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DIPLOMARBEIT

Titel der Diplomarbeit

Systematic Genetic Array (SGA) screening for stress-regulating factors in *Saccharomyces cerevisiae*

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Chinesisches Sprichwort:

Kein Tag soll ohne Leistungen verbracht werden, denn die Jugend erlebt man nicht zweimal.

Table of contents

Zusammenfassung

Das Leben kann stressig sein. Hefezellen, ähnlich wie die meisten lebenden Zellen, werden durch ihre Umwelt beeinflusst und antworten durch Anpassung von transkriptionellen Programmen und Wachstumsraten. Umweltsignale werden ausgelöst durch externe physico-chemische Parameter sowie der Verfügbarkeit von Nährstoffen. Etliche Transkriptionsfaktoren und Signaltransduktionswege führen zur Synthese von Proteinen, die für spezielle Aufgaben benötigt werden, wie zum Beispiel aktiver Transport von Nährstoffen, Verdrängung und Eliminierung von toxischen Substanzen, Reparatur von DNA und denaturierten Proteinen. Einer dieser Transkriptionsfaktoren ist Msn2. Msn2 ist ein Zn-Finger Transkriptionsaktivator der in bestimmten Stresssituationen aktiviert wird. Aktivierung führt zur Translokation von Msn2 vom Zytoplasma in den Zellkern.

Ziel dieser Arbeit war es Faktoren zu identifizieren, die im regulatorischen Weg für die Stressantwort durch Msn2 eine signifikante Rolle spielen. Diese Arbeit gliedert sich in 3 experimentelle Ansätze. Der erste Teil beschäftigt sich mit der Identifizierung von Msn2 Exportmutanten in einem High Content Screen. Der zweite Teil beinhaltet einen SGA Screen zur Identifizierung von Faktoren, die den Zellzyklusstopp einer hyperaktiven Msn2 Mutante inhibieren. Der dritte Teil ist ein chemogenomischer Screen der sich mit der Clusterung von Valinomycin induzierten Genen näher beschäftigt. Im High Content Screen konnten wir eine Mutante im *PHO13* Gen identifizieren, bei der eine Abweichung beim Export vom Nucleus in das Cytoplasma festgestellt wurde. Weiters konnte eine Rolle anderer Faktoren (zum Beispiel Rph1) nachgewiesen werden, die den Zellzyklusstopp einer hyperaktiven Msn2 Mutante inhibieren. Im dritten experimentellen Ansatz konnte für mitochondriale und Stressgene gezeigt werden, dass sie durch die Behandlung von Valinomycin induziert werden.
In dieser Arbeit wurde eine SGA Methode entwickelt, die bei der Durchführung von weiteren Screens sehr hilfreich ist.

Abstract

Life is stressful. Yeast cells, probably similar to most other living cells, are surprisingly aware of their environment and rapidly respond by adjusting transcriptional programs and growth rate to a number of external physico-chemical parameters and nutrient availability. Several transcription factors and signal transduction pathways are regulating the production of proteins required for specific tasks such as active transport of nutrients, extrusion and elimination of toxic substances, repair of DNA and denatured proteins, to name a few. One of these transcription factors is Msn2. Msn2 is a Zn-finger transcription factor and is activated in stress conditions. Activation of Msn2 results in translocation from the cytoplasm into the nucleus.

Aim of this work is to identify some factors, which have a significant role in this regulatory pathway. This work orders in three different parts. The first part is a high-content screen, which deals with the identification of Msn2 export mutants. The second part includes a SGA screen, which deals with the identification of factors that are downstream of a hyperactive Msn2 mutant and inhibits the cell cycle stop. The third part is a chemo-genomic screen, which deals with the clustering of valinomycin induced genes. In the high content screen an export mutant defective in the *PHO13* gene could be identified, which results in an aberration of the export from the nucleus into the cytoplasm. Likewise, some factors (e.g. Rph1) could be identified, which inhibit the cell grpwth defect of a hyperactive Msn2 mutant. In the third part mitochondrial and stress genes were found in cells treated with valinomycin.

In this work a SGA method could be developed, which is useful for further screens.

1. Introduction

Yeast Saccharomyces is one of the most common and studied model organisms in cell and molecular biology. It is also known as baker´s yeast and is in use for baking and fermenting alcoholic beverages for thousands of years. Yeasts are classified in the phylogenetic kingdom of ascomycete fungi. Many of the niches that fungi occupy are not constant, but rather their characteristics fluctuate frequently and suddenly, presenting stressfull situations for their inhabitiants. Free living fungi often encounter the stress of nutrient limitation, changes in external temperature, osmolarity, humiditiy, pH, exposure to toxins present in the environment and competition with microbial cohorts. Whole genome expression studies have now been conducted in model ascomycete fungi, including including the budding yeast *Saccharomyces cerevisiae*, the human commensal fungus *Candida albicans* and the fission yeast *Schizosaccharomyces pombe*. In addition to these specialized responses to environmental stress, some species also respond with a common gene-expression response, referred as the environmental stress response (ESR). This response has been clearly conserved between the distant relatives *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Gasch, 2007).

The ESR was originally described in *Saccharomyces cerevisiae* as a set of ~300 genes whose expression is induced and ~600 genes whose expression is repressed in response to diverse types of stress, such as heat shock, oxidative or reductive stress, osmotic shock, nutrient starvation, DNA damage and extreme pH (Causton *et al.*, 2001, Gasch *et al.*, 2000).

The majority of genes induced in the *Saccharomayces cerevisiae* ESR are regulated by the general stress transcription factors Msn2p and Msn4p, through the stress response element (STRE) found in the target genes upstream-regulatory regions (Estruch, 2000).

Adaption of gene expression via regulation of transcription is a key response to fluctuation environmental conditions. *Saccharomyces cerevisiae*, is a currently a versatile model system to study the underlying mechanisms of such processes in detail. Yeast cells, probably similar to most other living cells, are highly aware of their environment and rapidly respond by adjusting transcriptional programs and growth rate to a number of external physical and chemical parameters and nutrient availability. Multi-cellular organisms can use specialized organs and tissues to provide a relatively stable and homogenous internal environment. Unicellular organisms such as the yeast *S. cerevisiae* had to evolve autonomous mechanisms for adapting to drastic environmental changes. Yeast regularly withstands fluctuations in the types and quantities of available nutrients, temperature, osmolarity and acidity of their environment, and the variable presence of noxious agents such as radiation and toxic chemicals (Gasch et al., 2000).

Several transcription factors and signal transduction pathways are regulating the production of proteins required for specific tasks such as active transport of nutrients, extrusion and elimination of toxic substances, repair of DNA and denatured proteins (Gasch et al., 2000). With genome-wide mRNA profiling, we see how a genome reacts to its environment by modulation of often hundreds of genes.

1.1. Regulation of the yeast general stress response factors Msn2 and Msn4

The C_2H_2 Zn-finger transcription factor Msn2 is a key regulator of stress and nutrient response in the budding yeast *Saccharomyces cerevisiae*. Msn2, together with its paralog Msn4, influences expression of about 200 stress induced genes (Martinez-Pastor *et al.*, 1996, Gasch et al., 2000, Causton et al., 2001). Msn2 and Msn4 are required for activation of stress inducible genes in response to variety of stress conditions. They are activated during the diauxic transition, and have a role in both chronological and replicative ageing (Choo & Klug, 1994, Rep *et al.*, 2000, Hasan *et al.*, 2002, Boy-Marcotte *et al.*, 1998, Schüller *et al.*, 2004).

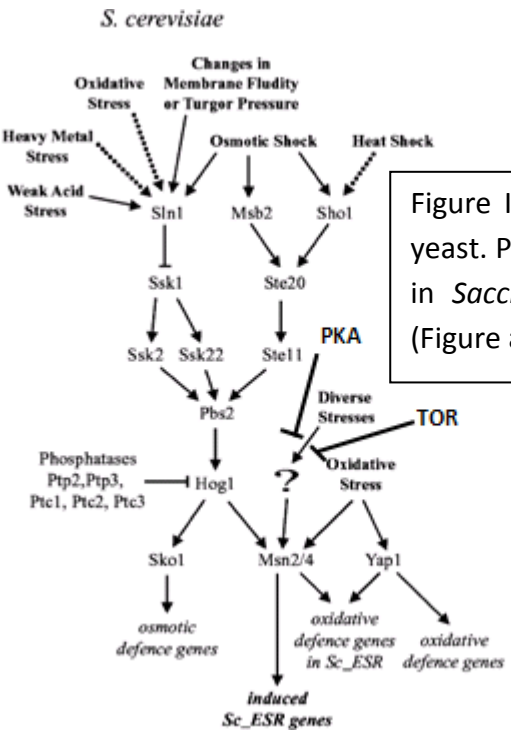


Figure I1: The Hog1 pathway in budding yeast. Proteins involved in Hog1 signalling in *Saccharomyces cerevisiae* are shown (Figure adapted from Gasch 2007)

Msn2 activity is regulated by at least four different signal transduction pathways. Hyperosmotic stress is generally controlled by the HOG pathway and activation of many genes by osmostress requires Msn2 (Rep et al., 2000). Two other pathways play a role in nutrient signaling to Msn2. Protein kinase A (PKA) inhibits Msn2 activity. Msn2 is the

mediator of most of the transcriptional response caused by inactivation of PKA (Boy-Marcotte et al., 1998, Garreau *et al.*, 2000, Görner *et al.*, 2002, Görner *et al.*, 1998).

