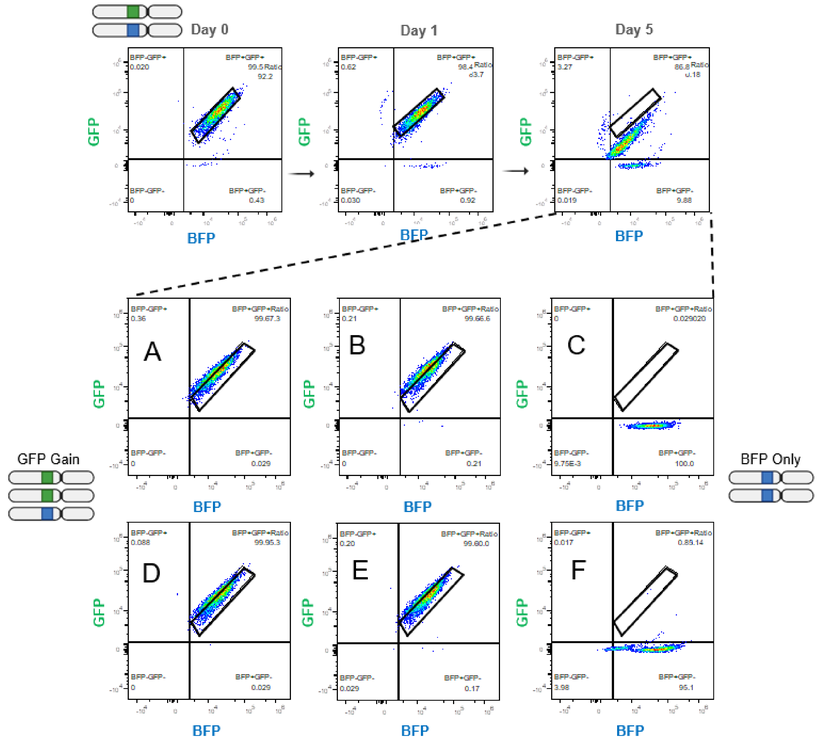


Fig. 3: During adaptation to 4-NOQ, isolate F4 exhibits LOH subpopulations after 24 hours. By Day 5, the population shows LOH and BFP CNV, demonstrating genome instability. This isolate was plated for single colonies, 2 of which are BFP LOH. The other 4 single colonies show GFP CNV. The increase in GFP fluorescence of these single colonies relative to the 1:1 ratio control is shown in the GFP histogram.

Rates and dynamics of populations after exposure to oxidative stress

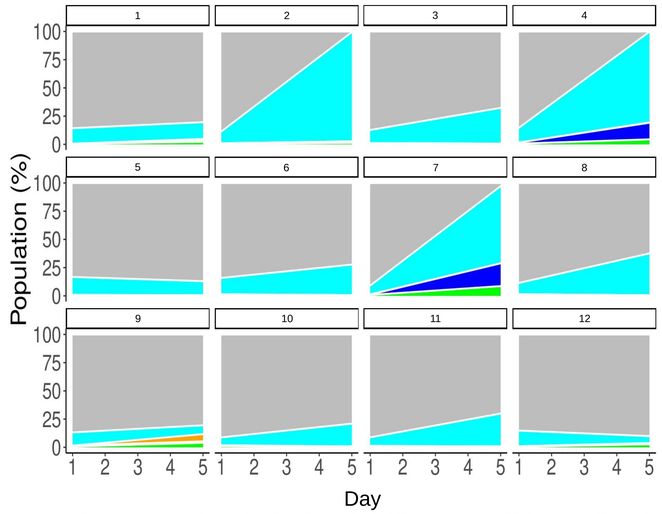
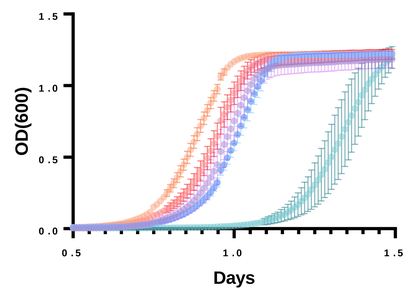


Fig. 4: Chromosome 3 isolates treated with 0.125 µg/mL 4-NOQ exhibit CNV BFP after 24 hours, with the frequency of this large genome change increasing in most isolates over 5 days.

Phenotypic analysis of single colonies to determine adaptation to oxidative stress

A) Oxidative Stress



B) Antifungal Drug

	Resistance	Tolerance
Chr5 1:1	0.5 µg/mL	0.14
Chr3 1:1	0.5 µg/mL	0.08
Chr3 2:2	0.5 µg/mL	0.20
Chr5 3:0	0.5 µg/mL	0.52
Chr5 1:0	0.5 µg/mL	0.20

Fig. 5: A) Single colonies from isolates treated with 4-NOQ demonstrate a range of adaptation to oxidative stress; B) Single colonies from isolates treated with 4-NOQ exhibit increased tolerance to antifungal drugs.

Conclusions

- Flow cytometry can be used to measure the rates and dynamics of mutations acquired during adaptation to oxidative stress
- Oxidative stress results in different phenotypes including increased tolerance to antifungal drugs

Acknowledgements

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References

Erwig, L., Gow, N. Interactions of fungal pathogens with phagocytes. *Nat Rev Microbiol* 14, 163–176 (2016). https://doi.org/10.1038/nrmicro.2015.21

Loll-Krippleber R, Feri A, Nguyen M, et al. A FACS-Optimized Screen Identifies Regulators of Genome Stability in Candida albicans. Eukaryot Cell. 2015;14(3):311-322. doi:10.1128/EC.00286-14

Dantas A, Day A, Ikeh M, Kos I, Achan B, Quinn J. Oxidative stress responses in the human fungal pathogen, Candida albicans. Biomolecules. 2015;5(1):142-165. doi:10.3390/biom5010142