Evolutionary capacitance driven by HSP90 during the *de novo* evolution of multicellularity

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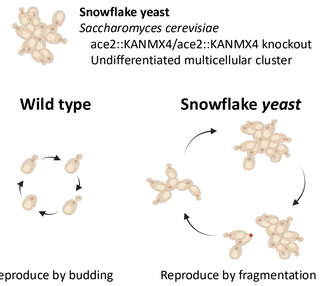
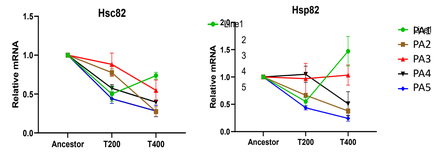
Introduction

Model system of nascent multicellularity which is capable of *in vitro* evolution [1, 2].

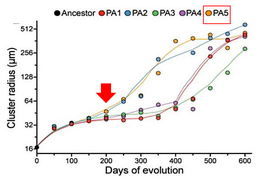


- Snowflake yeast has evolved into macroscopic size in a long-term evolution experiment of multicellularity (MuLTEE) in our lab [3].
- The expression of HSC82 and HSP82, two isoforms of heat shock protein 90 (HSP90), declines as the yeast clusters become larger over 600 transfers (~3,000 generations), with lineages evolving larger size losing HSP90 expression faster.



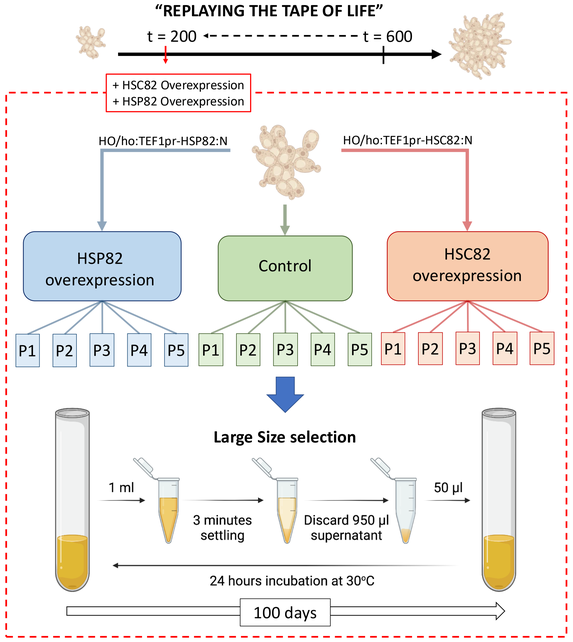
- HSP90 is a molecular chaperon that assists in folding and stabilizing cellular proteins. It is also known as an evolutionary capacitor that buffers cryptic genetic variations in other species such as *D. melanogaster* [4, 5].

Evolutionary replay experiment



Research question:

How is HSP90 involved in multicellular adaptation?



Based on the results of the original long-term evolution experiment [3, 6], we chose time point prior to the macroscopic transition (t = 200) to conduct our evolutionary replay experiment to examine the role of HSP90 in macroscopic evolution.

- HSC82 and HSP82, two isoforms of HSP90 in yeast, were overexpressed in evolved snowflake yeast at t = 200.
- Fitness of control, HSC82 overexpression (HSC82 OE), and HSP82 overexpression (HSP82 OE) lineages were characterized by fitness assay.
- The evolutionary replay experiment was conducted for 100 days with large size selection i.e., settling selection.
- Cluster size was measured using a Coulter counter.

Conclusion

- HSP90 overexpression reduced snowflake yeast fitness, demonstrating that declining HSP90 expression in the MuLTEE is adaptive (Fig. 1).
- Between the two HSP90 isoforms, HSP82 overexpression restrained the multicellular adaptation, but HSC82 overexpression surprisingly accelerated this transition (Fig. 2), over the next 100 rounds of selection.
- HSC82 OE populations evolved elongated cellular morphology in parallel (Fig. 3). Also, in a pilot gene expression assay, we observed a dramatic decrease in HSC82 expression level in macroscopic isolates of HSC82 OE populations at t = 60 (Fig.4). These observations mirror the observations in the original long-term experiment where the reduction of HSP90 expression was associated with elongated cells and macroscopic cluster size [3]. Thus, we find that the reduction of HSP90 plays a key role in elongated cellular morphology, which can lead to macroscopic transition via branch entanglement [6].
- This may be due to transient expression of HSP90 allowing for valley crossing during evolution, by allowing initially deleterious traits to fix via drift and then later be exposed by reduced HSP90 expression (Fig. 5).