Similarly the TOR (target of rapamycin) pathway (Beck & Hall, 1999) negatively regulates Msn2 activity. Treatment with rapamycin causes nuclear accumulation of Msn2 and activation of many Msn2-dependent genes (Görner et al., 2002, Hardwick *et al.*, 1999, Beck & Hall, 1999, Schmelzle *et al.*, 2004). The AMPKinase Snf1 phosphorylates Msn2 in response to glucose starvation. Initially, Msn2 and Msn4 were identified as multicopy suppressors of a *snf1*^{ts} allele. *SNF1* encodes an AMP activated protein kinase important for the switch from fermentative to respiratory growth in yeast (Hardie *et al.*, 1998, Carlson, 1999) (De Wever *et al.*, 2005). Msn2p and Msn4p do not have a role in Snf1p regulated processes and were most probably rescuing the *snf1*^{ts} allele indirectly. Instead, both transcription factors were shown to be required for activation of stress inducible genes (Estruch & Carlson, 1993, Schmitt & McEntee, 1996).

1.2. Msn2p and Msn4p shuttle between cytoplasm and nucleus

Several types of regulation have been found so far for Msn2. One dramatic effect is the rapid change of intracellular localization of Msn2 from cytoplasm to the nucleus in response to nutrient and stress conditions (Görner et al., 1998, Görner et al., 2002, Beck & Hall, 1999). Rapid change of localization has also been described for several other yeast transcription factors (Hopper, 1999). Within minutes of exposure to temperature shock, osmo stress, ethanol and weak acid exposure, and glucose starvation, the majority of Msn2p-GFP and Msn4p-GFP fluorescence accumulated in the nucleus. By returning the cells to their standard growth conditions, Msn2p-GFP and Msn4p-GFP relocalize to the cytosol. These localization effects are fully reversible and happen faster than production and subsequent folding of GFP, and thus do not require *de novo* protein synthesis. Therefore, Msn2p and Msn4p intracellular localization is a sensitive measure for the stress load of yeast cells (Görner et al., 1998).

1.3. The transport domains of Msn2p

The rapid change in intracellular distribution allowed identification of the domains important for this process, and to eventually identify the mechanisms leading to the response to multiple stress types. Since both Msn2p and Msn4p behave very similar, the structural requirements for regulated intracellular distribution should be conserved. By comparing the amino acid sequence of the two factors using sequence alignment several short stretches of homology could be identified. PKA affects intracellular localization in two ways. Immediately upstream of the DNA-binding domain a stretch of basic amino

acids is related to nuclear localization signals (NLS). It was also identified localization signal at the C-terminus of the protein in the region of amino acid 576 to 704. The NLS-region fused to GFP was constitutively localized to the nucleus. Msn2 has separate functional domains for nuclear import (nuclear localization signal, NLS), nuclear export (nuclear export signal, NES) and DNA binding. The C_2H_2 Zn finger DNA binding domain at the C-terminus recognizes the stress response element (STRE). The NLS is found adjacent to the DNA binding domain; it is phosphorylated and inactivated by PKA when glucose is available and rapidly dephosphorylated and activated by glucose starvation(Görner et al., 2002, De Wever et al., 2005).

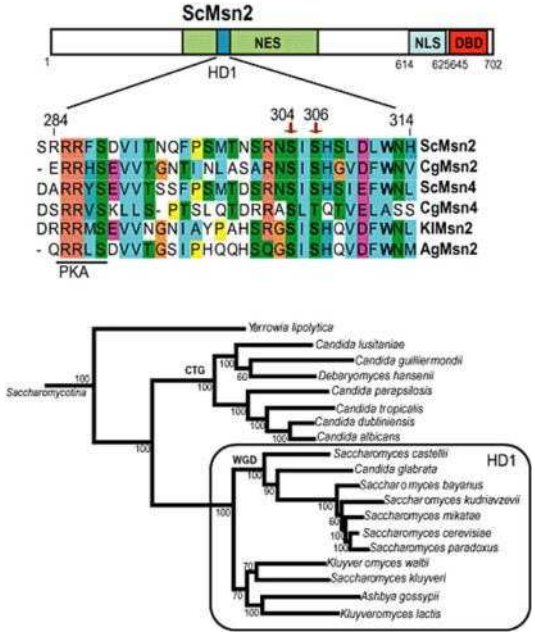


Figure I2: Alignment of Msn2 orthologous sequences including the HD1 (Roetzer *et al.*, 2008).

One further recognizable feature of ScMsn2 is its central region, which confers regulated nuclear export. This is conserved in the paralogue Msn4, and was previously described as HD1 (Görner et al., 1998). Two putative Msn2(CAGL0F05995g) and Msn4(CAGL0M13189g) orthologues with HD1 domains at synthenic positions in the *C. glabrata* genome (Roetzer et al., 2008). Roetzers results show which amino acid sequence is important for the NES.

1.4. PKA regulates Msn2p directly

Four PKA sites are present in the Msn2-NLS and no other region of Msn2p confers nuclear localization activity (Görner et al., 2002). In cells with low PKA activity, Msn2p and Msn4p are primarily nuclear, whereas artificially increasing the intracellular cAMP

levels, resulted in nuclear exclusion of the factors, even in the presence of environmental stress (Görner et al., 1998). Glucose starvation leads to dephosphorylation of PKA recognition sites in the Msn2 NLS and NES thus inhibiting nuclear export, activating nuclear import. Therefore, dephosphorylation of Msn2 converts it into an active form. On the other hand, stress conditions such as heat shock do not lead to dephosphorylation of Msn2 PKA but cause a similar nuclear concentration and chromatin recruitment of Msn2. Heat shock causes Msn2p hyperphosphorylation (Garreau et al., 2000, Chi *et al.*, 2001). Likewise, inhibition of the TOR pathway by rapamycin treatment also activates Msn2 without detectable change of its phosphorylation state. Furthermore, increase of PKA activity can suppress stress induced activation of Msn2. Stress treatment perhaps overrides this inhibition leading to Msn2 recruitment to promoter elements and activation of target genes.

1.5. Nuclear control of transcription factor activity

For several transcription factors regulation by nuclear import is considered a critical step for activation. However, careful analysis unveiled additional transport-independent layers of control for certain transcription factors (Chi et al., 2001). Examples are the yeast NFAT homologue Crz1 (Boustany & Cyert, 2002), Pho4 (Komeili & O'Shea, 1999), Mig1, and NFAT (Zhu & McKeon, 1999). Regulation of Msn2 which translocates rapidly from the cytoplasm to the nucleus and back turned out to be more complex than anticipated. Msn2 activity protects cells from stress damage, however, has also negative effects on yeast growth properties. The nuclear export region of Msn2 is required for its activation by stress (Boy-Marcotte *et al.*, 2006). PKA and Tor negatively influence the activity of Msn2 through this region.

PKA most probably regulates nuclear export indirectly. The phosphorylation pattern of the single PKA site (Ser288) within the NES region does not change during stress (Gasch et al., 2000). On the other hand high PKA activity triggers Msn2 nuclear export. Therefore, it was assumed that PKA regulates an additional factor also involved in Msn2 nuclear export. Inactivation of the TOR pathway by rapamycin also inhibits nuclear export of Msn2 but again does not change the phosphorylation pattern of Msn2 indicating also an indirect mode of action upstream of Msn2.

1.6. Hyperactive Msn2 Mutants

Previous work generated hyperactive Msn2 alleles by in vitro mutagenesis (Görner et al., 2002). For Msn2p six putative PKA phosphorylation sites have been identified and in one mutated Msn2p allele these six putative PKA phosphorylation sites were mutated from

Serine to Alanine, as shown in figure I3. Therefore, it should be similar to a non-phosphorylated, PKA independent Msn2 allele. Mutant strains with low protein kinase A activity grow poorly and total PKA deficiency is lethal for yeast. This lethality can be rescued by deleting the two genes encoding *MSN2* and *MSN4* (Smith *et al.*, 1998). This Msn2A1-6 cannot be modified by protein kinase A directly and its growth phenotype is very similar to a PKA deficient strain.

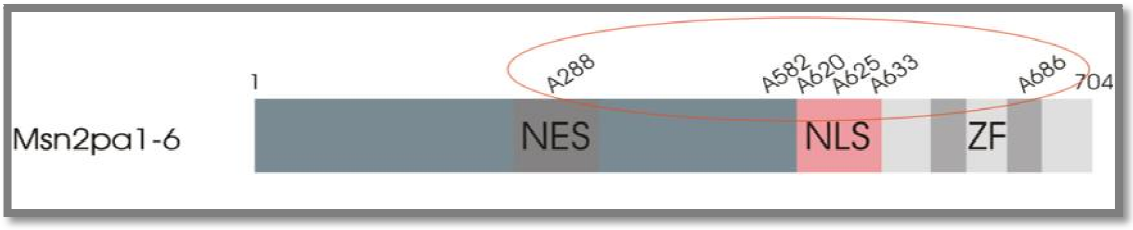


Figure I3: Schematic figure that represents the Msn2A1-6 mutant variant.

How stress and the mentioned signaling pathways regulate Msn2 is not completely understood, and therefore this work investigates in that question!

Main questions of this diploma thesis:

- What are the factors that regulate Msn2?
  Do cell cycle regulators exist downstream of Msn2 that mediate the Msn2A1-6 caused growth arrest?

