Investigating the genetic architecture of a Hsp90-dependent trait in the budding yeast *Saccharomyces cerevisiae*

Selena Ding[1], Hatim Amiji[1], Dipak Patel[1], Georgios I. Karras, PhD[1,2]
[1]Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.
[2]Genetics and Epigenetics Graduate Program, MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Introduction

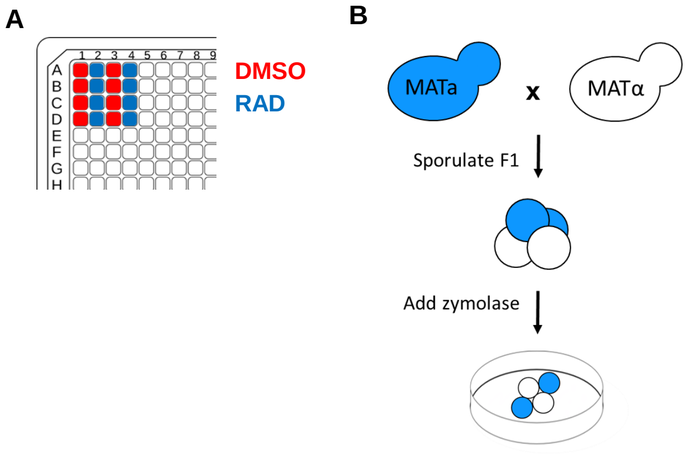
The heat-shock protein 90 (Hsp90) evolved to help cells maintain protein homeostasis under proteotoxic stress conditions. Yet, Hsp90 has important biological roles under basal conditions. In cancer cells, Hsp90 helps stabilize diverse oncogenic proteins, while in normal cells Hsp90 can buffer the effects of disease mutations. However, how traits become addicted to Hsp90 function remains unknown. To address this question in a simple system, we focused on the role of Hsp90 in palatinose utilization in the budding yeast Saccharomyces cerevisiae. Palatinose is a naturally occurring disaccharide in plants and a rich carbon source for diverse yeasts. Its utilization provides a simple model for studying Hsp90 dependent traits.

Methods

Growth curves
Yeast strains of diverse backgrounds were grown in 384 well plates and optical density (600 nm) was measured every 15 minutes for 37 hours.

Tetrad dissection
We crossed yeast strains harboring various degrees of robustness to Hsp90 inhibition to strains that were less robust or were unable to utilize palatinose. We then performed bulk sporulations and tetrad dissections to isolate spores for six independent crosses. We then determined the ability of each strain to utilize palatinose in the presence vs. absence of Hsp90 inhibitors (HSP90i).

Genetic screens
We utilized a gene deletion library and performed growth curve assays for each deletion strain under basal conditions vs. Hsp90 inhibition. Colony PCR was performed to confirm gene deletions were correct. As a control we used isolates of the original parental yeast strain.



Methodology (A) Set-up of 384 well plate. One yeast strained occupied 4 wells while DMSO or an Hsp90 inhibitor occupied 2 of those 4 wells. (B) Schematic depicting procedure for determining whether a trait follows a Mendelian pattern of inheritance.

Results

Figure 1: Robustness of palatinose utilization to Hsp90 inhibition by radicicol or NVP-HSP990 varies drastically across strains

