HEAT SHOCK RESPONSE, ER STRESS AND HETEROLOGOUS PROTEIN PRODUCTION IN *SACCHAROMYCES CEREVISIAE*

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Introduction. Secretion of recombinant proteins in yeast *Saccharomyces cerevisiae* can cause ER stress that can then become a limiting step for protein production. The accumulation of misfolded proteins in the endoplasmic reticulum causes ER stress and activates the unfolded protein response (UPR) mediated by Hac1p, whereas the proteostasis stress in the cytoplasm activates the heat shock response (HSR) that is mediated by Hsf1p. HSR is induced by environmental inducers such as heat shock, alkaline pH, oxidative stress, glucose starvation. The intracellular signal consists of misfolded membrane or cytosolic proteins that do not enter the secretory pathway and induce a subset of the heat shock response (HSR) targets genes (cytosolic unfolded protein response). UPR is activated by misfolded proteins in the ER and is regulated by the transcription factor (TF) Hac1p**.** Hac1p up-regulates the transcription of UPR-target genes, most of which are involved in the protein folding, vesicle trafficking and protein degradation. Activation of Hac1p is induced by the accumulation of misfolded proteins in the ER leading to oligomerization of the transmembrane kinase/nuclease Irep that initiates splicing of *HAC1* mRNA, resulting in its translation of the messanger and Hac1p synthesis.
The objective of our work is to find how the production of heterologous proteins in yeast influences the ER-stress management, more precisely is there overlap between HSR and UPR, during ER-stress?

Methods. For HSR induction we used constitutive HSF1-R206S mutant, expressed in lower or higher level. We did genome wide transcription analysis, and *in vivo* and *in vitro* follow up experiments. For the UPR induction we used the deltaHAC1 mutant and we stressed the cells with tunicamycin or DTT. We also used the genome wide transcription analysis, and *in vivo* and *in vitro* experiments to follow up the stress responses.

Results. We investigated the impact of HSR on protein secretion and found that constitutive activation of HSR improves secretion of tested heterologous proteins, a fungal α-amylase (aA) and human insulin precursor (IP). Interestingly, we found that constitutive activation of the HSR (mainly cytosolic response) by over-expression of a mutant HSF1 gene can relieve ER stress in both wild type and UPR-deficient hac1Δ cells. Unexpectedly, over-expression of the ER chaperone Kar2p alone was not able to rescue the growth deficiency of the hac1Δ strain. By using transcription analysis we found that HSR relieves ER stress through multiple pathways (up-regulation of genes in protein folding and secretion pathway, repression of genes for overall transcription and translation, and coordination with other stress responses, such as oxidative stress response). To further evaluate the role of these pathways, we deleted RPN4 in the hac1Δ mutant and found that HSR can still relieve the growth deficiency in cells with ER stress indicating that HSR can alleviate the ER stress mainly through facilitating protein folding and secretion and not through increasing the proteasome activity. Our study points towards new targets for engineering protein production in *S. cerevisiae*.

Conclusions.

1. Overexpression of HSF1R206S can rescue the UPR deficient strain under ER-stress conditions.
2. The significant changed genes in protein folding, secretion and degradation process in *HAC1* deletion and *HSF1* expression strains identifies KAR2, but the overexpression of *KAR2* cannot rescue the UPR deficient strain under ER-stress conditions
3. Top 10 Reporter TFs for *HSF1* overexpression identify YAP1 and SKN7 which are involved in oxidative stress response, we find that HSR reduces oxidative stress response
4. The reporter KEGG-pathways method identified the proteasome , and we find that the induction of HSR decreases all three proteasome activities

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