- Does disturbance of ion homeostasis induce specific stress responses?

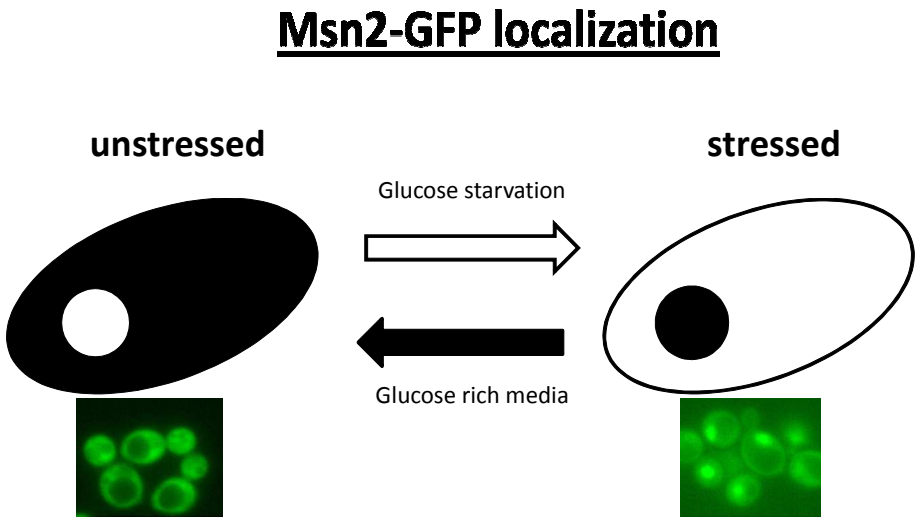
2. Msn2-GFP

2.1. Aim of this work

Activation of Msn2 is accompanied by nuclear accumulation. Two possible mechanisms could lead to nuclear accumulation of Msn2: The inactivation of the Msn2-NES (Nuclear export signal) or the loss of interaction with the exportin Msn5. Since activation of Msn2 and nuclear accumulation occur simultaneously both processes might be coupled. The nuclear export sequence of Msn2 might only be accessible to the exportin Msn5 in the unstressed state and if Protein Kinase activity (PKA) and Target of Rapamycin (TOR) activity is high. The aim of the conducted work is to identify factors that might be involved in the shuttling mechanism of Msn2.

To explore the possible connection between nuclear transport and activation we screened the systematic Euroscarf deletion library, for constitutive changes of the localization of a Msn2-GFP reporter construct. To introduce the Msn2-GFP construct into the library strains we used the SGA method and a Singer RoTor machine.

In this screen we wanted to identify mutants with aberrant regulation of the Msn2 NES. This type of mutants would lead to reduced nuclear export.



2.2. Results

In this screen we wanted to identify mutant strains with aberrant regulation of the Msn2 NES. First we transformed the vector AMG into the BY7092 yeast strain.

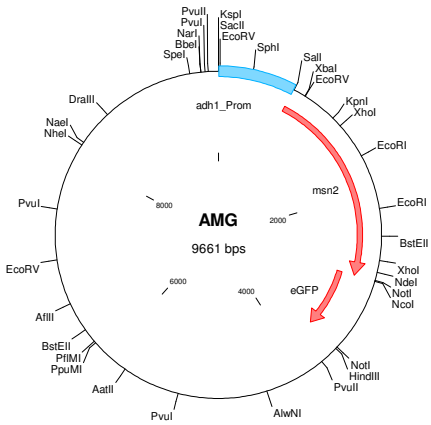


Figure R1.1: Plasmid map of vector AMG used in this work (Vector Database)

The AMG vector contains the *MSN2* gene with a GFP reporter construct under the control of an *ADH1* promoter and a *LEU2* gene as an auxotrophic marker. We used this yeast strain as the query strain for the whole screen. To analyze the possible connection between the nuclear export and import we screened the Euroscarf deletion library, which contains ~ 4950 non essential genes.

For that screen we used the SGA (synthetic genetic array analysis) method, which is described in the materials and methods part. After sporulation we pinned these cells onto SC-His-Leu plates to obtain cells which contain the Msn2-GFP reporter construct and the kanMX gene deletions.

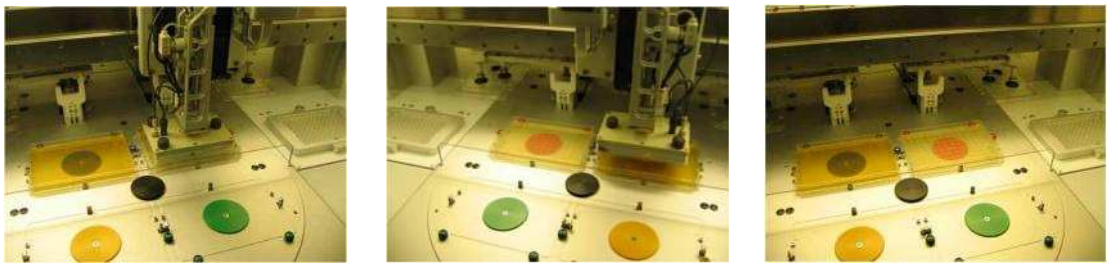


Figure R1.2: Pinning steps with the Singer RoTor yeast robot

Cells were grown to a diameter of about 1mm.

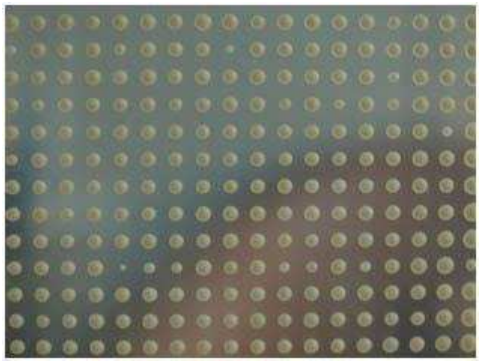


Figure R1.3: Part of an SC-H-L plate at the end of SGA

After growing the yeast cells on selective media we pinned them with Singer RoTor yeast robot into 96-well plates which contains SC-His-Leu liquid media with 2% glucose and let them grow for two days at 30°C to make sure that all glucose was used up. By using Diasticks we determined that the medium did not contain glucose after two days of growth. Accordingly, the Msn2-GFP was localized in the nucleus.

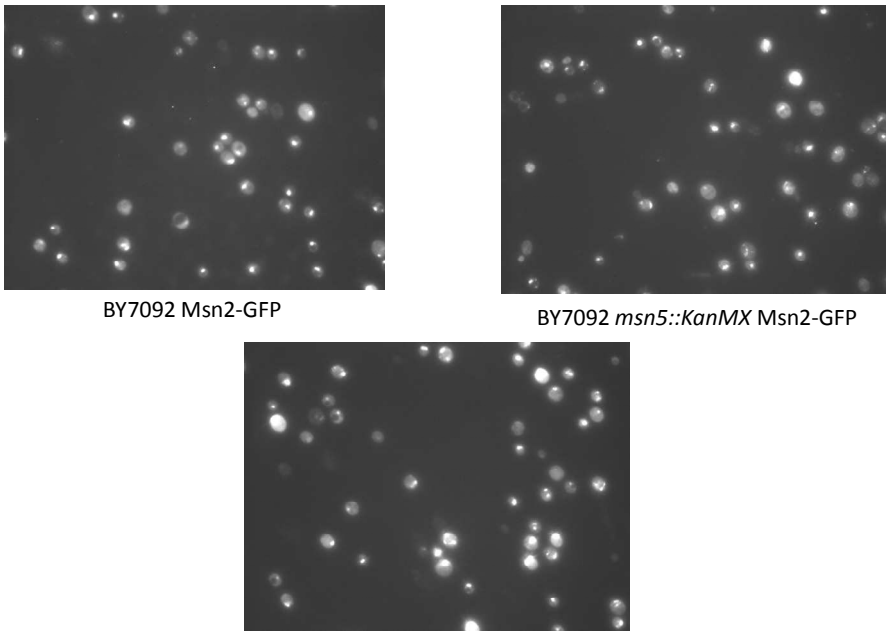


Figure R1.4: Microscope pictures of three different yeast strains (BY7092 Msn2-GFP WT; BY7092 *msn5::KanMX* Msn2-GFP; BY7092 Msn2-GFP R302) after two days of incubation at 30°C

The figure R1.4 shows, that the cells are stressed because of glucose starvation and consequently Msn2-GFP became localized in the nucleus. So this situation is a starting

point for Msn2-GFP screen to identify factors that regulate the export of Msn2 into the nucleus. The next step was to add glucose to a final concentration of 4% and incubate the cells after mixing for two hours at room temperature. Because of adding glucose export of Msn2-GFP from the nucleus into the cytoplasm started.

After these two hours of incubation in glucose we fixed the cells by adding formaldehyde solution (10% final concentration). After the formaldehyde treatment the cells were ready for microscopy.

