STUDY OF THE FUNCTION AND REGULATION OF PEROXISOMES IN RESPONSE TO SALT STRESS

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INTRODUCTION

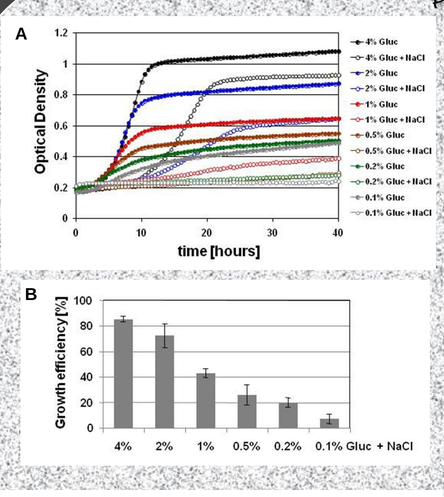
All living cells are constantly adapting to changes or stresses in their environment. Osmotic or saline stress causes a loss of water from the cells and disrupts ion homeostasis. These changes trigger adaptation programs in eukaryotic cells to repair damage, adjust cellular metabolism and allow proliferation. This research studies the mechanisms of metabolic adaptation in response to osmotic and salt stress in the yeast model (Saccharomyces cerevisiae), as it is one of the main models for understanding the mechanisms of adaptation to osmostress [1]. The HOG (high osmolarity Glycerol) MAP kinase pathway is the major signaling pathway under conditions of osmotic stress [2]. Its MAP kinase Hog1 coordinates a complex program of adaptation in cell cycle modulation, activation of gene expression and accumulation of osmolytes and ion transport [3]. Furthermore, adaptation to salt stress depends on the activation of mitochondria which are involved in ROS (Reactive Oxygen Species) balance during stress [4].

This study aims to investigate the regulation of peroxisomes (involved in catabolism of long chain fatty acids) during the adaptation to salt stress, with the following aims:

a. Possible levels of regulation of peroxisomes (expression of structural genes, biogenesis and number of peroxisomes).
b. Potential regulators of peroxisomes under stress conditions (signaling pathways, transcription factors).
c. Possible peroxisomal functions of protection against stress (compensation of respiratory metabolism, homeostasis of fatty acids).

The results obtained so far show that the correct function of peroxisomes is necessary for efficient adaptation to stress and that the organelle is specifically activated in response to stress. This is manifested by the activation of gene expression of peroxisome components and an increase in peroxisome number per cell. In addition, the Hog1 kinase is involved in the peroxisomal activation with Adr1 as a potential transcriptional activator operating upon salt stress.

PEROXISOMAL FUNCTION IS IMPORTANT FOR STRESS ADAPTATION UPON GLUCOSE LIMITATION



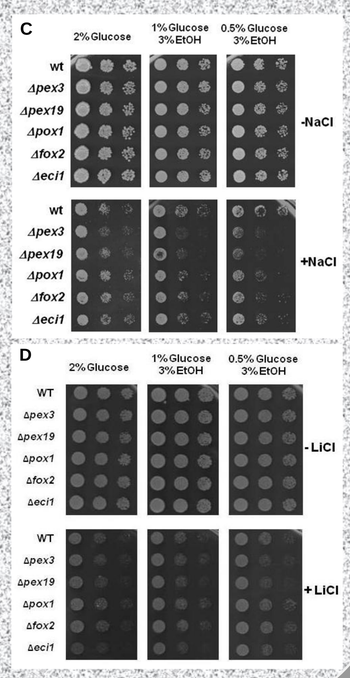


Figure 1. The glucose concentration influences the defense against osmotic stress. The continued growth of the wild-type strain BY4741 at different glucose concentrations with and without 1M NaCl, shows that the growth efficiency decreases with decreasing concentration of glucose (A and B). Growth assay in solid medium shows that the salt sensitivity of mutants in peroxisomal biogenesis or peroxisomal β-oxidation of fatty acids is aggravated at lower glucose concentrations (C and D).

