NISR RESEARCH GRANT
2004 Research Grant

Analysis and Application of Degradation Mechanism of Abnormal Proteins by the Yeast Rsp5 Ubiquitin Ligase

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Research aims

Yeast cells are exposed to environmental stresses during fermentations. Stress induces protein denaturation, generates abnormal proteins, and leads to growth inhibition or cell death. Therefore, stress tolerance is the key for application to industrial yeasts. We analyze the stress-tolerant mechanism and its application in yeast based on abnormal proteins[1–9].

Rsp5 is an essential E3 ubiquitin ligase containing the HECT-domain in budding yeast *Saccharomyces cerevisiae*. We previously isolated the *rsp5* mutant with a single amino acid substitution, Ala401Glu, in the third WW domain (a protein interaction module). Interestingly, when yeast cells were exposed to stresses such as toxic amino acid analogues, high growth temperature in a rich medium, ethanol, and oxidative treatments, the rsp5 mutant showed much more sensitivity to these stresses than the wild-type strain. These results suggest that *Rsp5* is required for selective degradation of abnormal proteins generated by these stresses, in addition to nitrogen-regulated degradation of Gap1[5].

In this study, focused on the degradation and repair systems of stress-induced abnormal proteins, we analyzed the function of Rsp5 under stress conditions.

Methods

1) Degradation system

To identify stress-induced substrates of Rsp5, we performed proteome analysis of the wild-type and the *rsp5* mutant strains using comparative 2D-PAGE and mass spectrometry.

2) Repair system

To analyze the effect of Rsp5 on the expression of stress proteins, we examined the transcription of three genes *HSP42*, *DDR2* and *HSP12*, using HSE-*lacZ*, STRE-*lacZ* and HSE+STRE-*lacZ*, respectively, as reporter in the wild-type and *rsp5* mutant cells under various stress conditions.

Results

1) Degradation system

The accumulation of abnormal proteins in cells is a serious problem. Rsp5 is believed to function as an ubiquitin ligase under various stress conditions, but its substrates remain unclear. Interestingly, some proteins such as Hsp12, Hsp78, Egd2 and Sug2 were accumulated in the *rsp5* mutant compared with those of the wild-type strain under high growth temperature in a rich medium (Fig. 1). The proteome analyses of wild-type and *rsp5* mutant strains suggested a possibility that the stress-induced proteins are substrates of Rsp5. Therefore, we investigated the interactions of Rsp5 and candidate substrates using two-hybrid assay. The stress-induced proteins interacted with Rsp5. Our results suggest that high temperature-induced abnormal proteins interact with the third WW domain of Rsp5, and are degraded selectively in proteasome or vacuole.

2) Repair system

The accumulation of abnormal or misfolded proteins in the cells under stress is a serious problem. To overcome it, two strategies can be considered: (*i*) to degrade the proteins through ubiquitin-proteasome system or (*ii*) to refold the proteins by molecular chaperones including stress proteins. In *S. cerevisiae*, two transcription factors, Hsf1 and Msn2/4, appear to be responsible for stress-induced gene expression. Hsf1 binds to heat shock elements (HSEs) and Msn2/4 binds to stress response elements (STREs) found in the promoter of many heat-inducible genes encoding stress proteins.

The β-galactosidase activities in the *rsp5* mutant were significantly lower than that in the wild-type strain when exposed to temperature upshift (Fig. 2), ethanol, sorbitol or heat shock treatment. Similar results were obtained from Northern blot analysis. The *rsp5* mutant fails to degrade and to refold stress-induced abnormal proteins. Overexpression of stress proteins complemented stress hypersensi-

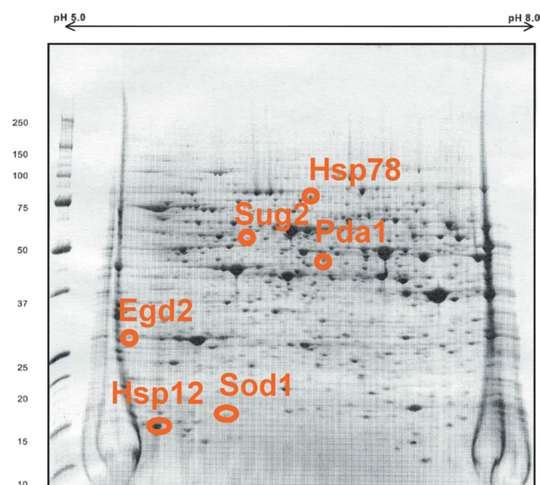


Fig. 1. 2D-PAGE pattern after growth at high temperature (37°C) in a rich medium.