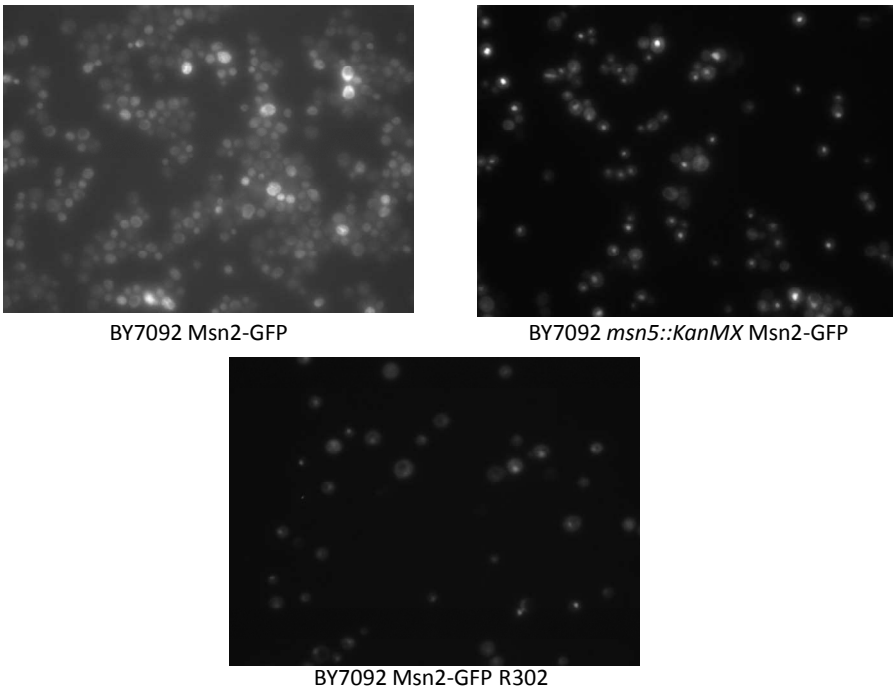


Figure R1.5: Microscope pictures of three different yeast strains (BY7092 Msn2-GFP WT; BY7092 *msn5::KanMX* Msn2-GFP; BY7092 Msn2-GFP R302) after incubation of glucose and treatment with formaldehyde.

In figure R1.5 it could be observed that in the wild type cells Msn2-GFP is in the cytoplasm, so the export is normal, whereas in the Msn5 deletion strain the export of Msn2 is inhibited. To make sure that approach was working correctly we tested the Msn2-R302 mutant generated by Andreas Roetzer. This mutant contains an Arginine instead of an Alanine at the amino acid position 302 which is located in the Msn2-NES. R302 mutant showed an aberrantly reduced nuclear export of Msn2 (Roetzer unpublished result). For the R302 mutant half of the cells show normal Msn2 export, whereas the other half of the cells had still partly nuclear localized Msn2.

These results demonstrated, that the setup and the incubation periods of the cells were appropriate. We took microscope pictures of all 4950 yeast strains and five mutants with aberrant regulation of the Msn2NES could be observed.

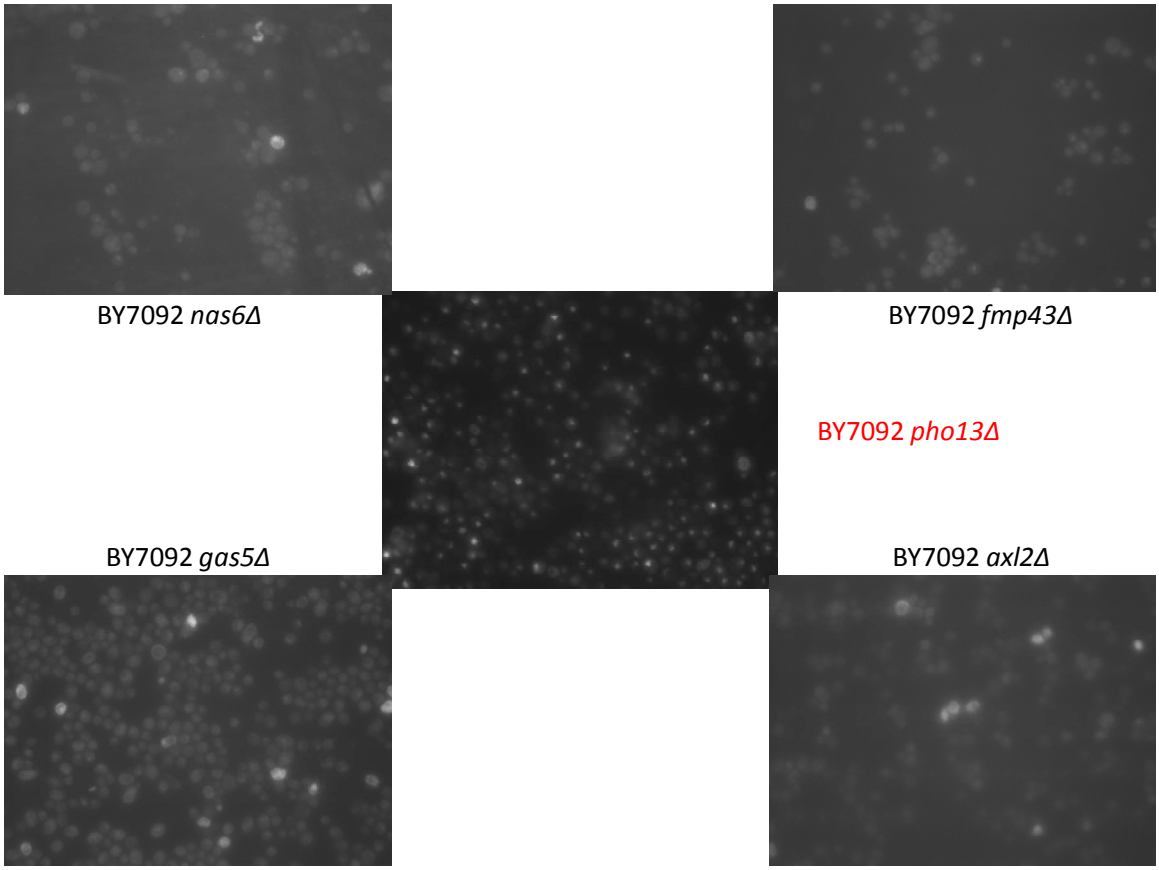


Figure R1.6: Five candidates of Msn2-GFP nuclear export screen

The figure above represents five yeast strains with different gene deletions with an aberrant regulation of the Msn2 NES.

Yeast strains BY7092 *nas6Δ*, BY7092 *fmp43Δ,* BY7092 *gas5Δ* and BY7092 *axl2Δ* showed a concentration of 20% of the cells localized Msn2 in the nucleus.

Yeast strain BY7092 lacking *PHO13* showed a rate of 90% of cells with Msn2 localized in the nucleus. We concluded that Pho13 was an interesting candidate being involved in the nuclear export of Msn2 and thus we wanted to confirm this hypothesis by another localization experiment.

We performed an experiment with the yeast strain BY7092 *pho13Δ* constructing a new setup. We plated out the candidate on a SC-His-Leu+Kan plate and incubated them for one day at 30°C. After that incubation step we inoculated the yeast strain in Sc-H-L+Kan liquid media and incubated them for one day at 30°C. We diluted the yeast culture to an OD_{600} of about 0,3 and let them grow to an OD_{600} of about 1,0.

In a first approach we stressed *pho13Δ* cells by glucose starvation.

We centrifuged the culture, resuspended the pellet in ddH_2O and analyzed the localization of Msn2-GFP in the cells by fluorescence microscopy. It could be observed that all of the Msn2-GFP reporter gene construct was in the nucleus.

After that step we added 4% of glucose and measured the time that the cells needed to export Msn2p. A small difference to the export time of Msn2 to a WT strain could be observed in the mutant. We repeated the export experiment using osmotic stress, because then the export of Msn2p is not as fast as with glucose starvation. We repeated cell growth as in the first control experiment and added NaCl to a final concentration of 0,5M and took pictures after diverse time points to follow the localization of Msn2-GFP, as seen in figure R1.7.