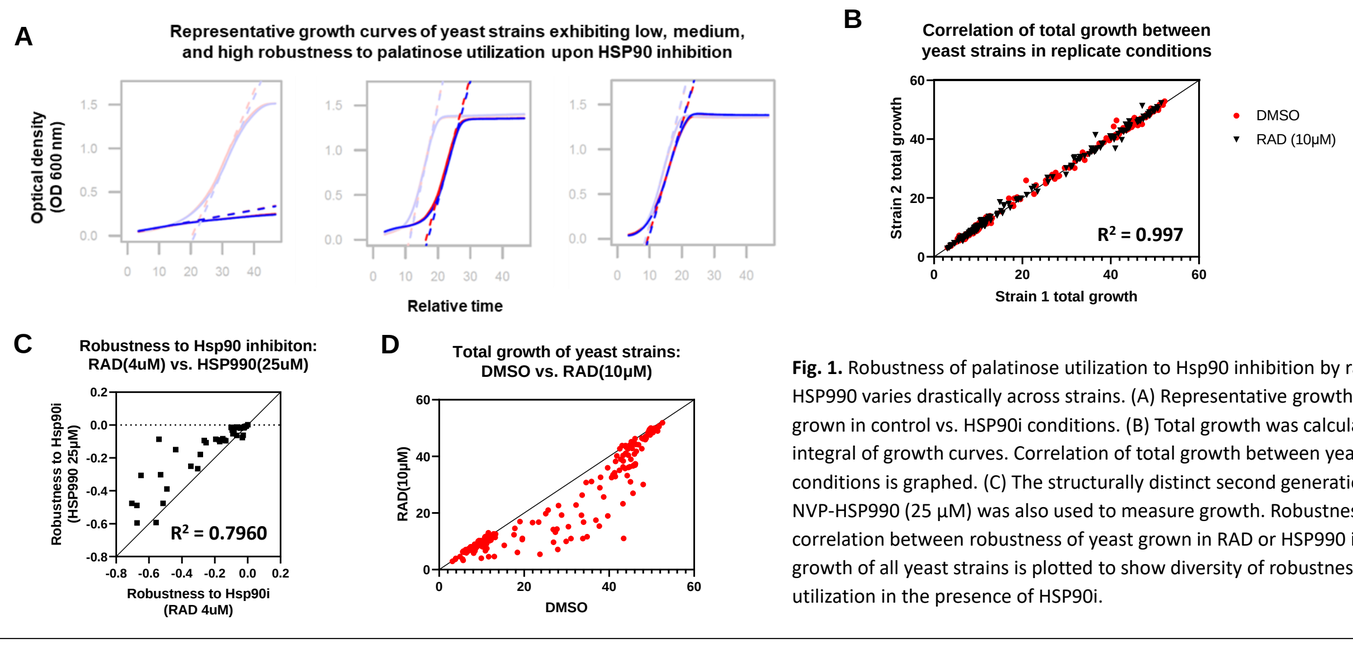


Fig. 1. Robustness of palatinose utilization to Hsp90 inhibition by radicicol or NVP-HSP990 varies drastically across strains. (A) Representative growth curves from yeast grown in control vs. HSP90i conditions. (B) Total growth was calculated by taking the integral of growth curves. Correlation of total growth between yeast grown in replicate conditions is graphed. (C) The structurally distinct second generation Hsp90 inhibitor NVP-HSP990 (25 μM) was also used to measure growth. Robustness was calculated and correlation between robustness of yeast grown in RAD or HSP990 is graphed. (D) Total growth of all yeast strains is plotted to show diversity of robustness to palatinose utilization in the presence of HSP90i.

Figure 2: Genetic crosses reveal a genetic basis for differences in robustness to palatinose utilization and imply a role for epistasis

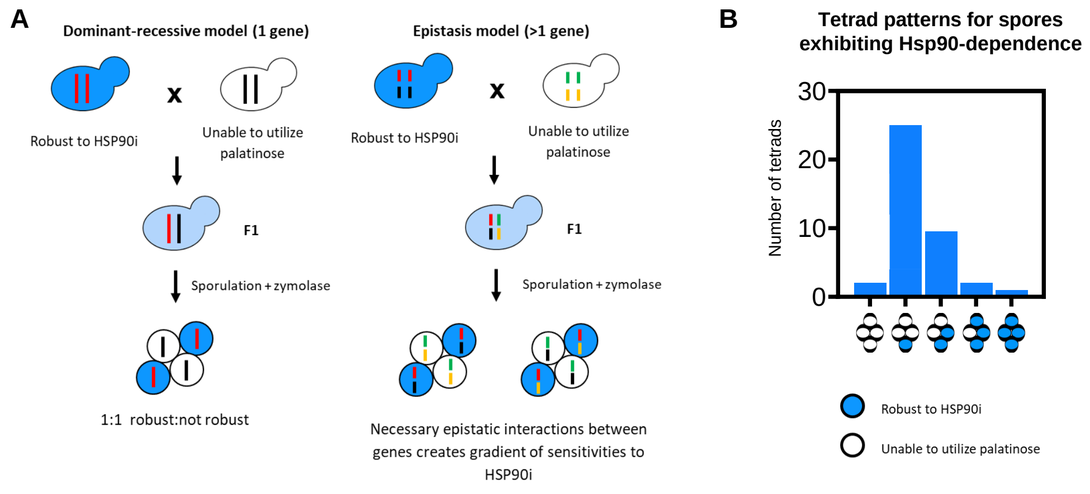


Fig. 2. Genetic crosses reveal a genetic basis for differences in robustness to palatinose utilization and imply a role for epistasis. (A) Potential genetic models for Hsp90 dependence trait. In dominant-recessive model, genetic crosses would create a 1:1 robust:not robust ratio in presence of HSP90i. In the epistatic model, interactions between genes create a gradient of sensitivities to HSP90i. (B) Results from tetrad dissection and growth curve analysis of separated spores shows that most tetrads contained only one spore that was robust to HSP90i.

Figure 3: Genome-wide genetic screen identifies potential candidates regulating palatinose utilization and robustness to Hsp90 inhibition

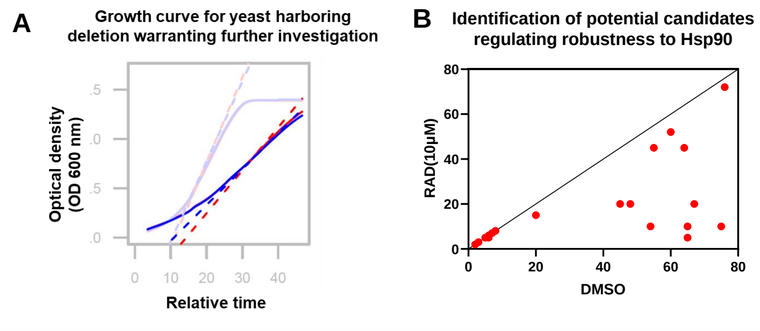


Fig. 3. Genome-wide genetic screen identifies potential candidates regulating palatinose utilization and robustness to Hsp90 inhibition. (A) Example growth curve for yeast strain harboring deletion that warrants further mechanistic investigation. (B) Expected plot from compilation of growth curve data from different deletion strains. We expect to see a diversity of sensitivities to HSP90i. Further mechanistic investigation will be performed on these strains.

Conclusion

Our experiments demonstrate a unique role for Hsp90 in palatinose utilization in yeast. Additionally, genetic variation influences this trait and epistatic interaction between genes point to its Hsp90 dependence. In conclusion, our results establish a model for the genetic basis of an Hsp90-dependent trait, identify specific genes involved, and lay a foundation to characterize the evolutionary significance of underlying mechanisms.