Acknowledgment

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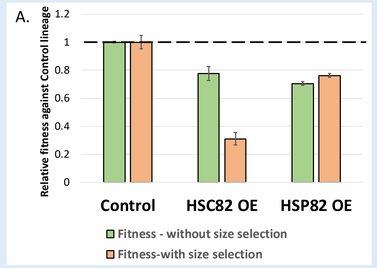


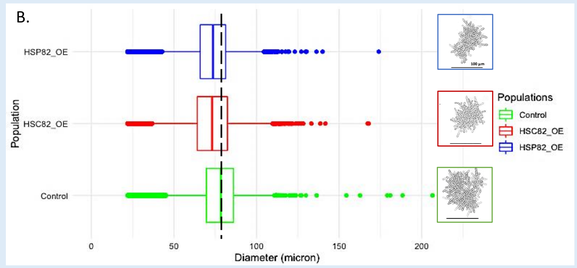
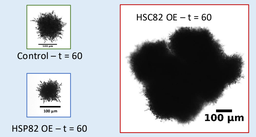
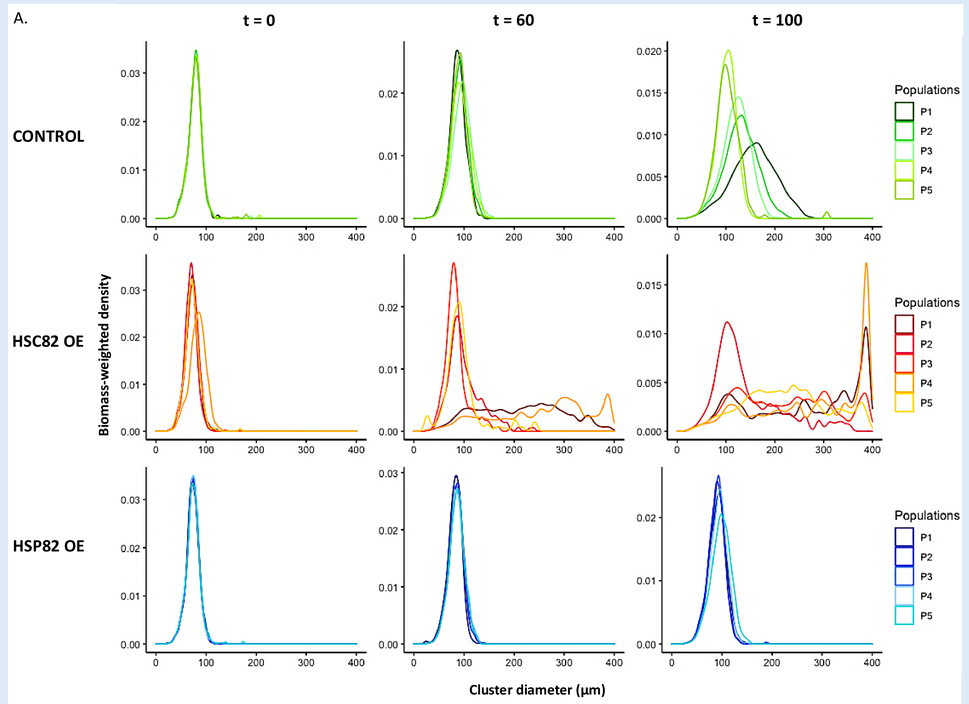


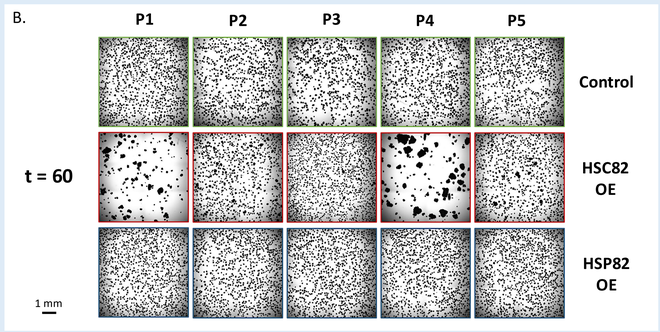
Figure 1. HSC82 and HSP82 overexpression in t200 yeast reduces size and fitness.
As shown in Fig. 1A, fitness of overexpression strains were relatively lower than the control, especially in large size selection condition.
Median cluster diameter of overexpression strains were approximately 6% smaller as compared to the control (Fig. 1B).