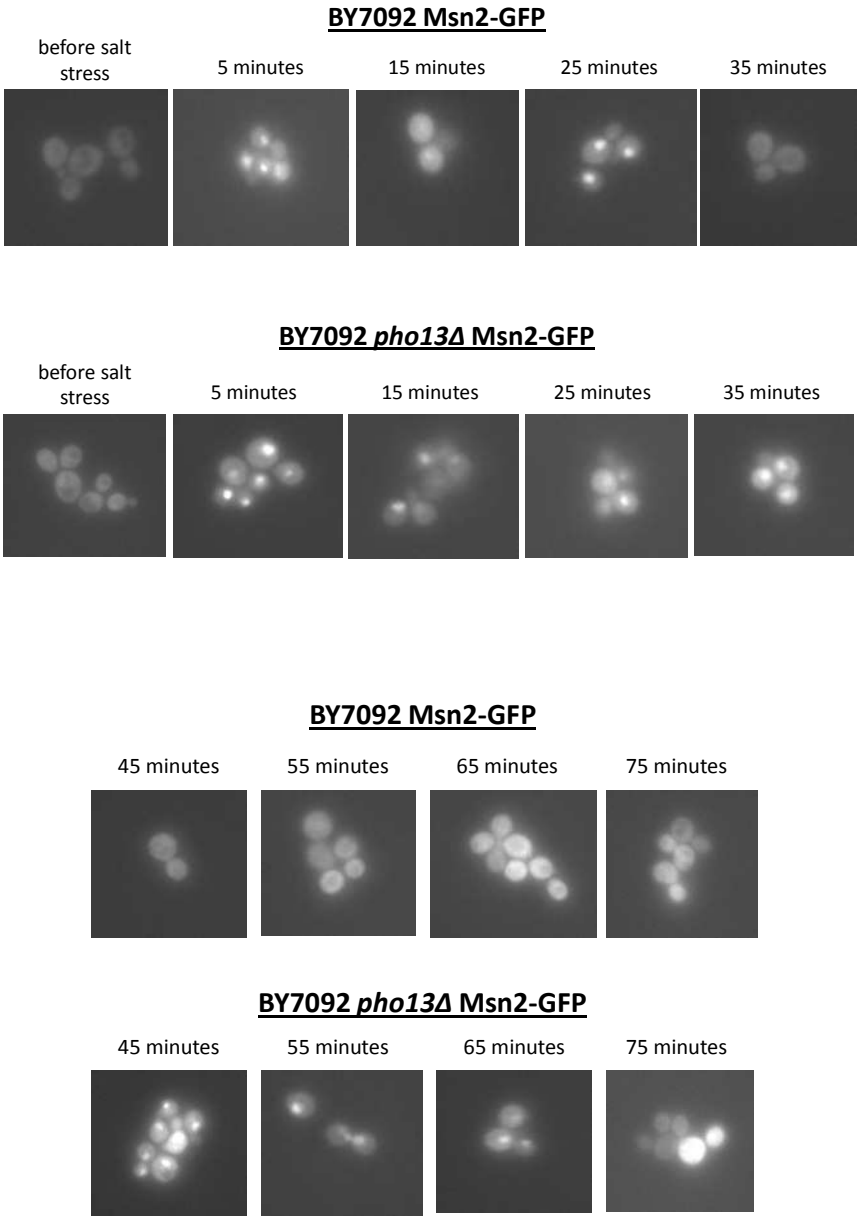
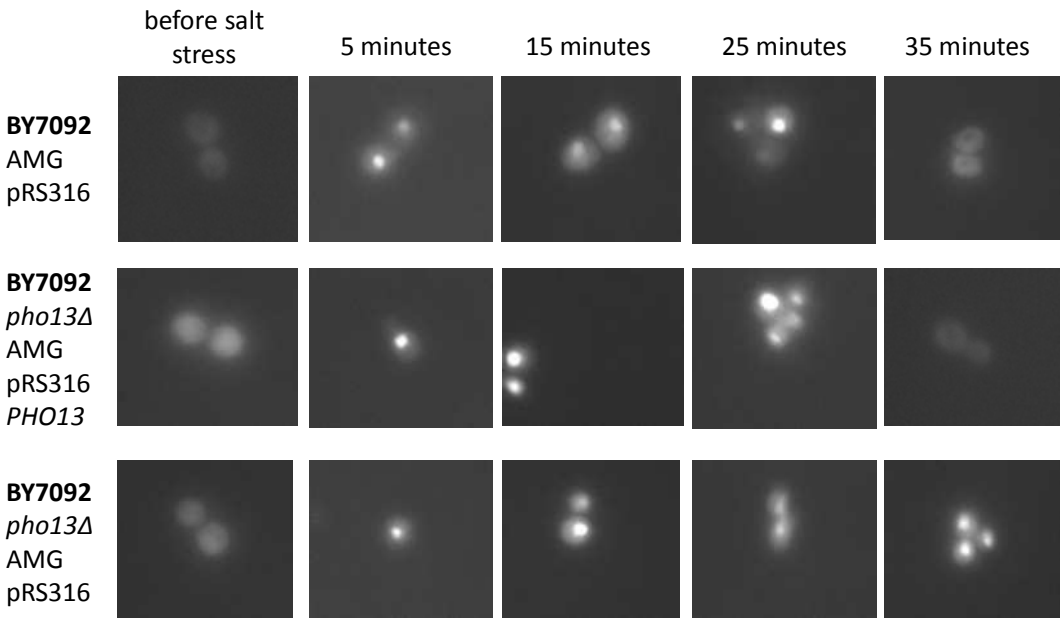


Figure R1.7: Salt stress experiment with documentation of the export of Msn2-GFP in different yeast strains

It could be observed that the export of Msn2p in the control yeast strain BY7092 Msn2-GFP occurred after 35 minutes incubation with 0,5M NaCl, whereas the strain BY7092 *pho13Δ* Msn2-GFP the export time of Msn2p from nucleus to the cytoplasm was duplicated. However, after 75 minutes export of Msn2p also occurred in the deletion mutant strain.

Thus Pho13 could be a novel regulator of the export of Msn2p from the nucleus into the cytoplasm. To show that this Msn2 localization phenotype is significant for Pho13 we performed a complementation assay. First a PCR was performed with the *PHO13* forward and reverse primers mentioned in the Materials and Methods part. Afterwards we digested this fragment and the pRS316 vector with the same enzymes (HindIII and Xho1) and ligated the fragment into the multiple cloning site of the vector. The last step was to transform this construct into the yeast strain BY7092 *pho13Δ* Msn2-GFP. We also transformed the empty pRS316 vector into the BY7092 Msn2-GFP (control strain) and BY7092 *pho13Δ* strain.

We repeated the experiment as described above.



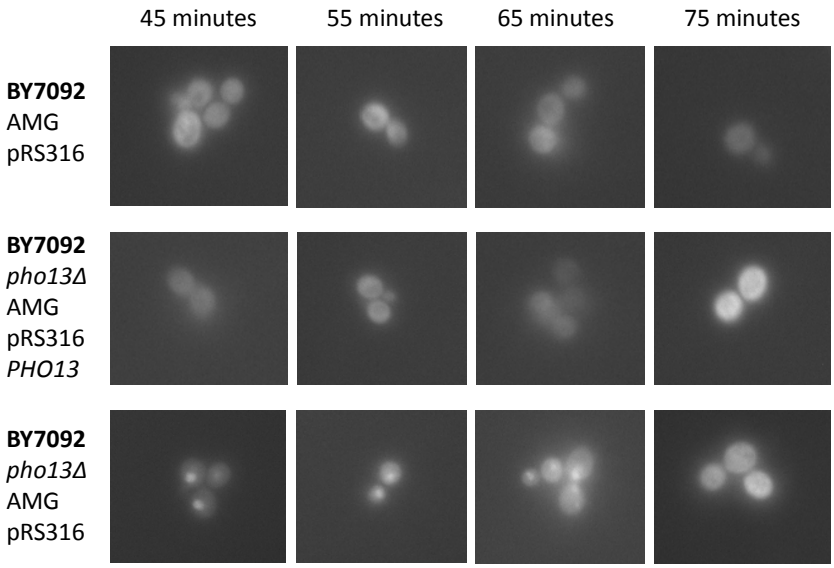


Figure R1.8: Salt stress complementation experiment with documentation of the export in different yeast strains

We found no difference in localization of Msn2-GFP between WT strain and BY7092 *pho13Δ* transformed with pRS316-*PHO13*. We observed complementation and thus we confirmed that *PHO13* is possibly a gene of interest. After 35 minutes, Msn2-GFP was localized in the cytoplasm in the BY7092 AMG pRS316 and BY7092 AMG *pho13Δ* pRS316-*PHO13* strain, whereas yeast strain BY7092 AMG *pho13Δ* pRS316 takes two times longer. We also counted (n=~50) cells with nuclear localization at each time point.

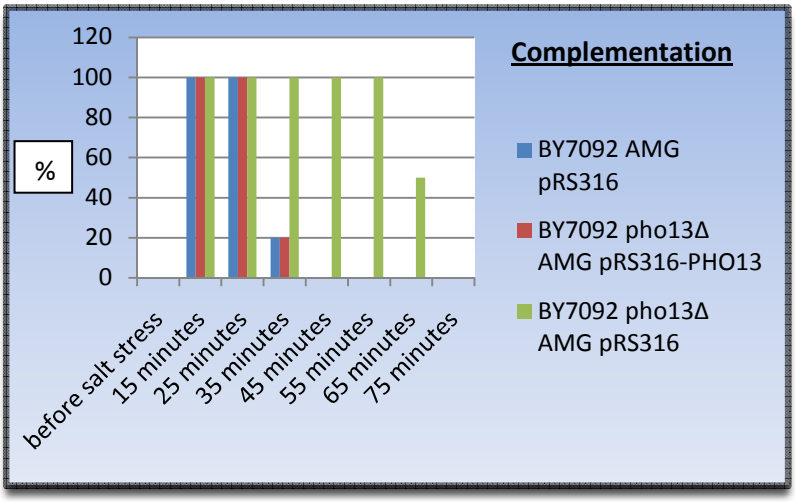


Figure R1.9: Cell counting experiment with nuclear localization

Further experiments will first measure the expression of Msn2 target genes (*CTT1, HSP12*) by Northern Blot approach with yeast strain BY7092 *pho13Δ*.

2.3. Discussion

Activation of Msn2 is accompanied by nuclear accumulation. The activation of Msn2 and nuclear accumulation occurs simultaneously and both processes might be coupled. Using the localization screen, with the Msn2-GFP reporter construct, we searched for mutant strains that resulted in a delayed export of the C_2H_2 Zn-finger transcription factor Msn2.

As an interesting factor we found Pho13. Pho13 (YDL236W) is an alkaline phosphatase and is specific for p-nitrophenyl phosphate. It also has protein phosphatase activity. The cellular components in which Pho13 is active is in the cytoplasm and the nucleus (Saccharomyces Genome Database; www.yeastgenome.org).