INDUCTION OF PEROXISOMAL STRUCTURAL GENES UPON SALT STRESS OCCURS VIA Hog1 MAPK AND THE Adr1 TRANSCRIPTION FACTOR

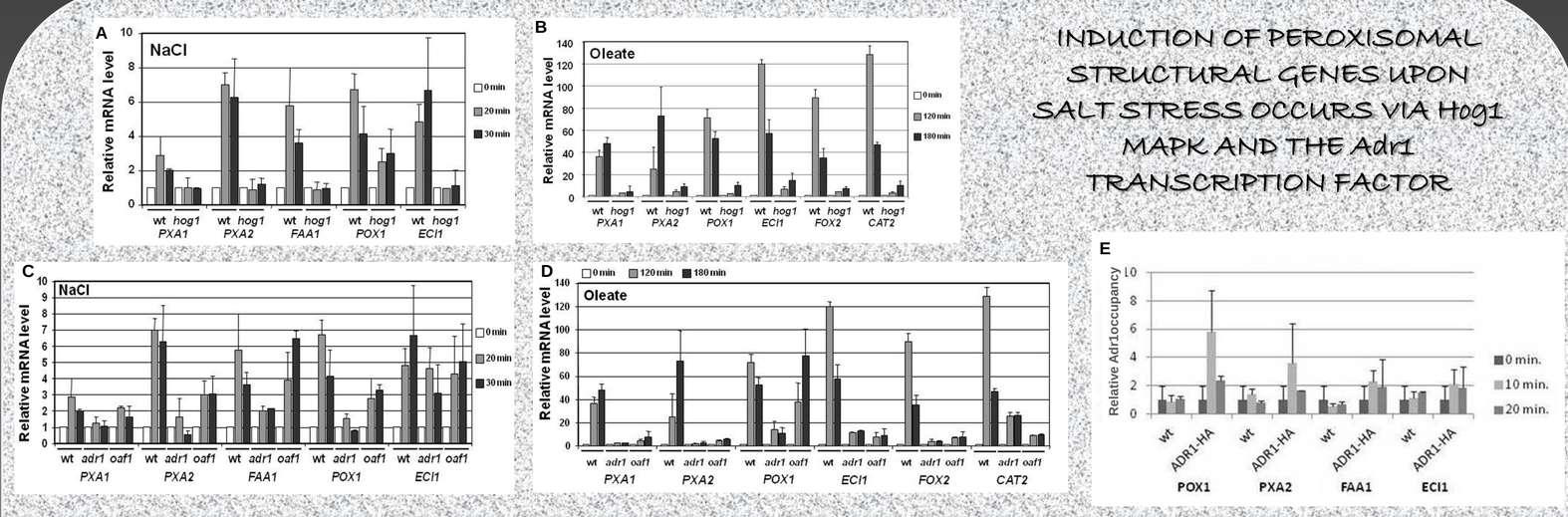


Figure 3. The MAP kinase Hog1 is important for the induction of peroxisomal genes under salinity and oleate conditions. The transcripction factor Adr1 is more important for peroxisomal induction than Oaf1 in response to osmotic stress. By quantitative real-time PCR the levels of the indicated peroxisomal genes were measured in the wild-type and in the mutant *Δhog1*. The results show that in response to 0,4M NaCl, transcriptional activation is impaired in the absence of Hog1 function (A). A reduction of gene induction is also observed upon growth with oleate (B). With the same technique we compared the transcriptional factor mutants *Δadr1* and *Δoaf1* to the wild-type and found that in response to salinity, the Adr1 factor has a much more important role in activating peroxisomal genes than Oaf1 (C). In response to oleate we show that both factors are important for activation of peroxisomal genes as previously described (D). The ADR1-3xHA strain was used in chromatin immunoprecipitation assays (ChIP) and compared with the wild-type strain (E). Adr1 binding to peroxisomal gene promoters is stimulated by osmotic stress in a Hog1 dependent manner.