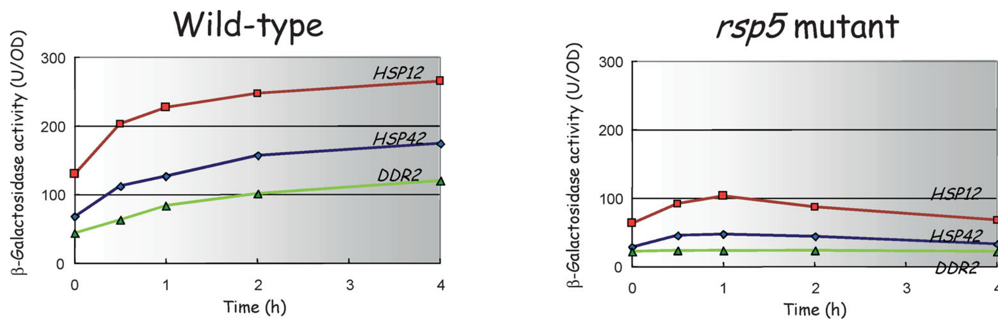


Fig. 2. Transcription analysis of stress proteins when exposed to temperature-upshift in the wild-type and *rsp5* mutant strains.

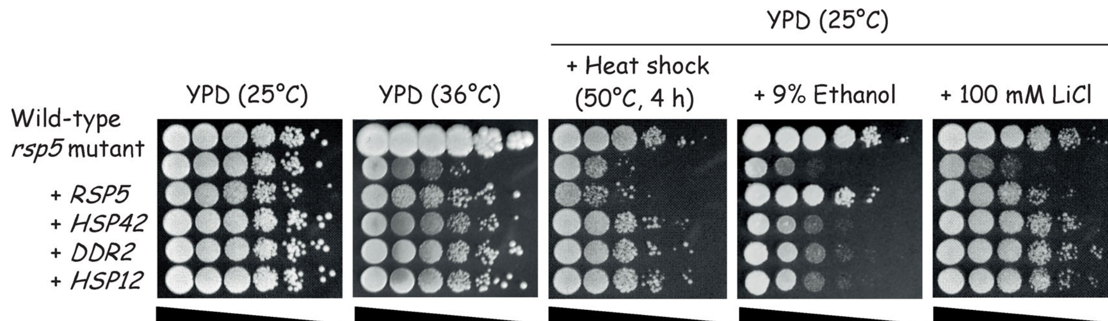


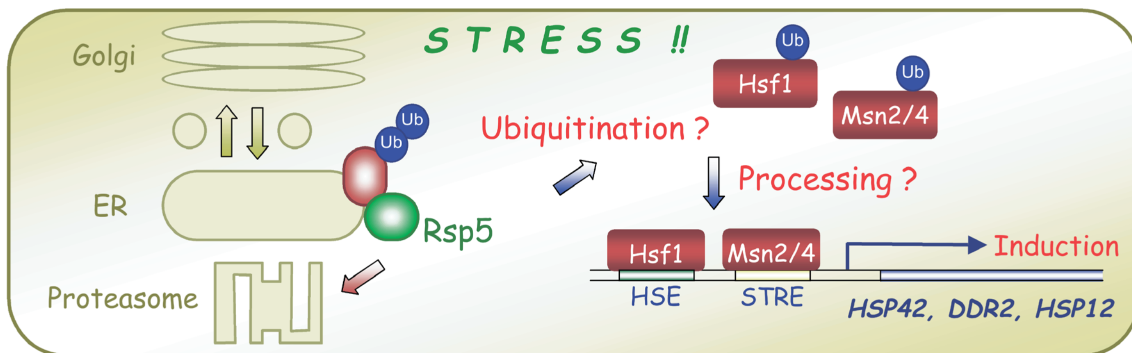
Fig. 3. Overexpression of *HSP42*, *DDR2* and *HSP12* in the *rsp5* mutant.

tivity of the *rsp5* mutant (Fig. 3). These results indicate that Rsp5 regulates the expression of stress proteins.

Conclusions

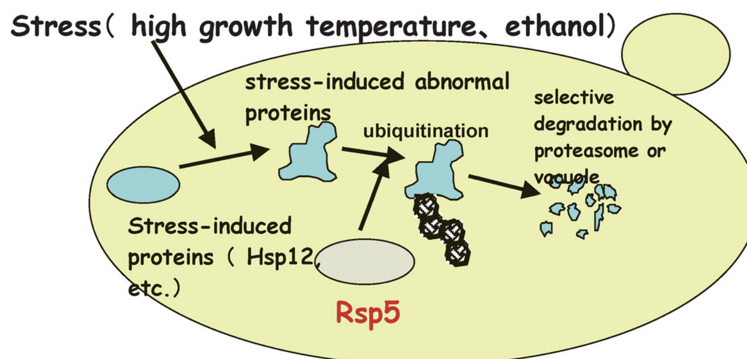
1) Degradation system

Some proteins such as Hsp12, Hsp78, Egd2 and Sug2 were accumulated in the *rsp5* mutant compared with those of wild-type strain under high growth temperature in a rich medium. Some proteins including Hsp12, Hsp78 and Sod1 were also accumulated under ethanol stress. Two-hybrid assay showed the weak interaction of Rsp5 and stress-induced proteins, suggesting that these proteins are substrates of Rsp5.



2) Repair system

Under stress conditions (temperature upshift, ethanol, sorbitol and heat shock), the *rsp5* mutant resulted in decreased expression of stress proteins (Hsp42, Ddr2 and Hsp12). Rsp5 may have a novel function that regulates the expression of stress proteins through ubiquitination of two transcription factors (Hsf1 and Msn2/4).



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