In mutants lacking Pho13, the nutrient utilization is increased and the competitive fitness is decreased. Alkaline phosphatases (ALPases) in *Saccharomyces cerevisiae* are the products of two structural genes (*PHO8* and *PHO13*) (Vogel & Hinnen, 1990). The enzyme product of *PHO13* is a monomeric protein and is specific to p-nitrophenyl phosphate (pNPP) (Attias & Bonnet, 1972, Toh *et al.*, 1976) or histidinyl phosphate. The physiological role of that enzyme is not clear and its natural substrate has not been identified (Tuleva *et al.*, 1998).

In one scenario Pho13 an alkaline phosphatase could act upstream of Msn2 as an inhibitor of nuclear export. The nuclear export sequence of Msn2 might only be accessible to the exportin Msn5 in the unstressed state and if PKA and TOR activity is high.

In the Msn2-GFP localization experiment we used the yeast mutant strain with an *MSN5* deletion as a negative control. In these cells Msn2 is always localized in the nucleus, comparable in cells with stressed conditions. Pho13 could be a factor that stabilizes the Msn2-Msn5 interaction.

One scenario that could exist is that in stressed conditions such as heat shock or osmotic stress the interaction of Msn2 and Msn5 is disrupted, and Msn2 is localized in the nucleus. Addition of glucose in wild type cells will result in Msn2-Msn5 interaction (stabilized by Pho13) followed by export of Msn2 from the nucleus into the cytoplasm.

In the yeast strain BY7092 Msn2-GFP *pho13Δ*, Pho13 does not exist and the interaction between Msn2 and Msn5 (the exportin) is not as stable as in the wild type cells. One result could be a delayed export of the C_2H_2 Zn-finger transcription factor Msn2.

In our case BY7092 Msn2-GFP *pho13Δ* needs 75 minutes for the export of Msn2 from the nucleus into the cytoplasm, whereas BY7092 Msn2-GFP only needs 35 minutes.

3. β-Estradiol induced expression of Msn2A1-6

3.1. Aim of this work

Msn2p contains six putative PKA (Protein kinase A) phosphorylation sites. Serine to alanine substitution of all protein kinase A consensus sites in Msn2p generates a constitutive hyperactive *MSN2-A1-6* allele. Expression of this hyperactive *MSN2-A1-6* allele causes G1 arrest and these cells have a similar phenotype as PKA deficient strains or cells that have been treated with glucose starvation. These observations indicate that Msn2p is part of the regulatory pathway that influences the response to stress and starvation conditions and that a cell cycle regulator downstream of Msn2p could exist. In previous experiments different candidate genes have been described in this context but none of them could rescue the Msn2A1-6 caused growth phenotype.

Previous work generated a conditional version of the Msn2A1-6 encoding gene, driven by the *CUP1* promoter (Durchschlag, Reiter, unpublished observation). The *CUP1* promoter can be induced with a low concentration of copper ions. Cells expressing the Msn2A1-6 protein had a severe growth defect.

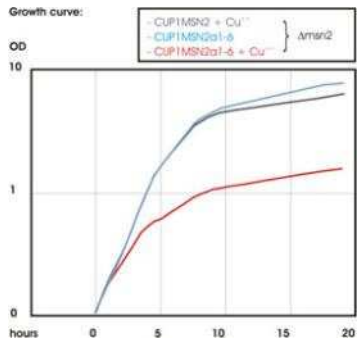


Figure A2.1: Growth curve of three different yeast strains (W. Reiter unpublished observation). The growth of cell cultures expressing either Msn2, or Msn2A1-6 was monitored by OD_{600} measurements.

The figure A2.1 above shows, that after one hour of induction with copper, cells expressing Msn2A1-6 reduce their growth rate in liquid cultures. Cultures expressing Msn2A1-6 had an increased number of cells in G1 phase of the cell cycle. Msn2A1-6 expressing cells showed also an increase in cell size even at lower copper concentration.

In our experiments with the hyperactive mutant of Msn2 expressing cells, we did not use a copper regulated but a β-Estradiol induced conditional system, because there are no side effects of copper and the system is tuneable.

The aim of this part of my Diploma thesis was to identify factors that rescue the Msn2A1-6 caused growth arrest.

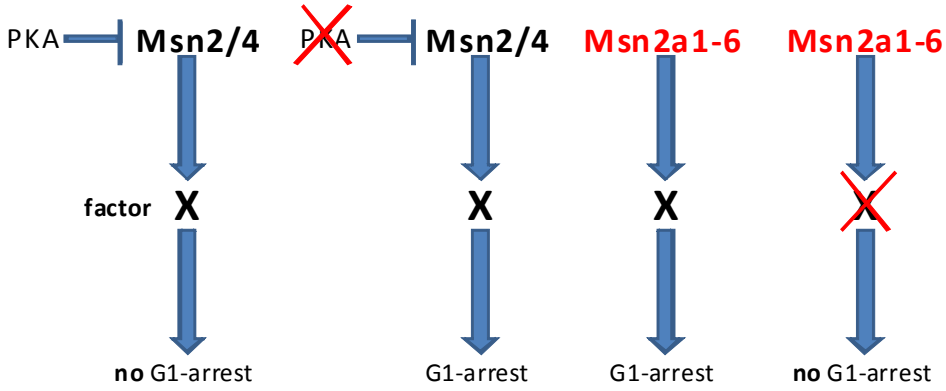


Figure A2.2: 4 different ways of regulation Msn2 are presented. PKA inhibits Msn2/4 and results in normal proliferation, whereas deletion of PKA or expression of the hyperactive Msn2A1-6 causes G1-arrest. The aim of the screen was to identify factors (X) that rescue the Msn2A1-6 caused growth phenotype.

3.2. Results

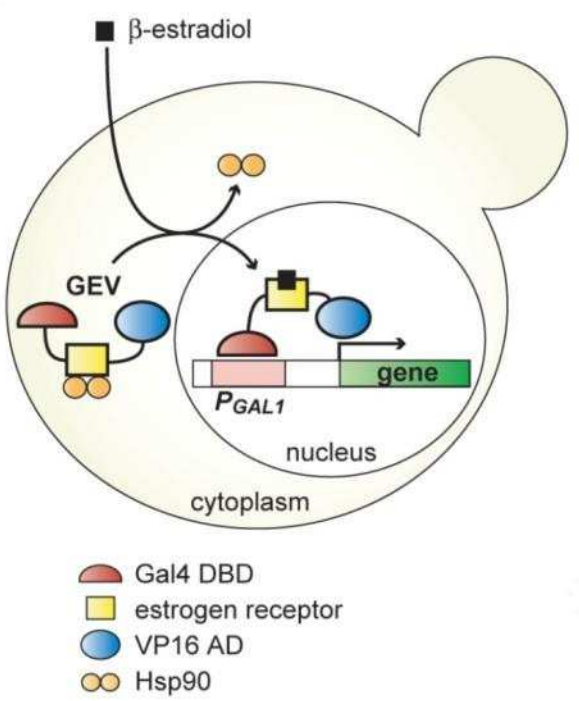


Figure R2.1: β-Estradiol inducible system (Takahashi & Pryciak, 2008)

Because they are hydrophobic molecules, estrogens easily diffuse across cell membranes. When inside a cell, estrogens bind to a highly specific, soluble estrogen receptor. The β-Estradiol inducible system consists of two different constructs. ADGEV contains the DNA binding domain of Gal4, the ER (Estrogen Receptor) Hormone binding domain and the VP16 (viral protein 16) transcriptional activator domain. In the absence of β-Estradiol, Hsp90 (heat shock protein 90), in yeast Hsp82 respectively, keeps the ADGEV construct inactive by binding to the estrogen receptor domain. In the presence of β-Estradiol, the hormone competes for binding to its receptor with Hsp90 and the ADGEV construct can enter the nucleus. The other construct contains a GAL1 promoter with the gene of interest, in this case Msn2A1-6.

3.2.1. Cloning steps for the β-Estradiol inducible system:

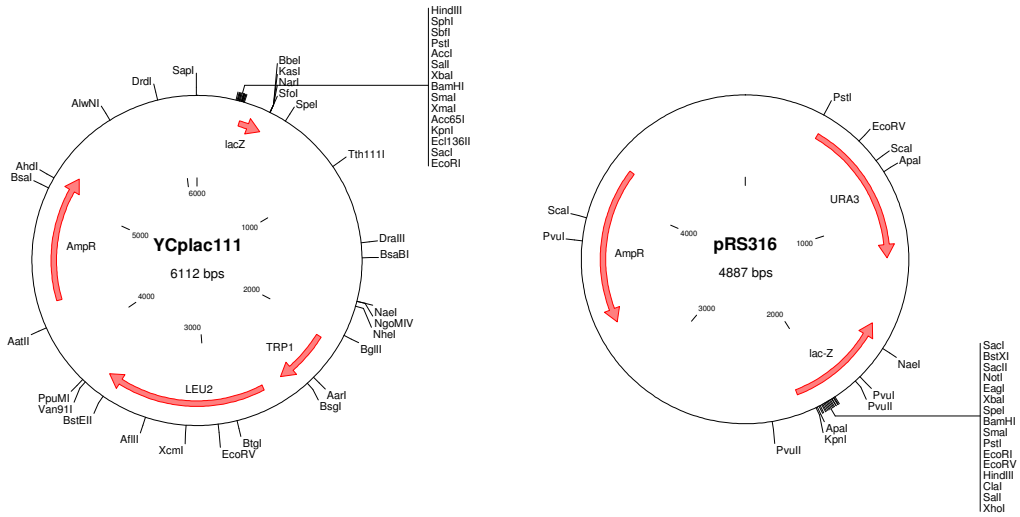


Figure R2.2: Plasmid map of YCplac111 and of pRS316 used in this work (Vector Database)

The first step was to transform the reporter construct p*GAL1*prMsn2A1-6 (source Dr. Wolfgang Reiter) into the BY7092 yeast strain to see if the expression of Msn2A1-6 works.