SALT STRESS INDUCES PEROXISOMAL STRUCTURAL GENE EXPRESSION AND THE NUMBER OF PEROXISOMES

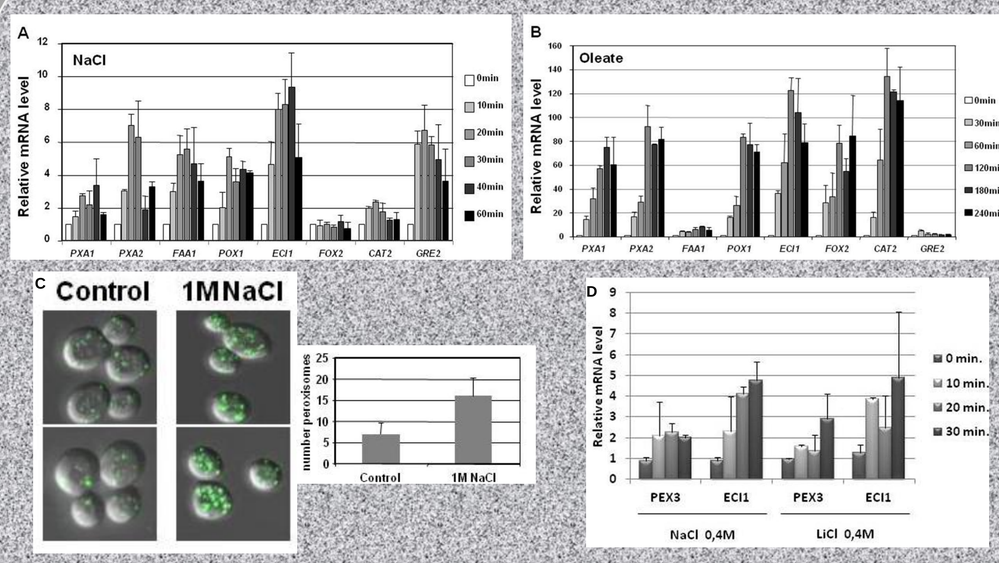


Figure 2. Salt stress induces peroxisomal gene expression and increases the number of peroxisomes. By quantitative real-time PCR, relative levels of mRNA of the peroxisomal genes *PEX1* and 2, *FAA1*, *POX1*, *ECI1*, *FOX2*, *CAT2* and *GRE2* as a control, were measured. All genes except *FOX2* show an induction in response to 0,4M NaCl (A). In response to oleate all genes show an induction as expected, because peroxisomal metabolism is essential for growth on oleate on the sole carbon source (B). Confocal microscopy shows that the number of peroxisomes per cell increase from 7 to 15 approximately, under salt conditions (C). Relative levels of mRNA of *PEX3* were also measured under salt stress conditions and results show that this gene induce under that conditions. Since *PEX3* is a peroxisomal biogenesis gene, this could mean that osmotic/salt stress could activate peroxisomal biogenesis (D).

