Analysis of a novel stress tolerance mechanism in yeast

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Report: We discovered a novel stress tolerance mechanism in yeast. Our results suggest that methionine metabolism might be linked to stress tolerance.

Research aims

Nutrient balance is a critical determinant of lifespan. Nutrient limitation and genetic or pharmacologic inhibition of nutrients and energy metabolism extend lifespan in diverse model organisms. In our laboratory, a mutation of *S*-adenosylhomocysteine (SAH) hydrolase, *sah1-1*, was previously found to affect cell growth and chronological lifespan in budding yeast, *Saccharomyces cerevisiae*. The *sah1-1* mutant demonstrated a growth defect and a short lifespan. We also isolated the long-lived mutant by screening for suppression of the growth defect in the *sah1-1* mutant. This mutation was named *SSG1-1* (Spontaneous Suppressor of Growth-delay of the *sah1-1*). The *SSG1-1* single mutant was shown to have a longer lifespan than a wild-type yeast strain. Interestingly, *SSG1-1* mutants also showed increased oxidative and thermal stress resistance. However, the molecular mechanism(s) underlying these functions are unknown. In this study, we examined this novel stress tolerance mechanism by using *SSG1-1* yeast mutants.

Methods

Stress-resistance assay. Oxidative stress-resistance was measured by spot assays. Cells suspended in water (5×10^7 cells/ml) were spotted onto SDC plates containing indicated concentrations of hydrogen peroxide. The cells were then grown at 25°C for 3 days. For heat shock-resistance assays, cells were diluted to 5×10^7 cells/ml and spotted onto YPD plates. The plates were then incubated at 55°C (heat-shock) for 35 or 40 minutes. After the heat-shock treatment, the plates were transferred to 25°C and incubated for 3 days.

Results

1) *SSG1-1* cells increased stress resistance

S-adenosyl-L-methionine (SAM) is a central coenzyme in the metabolism occurring in

a majority of biological methylation reactions.[1)] Because SAH competitively inhibits the methylation reaction by means of SAM, hydrolysis of SAH to adenosine and homocysteine by *SAH1* is essential (Fig. 1). Previously we found that a mutation of *sah1-1* showed slow growth and accumulated both SAH and SAM. [2)] Our genetic screening identified the *SSG1-1* mutation as a suppressor of *sah1-1* in yeast. [3)] We showed that Ssg1-1 is involved in the increased production of SAM and SAH. Interestingly, we found that the *SSG1-1* single mutant is long-lived and shows increased resistance to oxidative and heat stress (Fig. 2). We further demonstrated that stimulating SAM synthesis is sufficient for stress resistance.

2) Ssg1-1 protein localized at vacuolar membrane

To investigate the function of Ssg1-1 protein, the subcellular localization of this protein was observed using fluorescence microscopy. The Ssg1-1 protein was found to be strongly localized in the vacuolar membrane during the log phase (Fig. 3). Next, we examined whether the localization of Ssg1-1 protein the vacuolar membrane was functionally significant. The stress tolerance effect of the *SSG1-1* mutation was found to be canceled by the deletion of *VPS33*, a gene that is necessary for vacuole fusion to form normal vacuoles, even though the Ssg1 protein was produced at roughly the same level as that in the *SSG1-1* single mutant. This suggests the importance of the vacuolar membrane in the function of the Ssg1-1 protein.

Conclusion

Our results predicted Ssg1-1 protein as a factor in stress tolerance as well as longevity in yeast, and suggested that the Ssg1-1 protein functions by localization in the vacuolar membrane. We thus speculate that *SSG1-1* and SAM/SAH homeostasis might be linked to stress tolerance.

References

1) Thomas, D. and Surdin-Kerjan, Y. (1997) Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* **61**:503-532.

2) Mizunuma, M., Miyamura, K., Hirata, D., Yokoyama, H., and Miyakawa, T. (2004) Involvement of S-adenosylmethionine in G1 cell-cycle regulation in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* **101**:6086-6091.

3) Ogawa, T. *et al.* (2016) Stimulating *S*-adenosyl-l-methionine synthesis extends lifespan via activation of AMPK. *Proc. Natl. Acad. Sci. USA*, **113**:11913-11918.

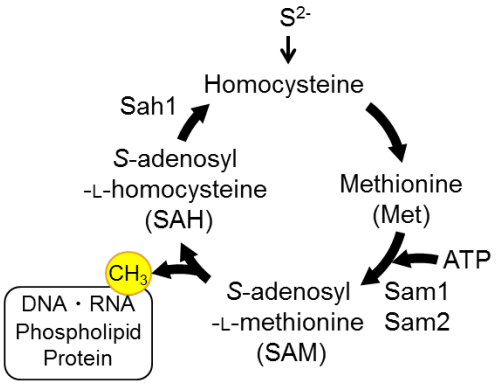

Fig. 1. Schematic diagram of methionine metabolism in budding yeast

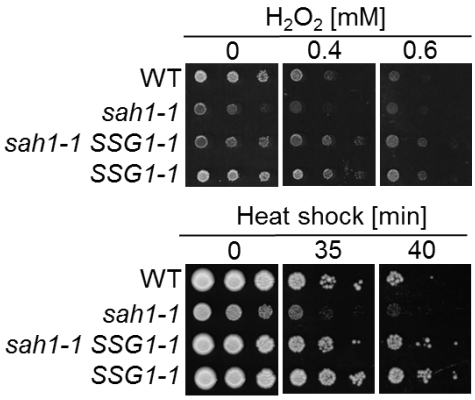

Fig. 2. Hydrogen peroxide (H_2O_2) stress and heat-shock stress tests. Ten-fold serially diluted cells were spotted onto solid medium. Details are described in methods.

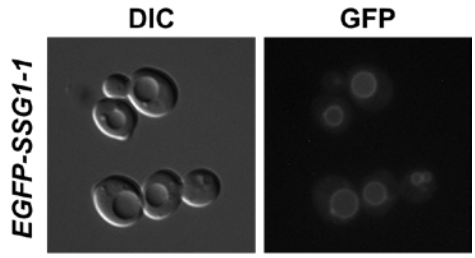

Fig. 3. Cellular localization of EGFP-SSG1-1 protein. Localization of EGFP-tagged SSG1-1 in wild type cells grown at 25°C.