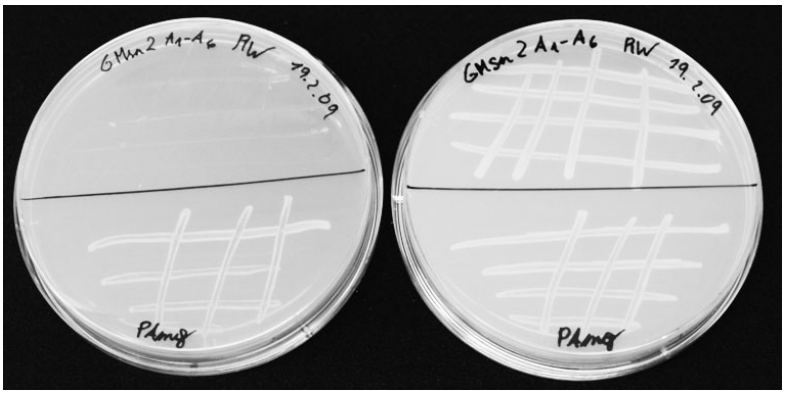


Figure R2.3: Control for Msn2A1-6 expression. On the right there is the SC-Leu control and on the left the SC-Leu+ galactose plate shown. The upper half of the plate represents the BY7092 p*GAL1*prMsn2A1-6 strain and the lower half of the plates represents the BY7092 p*GAL1*prMsn2 control strain.

In figure R2.3 the BY7092 p*GAL1*prMsn2A1-6 strain didn´t grow on SC-Leu+ Galactose plates, whereas the BY7092 p*GAL1*prMsn2 control strain grew on both the control plate and the Galactose containing plate. This result indicates, that Msn2A1-6 is expressed from these constructs.

I cloned ADGEV sequence (which contains the *GAL4* DBD, ER HBD and the VP16) into the multiple cloning site (HindIII and BamHI) of a centromeric vector pRS316 and transformed both constructs, the ADGEV and p*GAL1*prMsn2A1-6 reporter gene construct into the yeast strain BY7092.

3.2.2. Adaptation of the β-Estradiol-inducible system:

In a next step we determined the working concentration for the β-Estradiol inducible system for Msn2A1-6. A hormone concentration to 15nM and lower and came to the reference region.

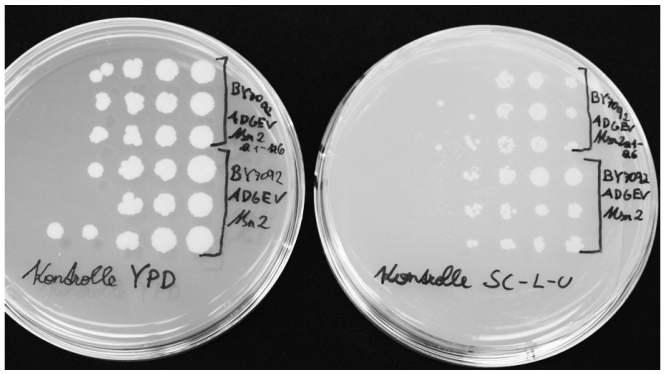


Figure R2.4: Growth control of both strains, BY7092 p*GAL1*prMsn2a1-6 (upper three lanes) and BY7092p*GAL1*prMsn2 (lower three lanes).

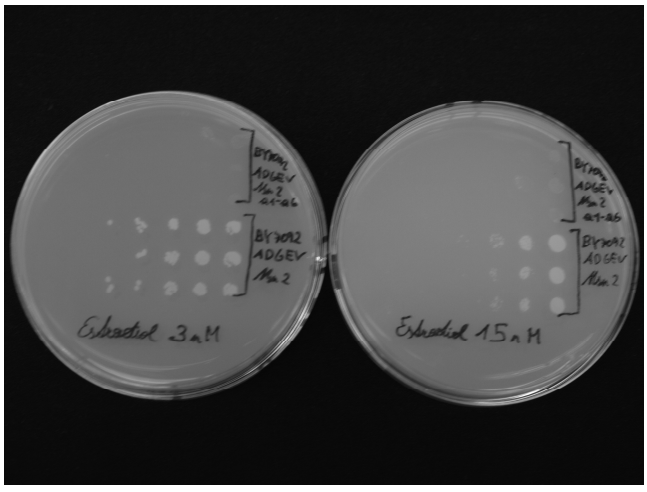


Figure R2.5: Optimization of the β-Estradiol inducible system. (3nM and 15nM)

On SC-Leu-Ura plates with a β-Estradiol concentration of 3nM and 15nM the strain with hyperactive Msn2 (Msn2A1-6) did not grow, whereas the control strain grew on both

plates. Interestingly growth of the control strain was also markedly reduced at higher concentration of β-Estradiol (15nM).

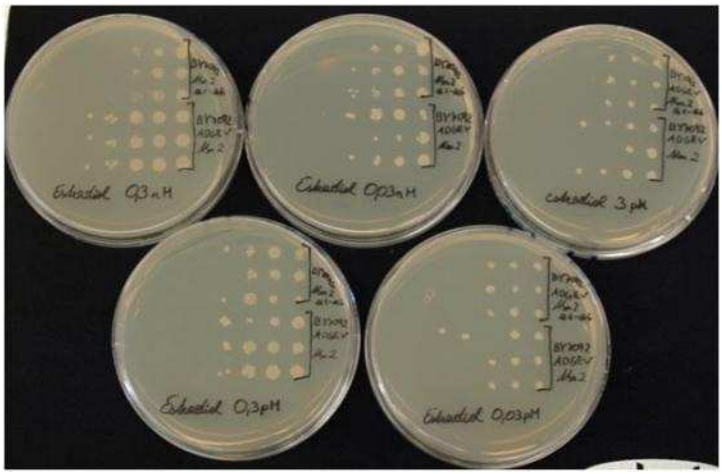


Figure R2.6: 0,3nM, 0,03nM, 3pM, 0,3pM, 0,03pM

Figure R2.6 represents growth results of strains with lower concentrations of β-Estradiol.

It illustrates that BY7092 strain carrying both ADGEV and p*GAL1*prMsn2A1-6 is sensitive to 0,3nM β-Estradiol in the medium but not completely inhibited. Therefore, this concentration was chosen to screen with the hyperactive Msn2A1-6 allele.

3.2.3. Gene expression levels of Msn2A1-6

After optimizing the β-Estradiol concentration we wanted to verify the gene expression levels of Msn2A1-6, for that we performed a Northern Blot. I used four different strains (BY7092 ADGEV Msn2; BY7092 ADGEV Msn2A1-6; BY7092 ADGEV Msn2A6; BY7092 ADGEV YCplac111) to show if target genes of Msn2A1-6 are expressed. The empty plasmid YCplac111 was used as a negative control.

Northern Blot

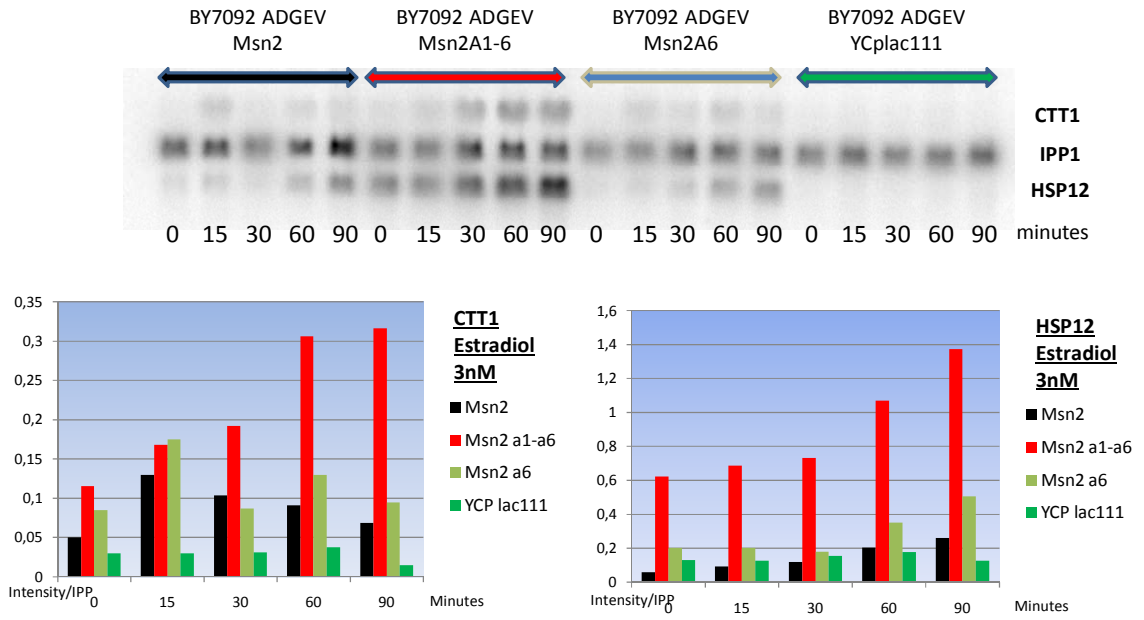


Figure R2.7: Northern Blot of Msn2 target genes with different strains and constructs.

