# Study on preferential translation under pronounced translation repression caused by brewing-related stress in *Saccharomyces cerevisiae*

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**Report:** We have investigated RNA-binding proteins (RBPs) involved in preferential translation under pronounced translation repression caused by severe ethanol and vanillin stress in *Saccharomyces cerevisiae*. We have succeeded to identify several factors that interact with the RBPs and other mRNAs that may be effectively translated under the tested stress conditions.

**Research aims**

Yeast cells are exposed to various kinds of stress during the process of alcoholic fermentation. Ethanol, which is produced by yeast, causes severe repression of overall protein synthesis in yeast cells at high concentrations (>9% v/v). Representative fermentation inhibitors derived from lignocellulosic biomass such as vanillin, furfural, and 5-hydroxymethylfurfural (HMF) also cause pronounced translation repression and prevent the efficient and economical production of second-generation bioethanol[1)].

Recently, Zid and O'Shea found that *HSP26* and *HSP30* mRNAs are selectively and efficiently translated despite the pronounced translation repression caused by glucose depletion, suggesting that crucial mRNAs that are essential for survival under stress conditions can be preferentially translated[2)]. We have also reported that the *BTN2* mRNA can be preferentially translated under severe ethanol stress and *ADH7* and *BDH2* mRNAs are efficiently translated under severe vanillin stress[1, 3, 4)]. Very intriguingly, such preferential and selective translation under pronounced translation repression is enabled by DNA promoter regions but not by mRNA sequences[1-4)]. Therefore, it is considered that RNA-binding proteins (RBPs) are involved in the preferential translation. In order to get a clue on the mechanisms underlying preferential translation, we identified the RBPs that respond to severe ethanol and vanillin stress and carefully examined them in this study.

**Methods**

In order to identify key factors for the preferential translation under severe ethanol and

vanillin stress conditions, expression of *BTN2* and *ADH7* in RBP-deficient mutants (205 strains) was examined by qPCR and western blot analyses. Proteins and mRNAs that interact with the RBPs upon stress were identified by co-immunoprecipitation (co-IP) and DNA microarrays.

**Results**

Through the careful examination of 205 RBP-deficient mutants, we identified 7 RBPs involved in the efficient protein synthesis of Btn2 or Adh7 under pronounced translation repression caused by severe ethanol or vanillin stress. Although the deficient mutants of these RBPs showed almost identical levels of mRNA synthesis in the wild type, the protein synthesis of Btn2 and Adh7 was severely repressed. *rbp26*Δ cells hardly induced Bnt2 protein synthesis under 10% ethanol and showed hypersensitivity to ethanol stress. Co-IP analysis indicated that Rbp26 interacted with Hsp70s and translation elongation factors (eEFs) in the presence of 10% ethanol. It is known that the mammalian eEF-1A moves to the nucleus from the cytoplasm and then escorts *HSP* mRNAs to the translation machinery for efficient translation under heat shock conditions[5]. We currently speculate that yeast cells might have a similar system that facilitates translation under severe ethanol stress using eEFs. We also analyzed mRNAs bound to Rbp26 in the presence of 10% ethanol, as they are candidates that can be efficiently translated under severe ethanol stress. We are now verifying for their preferential translation under 10% ethanol stress.

Rbp15 seems to be critical for the efficient translation of *ADH7* under severe vanillin stress, and a similar analysis to that performed for Rbp26 was carried out. We found that *YLL056c* mRNA bound to Rbp15 in the presence of vanillin. Although the expression of *YLL056*c was strongly repressed at the transcriptional level under non-stress conditions, it was significantly activated not only at the transcriptional level but also at the translational level under severe vanillin stress[6]. The induced expression levels of *YLL056c* were markedly higher than those of *ADH7* and *BDH2* upon vanillin stress. Expression of *YLL056c* was also induced by furfural and HMF, and the *yll056c*Δ cells presented hypersensitivity to vanillin, furfural, and HMF. The *YLL056c* promoter could induce the efficient expression of other non-related genes such as *ALD6* and *GFP* under pronounced translation repression caused by vanillin, furfural, or HMF. Since the gene expression under the control of the *YLL056c* promoter was not induced under non-stress conditions, the *YLL056c* promoter seems to have an advantage over other constitutive strong promoters to reduce the burden on yeast cells after a reduction in vanillin concentration. We think that the *VFH1* promoter constitutes a useful tool for improving the stress tolerance to vanillin, furfural, and HMF[6].

## Conclusion

Our data suggest that Rbp26 facilitates the preferential translation of *BTN2* mRNA via the formation of a complex with Hsp70s and eEF under severe ethanol stress conditions. By analyzing Rbp26-bound mRNAs, we also identified other candidates that can be preferentially translated in the presence of 10% ethanol. Regarding the response to severe vanillin stress, we found that the *YLL056c* promoter is able to modify gene expression in the presence of fermentation inhibitors derived from lignocellulosic biomass. There is strong indication for the superior utility of the *YLL056c* promoter for breeding optimized yeast strains in bioethanol production.

## References


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