HOG1 FUNCTION IN PEROXISOMAL BIOGENESIS

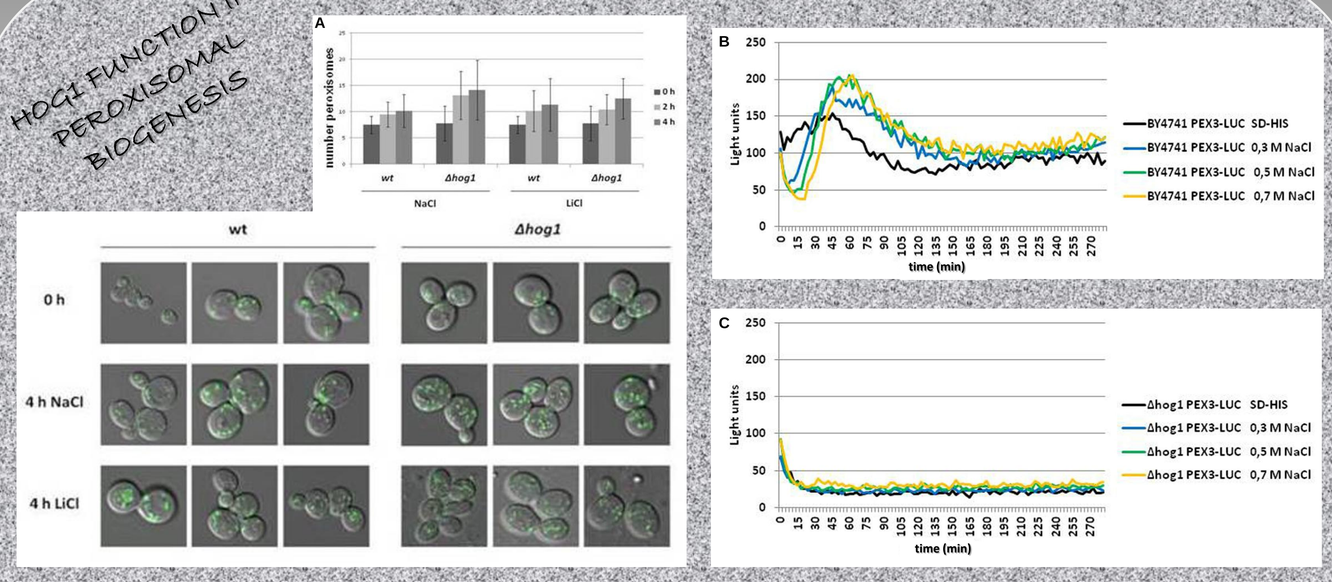


Figure 4. Function of Hog1 in the induction of peroxisomal biogenesis in response to salt stress. Confocal microscopy shows that a *Δhog1* mutant increases its number of peroxisomes per cell at the same level as the wild-type, upon osmotic stress (A). A *PEX3-luciferase* reporter assay in vivo shows that *PEX3* expression is transiently induced upon salt stress in a Hog1 dependent manner (B and C). This could mean that Hog1 is involved in the induction of peroxisomal biogenesis in response to osmotic stress. Work is in progress to decipher whether peroxisomal increase upon stress occurs via biogenesis de novo or from existing peroxisomes by fission.