Reduced HSP90 expression is adaptive.
What happens if we go back to time point t = 200, overexpress each isoform of HSP90, and then re-evolve them? Will this inhibit multicellular adaptation?

HSP82 overexpression restrained multicellular adaptation while, surprisingly HSC82 overexpression accelerated it.







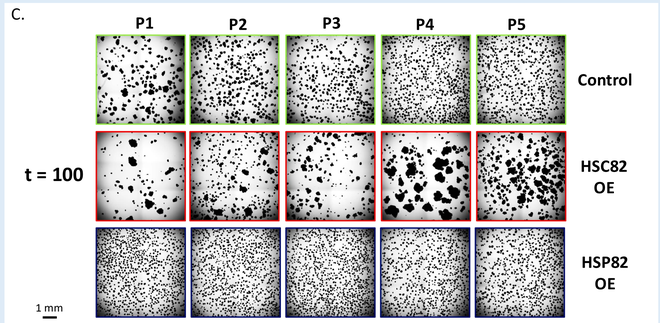


Figure 2. HSP82 overexpression keep cluster size small while HSC82 overexpression populations rapidly evolved into macroscopic clusters over the course of evolutionary replay experiment.
The cluster size distribution of ancestral (t = 0) and evolved (t = 60 and t = 100) populations of the control, HSC82 OE, and HSP82 OE lineages is shown in biomass-weighted density plots(Fig. 2A). Cluster diameter and density is respectively shown in x-axis and y-axis of the individual plot. Colors represent different populations. Under the large size selection pressure, **all populations gradually became larger in cluster diameter, except HSC82 OE lineage rapidly evolved into macroscopic clusters**. Starting from time point t = 60, some HSC82 OE populations exhibited heterogeneous size distribution, and macroscopic clusters appeared in the population (Fig. 2B). **After 100 days of evolutionary replay experiment, macroscopic clusters appeared in all HSC82 OE populations while the cluster size stayed relatively small in HSP82 OE populations.** Meanwhile, some of the control populations started to become macroscopic as they were in the MuLTEE (Fig. 2C).

What leads to the rapid macroscopic transition in HSC82 OE lineages?

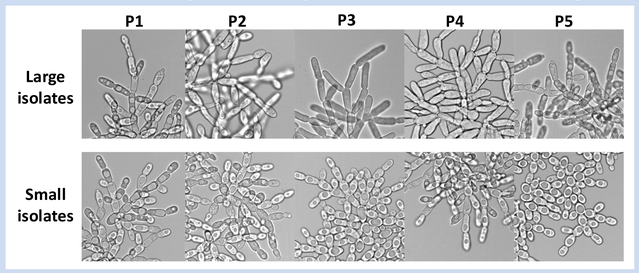


Figure 3. Elongated cellular morphology was observed in macroscopic isolates
Both large and small isolates from HSC82 OE populations (t = 60) continued the branched growth. However, elongated cell shape was observed in large isolates while small isolates remained in oval shape.

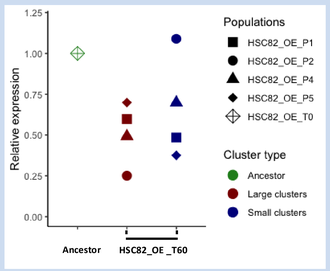


Figure 4. HSC82 expression level was reduced in the macroscopic evolved populations.
Pilot gene expression assay showed a dramatic decline in the level HSC82 expression in macroscopic evolved isolates at t = 60.

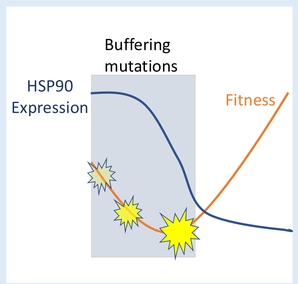


Figure 5. Valley crossing hypothesis
The loss of HSP90 expression reduces the efficacy of chaperone protein function, exposing previously neutral variation to selection. Our experiment suggests that transient expression of HSP90 may have played a role in evolutionary valley crossing in our experiment. Specifically, that mutations that would have been selected against in a low HSP90 environment instead arose to a high frequency via drift, and, through either subsequent compensation or epistasis, were later adaptive.

Future directions

- Examine the expression of HSC82 and HSP82 over the course of the replaying evolution.
- Identify the molecular basis of phenotypic differences between macroscopic and microscopic snowflake yeast in the evolutionary replay experiment.
- Testing the valley crossing hypothesis by conducting genome analysis.

References:

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