Variability of hyperosmotic stress response via the HOG pathway in wild yeast strains

Sidney Kuo¹,², Ruth Schwartz¹, and Scott A. Rifkin¹

Abstract — To respond to hyperosmotic stress conditions, Saccharomyces cerevisiae activates a MAP kinase pathway, in which Hog1 is a core regulator. Although many of the molecular details of the pathway are known, the extent of natural genetic variation on signaling dynamics is not. After screening several strains from the SGRP collection, we have found one that differs in response toward hyperosmotic stress. Here, we attempt to elucidate the molecular mechanisms leading to altered signaling dynamics as well as resulting phenotypic consequences. We also evaluate the role of selection in generating this variation by considering whether fitness is affected in hyperosmotic conditions.

The budding yeast Saccharomyces cerevisiae is found in a variety of ecological niches such as on decomposing fruits. While fruits provide nutrients to the microbe in the form of sugars, one challenge that yeast face is the increasing osmolarity of the surrounding environment as the fruits dry. In response to hyperosmotic conditions, S. cerevisiae utilizes a MAP kinase (MAPK) signaling cascade to respond to the changing environment. The signaling cascade is conserved in all fungal species, and Hog1, the core regulator, is the homolog to mammalian p38 stress response protein.[1]

There are two branches for the upstream components of the HOG signaling cascade, the Sln1 and the Sho1 branches. The two branches are redundant as well as functionally independent, and they both converge on the MAPK kinase (MAPKK) Pbs2.[2] Pbs2 then phosphorylates Hog1, which then transiently translocates from the cytoplasm into the nucleus in order to activate the appropriate stress response genes. These genes include Gpd1, Gpp1, and Gpp2 which up-regulate glycerol production allowing the cells to adapt to high osmolarity environments.[3] Even though much detail is known about the pathway, one question that remains is how variable are the dynamics of the signaling event across the species.

In order to investigate this variation, we evaluated a collection of wild yeast from the Saccharomyces Genome Resequencing Project (SGRP).[4] To capture potential single-cell variability, we adopt a microfluidic approach, performing live microscopy on cells in a variety of different conditions. After screening a few strains from the collection, we narrowed in on one, L-1374, that exhibits a pronounced difference in Hog1 dynamics after hyperosmotic shock. Hog1 delocalization occurs much less rapidly in L-1374 compared with the lab strain, S288c and the other strains we screened. Further experiments reveal possible growth rate differences in hyperosmotic conditions, suggesting that the different Hog1 delocalization pattern may tie into alternative life-history strategies between the two strains.

To understand the underlying molecular mechanism of the divergent phenotype, we examined the genome sequence differences between the two strains. We found several substitutions in regulatory regions as well as non-synonymous nucleotide substitutions in coding regions throughout the network, in particular in the Sln1 gene, which functions as an osmolarity sensor for the cell. It is a strong candidate for causing differences in Hog1 dynamics, and we test the effects of this genotypic variation by swapping alleles between L-1374 and S288c. We also take a complementary quantitative genetic approach by mating the two strains, phenotypically characterizing growth rate, survival, and signaling dynamics in the progeny, and then using deep sequencing to isolate quantitative trait loci affecting the dynamics.

With the rise of single-cell techniques, we now have the ability to detect potentially subtle differences in dynamics of signaling pathways. This allows us to uncover molecular mechanisms of phenotypic variability and to determine how this variation shapes the direction of evolution.

References

¹Division of Biological Sciences, UC San Diego, Email: siko@ucsd.edu
²Cell and Molecular Training Program, UC San Diego