Mechanisms involved in transcriptional regulation of the *GPD1* gene during hyper-osmotic stress in *Saccharomyces cerevisiae*

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Abstract

Exposure of *Saccharomyces cerevisiae* to osmotic stress leads to increased production and accumulation of glycerol. The increase of glycerol production is accompanied by enhanced transcription of the *GPD1* gene, encoding cytoplasmic NAD^+-dependent glycerol 3-phosphate dehydrogenase. The molecular mechanisms involved in the transcriptional regulation of the *GPD1* gene during osmotic challenge has been investigated in this thesis.

To identify the promoter elements involved in regulating the enhanced expression during osmotic stress, different parts of the promoter were fused to a *cat* reporter gene. A 161bp (-483 to –322) fragment was identified by internal deletions as being highly important for the activity of the *GPD1* promoter. Electrophoretic mobility shift assay (EMSA) and footprint analysis demonstrated that this region contains at least two protein binding sites with sequences similar to Rap1p (Repressor Activator Protein 1) consensus binding sites. By site directed mutagenesis of essential nucleotides in these binding sites it is demonstrated that the binding of Rap1p is important for osmotic induction of *GPD1* during steady-state growth.

I also investigated the global expression pattern during steady-state growth on 1M NaCl using DNA micro arrays in order to identify co-regulated genes that may share regulatory elements with *GPD1*. The mRNA levels of 131 genes were altered significantly at least 2-fold. We also investigated the global protein expression changes by 2D-PAGE, also during steady-state growth on 1M NaCl. The calculated Pearson correlation of mRNA and protein changes during osmotic stress is 0.66. Moreover, functional analysis of 91 deletion mutants from the set of 138 genes with altered expression revealed that gene expression is a poor indicator of functional importance, since there was no growth defect when growing the mutants in 1M NaCl.

I present evidence of a 161bp fragment (-483 to –322) within the *GPD1* promoter to contain the important elements for its osmotic regulation during adaptation to NaCl. A detailed analysis of this fragment revealed a 50bp fragment (-450 to –401) upstream of the Rap1p binding sites, which has the kinetics of the native *GPD1* transcript during adaptation to salt, however it displayed only 40% of the maximum level of induction.

In addition we have data supporting that the osmotic induction of the *GPD1* gene is not regulated via the general stress response element (STRE), the penta nucleotide CCCCT.

Keywords: glycerol, osmotic stress, *GPD1*, *RAP1*, transcription, global gene expression.

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