Autophagy contributes to the ethanol response in the Saccharomyces cerevisiae cells

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Baker's yeast Saccharomyces cerevisiae is the valuable ethanol producer important for biofuel manufacturing. During fermentation, yeast cells cope with several stresses induced by high ethanol concentration. Among these are a disturbance in membrane integrity, oxidative stress, and proteotoxic stress caused by intracellular protein aggregation. The ubiquitin-proteasome system (UPS) and autophagy are the main proteolytic systems that degrade intracellular protein waste. How crosstalk between these systems helps the cells to tolerate ethanol-induced stresses is not fully understood. We have shown that impaired proteasome function sensitizes yeast to high ethanol, isopropanol, and butanol concentrations. Surprisingly, the proteome analysis showed that yeast proteasomal subunits are not upregulated upon short-term ethanol treatment. However, the UPS and autophagy protein substrates' level is decreased in the mutant strain with the impaired Rpn4-dependent regulation of the 20S proteasomal subunit Pre1 (YPL), while less significant changes are observed for the RPN4 deletion mutant (rpn4-Δ) compared with the wild-type strain. Next, ethanol stress induces autophagy hyperactivation in the YPL strain, while no autophagy hyperactivation is seen in the rpn4-Δ strain compared with wild-type. Accordingly, the expression of important autophagy genes ATG7 and PRB1 is induced in YPL upon ethanol stress. PRB1 as an Rpn4 target is overexpressed due to Rpn4 stabilization in the YPL. Deletion or CRISPR/dCas9-mediated repression of Rpn4-dependent regulation of the PRB1 renders yeast cells more sensitive to ethanol than wild-type. We conclude that both ubiquitin-proteasome and autophagy systems are required for ethanol resistance in yeast cells. The compensatory autophagy induction upon proteasome dysfunction partially depends on the Rpn4. This work is supported by the Russian Science Foundation grant no. 17-74-30030.