RESPIRATORY AND OSMOTIC STRESS CONDITIONS AND ITS IMPACT ON PEROXISOMAL REGULATION

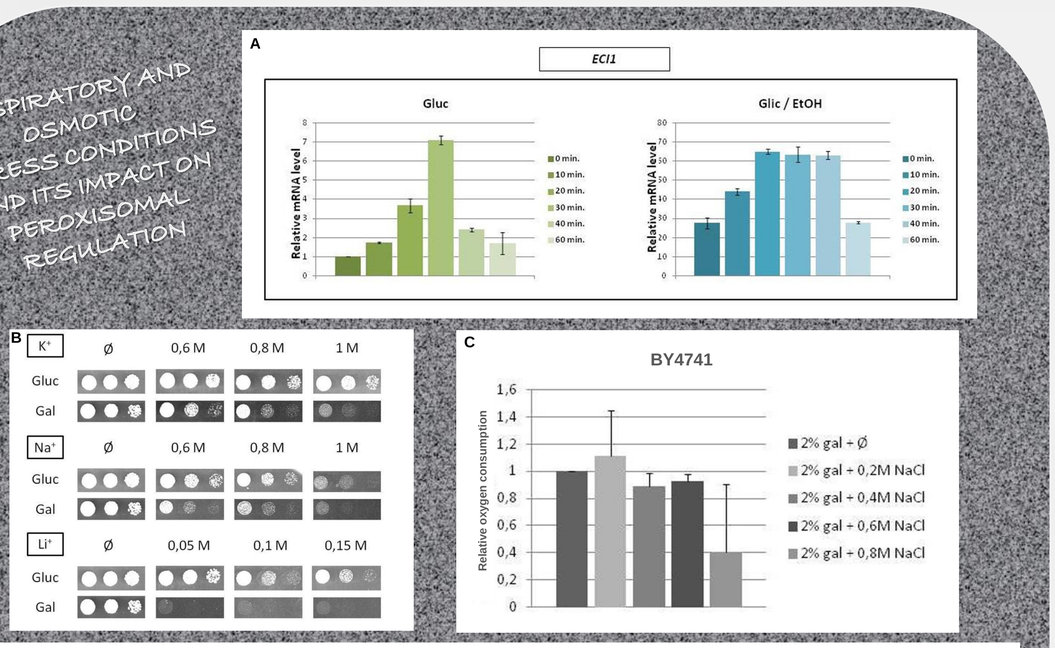
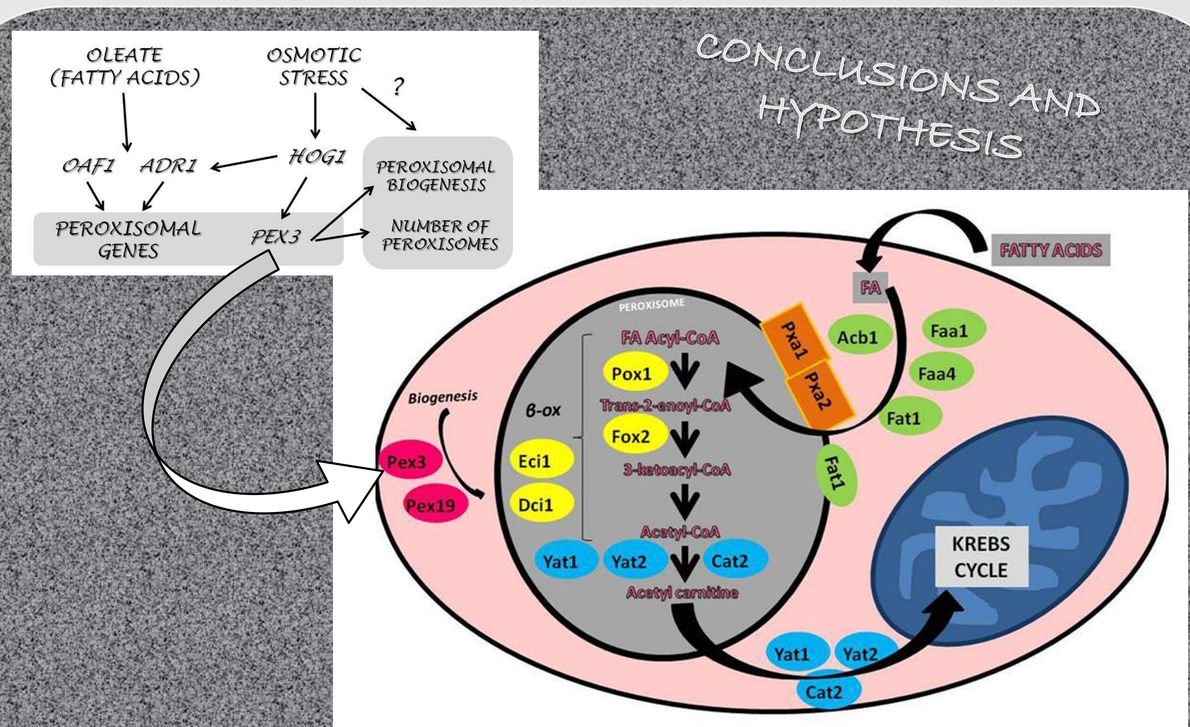


Figure 5. The peroxisomal response and to osmotic stress, changes in respiratory media. The levels of relative mRNA of different peroxisomal genes in the wild-type have been measured in response to 0,4M NaCl, using quantitative real-time PCR. These measurements were performed in fermentative media with glucose (gluc) and in respiratory media with glycerol and ethanol (Glic/EtOH) as the energy source. Growth in respiratory media generally increases the expression of peroxisomal genes. However, an additional induction by NaCl stress can still be observed, which is less transient as compared to fermentative growth conditions (A: data shown only for ECI1 expression). The inhibitory effect of different toxic cations is enhanced by partially respiratory growth conditions (gal) and increases from $K^+ < Na^+ < Li^+$ (B). The oxygen consumption measured with an oxygen electrode of the wild-type decreases as the concentration of NaCl increases (C).

CONCLUSIONS AND HYPOTHESIS



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