Expression of genes was induced by adding 3nM of β-Estradiol. At 5 time points samples were taken and RNA levels analyzed with Northern Blots. I used three labeled probes recognizing *CTT1*, *IPP1* as a reference and *HSP12*. As expected, the hyperactive Msn2A1-6 allele leads to increased expression of *CTT1* and *HSP12* mRNA. *HSP12* is a plasma membrane localized protein that protects membranes from desiccation. *HSP12* is induced by heat shock, oxidative stress, osmostress, stationary phase entry, glucose depletion, oleate and alcohol. *CTT1*, named as cytosolic catalase T, has a role in protection from oxidative damage by hydrogen peroxide (Gasch et al., 2000).

This result also shows, that transcriptional control in hyperactive strain is partially leaky, because we observed a low but detectable expression of *CTT1* and *HSP12* even if there was no hormone added. To get a deeper insight into the global role of Msn2A1-6 we performed global transcript profiling.

3.2.4. Microarray analysis of the Msn2A1-6 transcript profile:

Transcriptional profiles were determined by *Saccharomyces cerevisiae* microarrays with BY7090 ADGEV p*GAL1*prMsn2A1-6 strain with a 3nM β-Estradiol treatment.

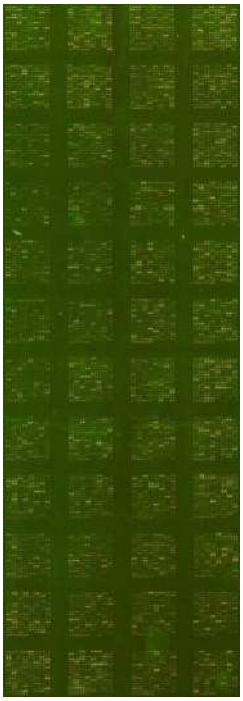


Figure R2.8: Microarray

Samples were taken of a control strain (Msn2) and the hyperactive Msn2A1-6 strain at 90 minutes time point. The total mRNA levels of cells expressing ADGEV Msn2 or ADGEV Msn2A1-6 was compared by hybridization of the labeled cDNA to 2 microarrays.

Microarray data of Msn2A1-6 induced genes (with 3nM β-Estradiol) is shown in the Appendix part.

GO_term	Cluster frequency	P-value
response to stimulus	79 out of 365 genes, 21.6%	3.64e-06
response to stress	59 out of 365 genes, 16.2%	1.11e-05
response to oxidative stress	17 out of 365 genes, 4.7%	0.00044

Table R2.9: GO terms of the first 365 induced genes (3nM β-Estradiol)

Microarray results show that 59 out of 365 genes belong to the response to stress, such as *HSP30* and *HSP42*. 17 out of 365 genes belong to response of oxidative stress, such as *HSP12*, *CTT1* (we expected those two) and *HSP104*.

Interestingly 14 out of the 100 strongest repressed are involved in the galactose metabolism, such as *GAL7*, *GAL10*, *GAL3*, *GAL4*.

3.2.5. SGA (Synthetic genetic array analysis):

To identify factors that rescue the G1-arrest, I used the SGA method.

The SGA method is in detail described in the materials and methods part. The last step of the SGA method is to get *MATa* cells with two constructs (ADGEV; pGAL1prMSN2A1-6) and different Euroscarf deletions. To identify some factors that rescue the G1-arrest we wanted to perform some β-Estradiol screens. So I started to pin the resulting cells on two different plates: SC-H-L-U+Kan plates as a control and SC-H-L-U+Kan plates with different concentrations of β-Estradiol (1nM, 2nM, 3nM).

We let them grow on room temperature for at least five days and scanned these cells every day. We compared the growth of all colonies with the Gene Pix Pro program. After sorting out all the false positive candidates, I identified 6 positive candidate deletion mutants that rescued the G1-arrest.

All primary isolated yeast strains and their catalase results are shown in the Appendix part (Table A3).

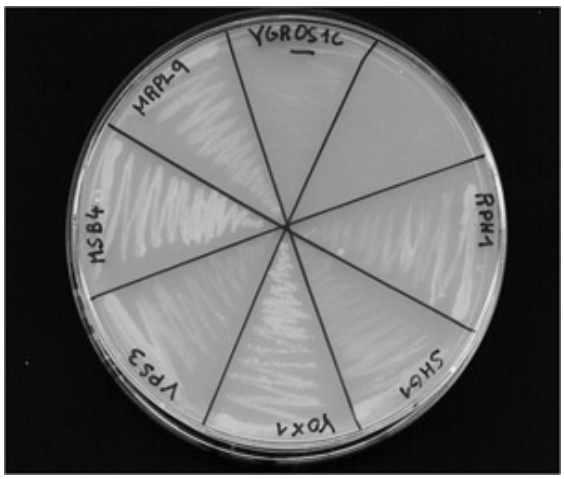


Figure R2.10: 6 positive candidates on SC-H-L-U+Kan +2nM β-Estradiol plates

In Figure R2.10 we used YGR051C, which is the deletion strain eliminating a dubious ORF, as a negative control. Accordingly, this strain shows no growth in the screen.

Gene	Function
SHG1	Subunit of the COMPASS (Set1C) complex, which methylates histone H3 on lysine 4 and is required in transcriptional silencing near telomeres
YOX1	Homeodomain-containing transcriptional repressor, binds to Mcm1p and to early cell cycle boxes (ECBs) in the promoters of cell cycle-regulated genes expressed in M/G1
VPS3	Component of CORVET tethering complex; cytoplasmic protein required for the sorting and processing of soluble vacuolar proteins, acidification of the vacuolar lumen, and
MSB4	GTPase-activating protein of the Ras superfamily that acts primarily on Sec4p, localizes to the bud site and bud tip, has similarity to Msb3p; msb3 msb4 double mutation causes
MRPL9	Mitochondrial ribosomal protein of the large subunit
RPH1	JmjC domain-containing histone demethylase which can specifically demethylate H3K36 tri- and dimethyl modification states; transcriptional repressor of PHR1; Rph1p

Table R3.11: 6 positive candidates; Description of functions are taken from the Saccharomyces Genome Database

3.2.6. Catalase measurement of β-Estradiol induced expression of Msn2A1-6

To control if the expression and constructs are working and to exclude false positive candidates we wanted to accomplish some catalase measurements.

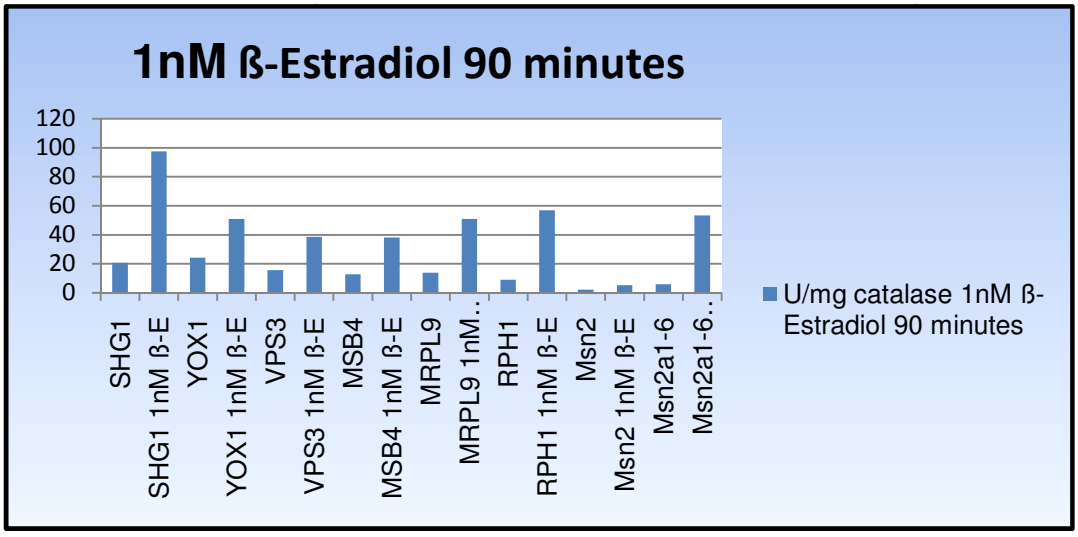


Figure R2.12: Catalase measurements of 6 candidates

For catalase measurements samples were taken with and without 1nM β-Estradiol induction of 6 positive candidates. The strain BY7092 plus ADGEV p*GAL1*prMsn2 was used as a negative control and ADGEV p*GAL1*prMsn2A1-6 as a positive control. Catalase

measurements show, that in all candidate strains the constructs are working because nearly the same catalase expression was measured as in the positive control.

3.3. Discussion (β-estradiol induced expression of Msn2A1-6):

Msn2p is part of the regulatory pathway that influences the response to stress and starvation conditions. A cell cycle regulating factor downstream of Msn2p could exist.

In our approach, we searched for factors that rescue the Msn2A1-6 caused growth arrest and identified six candidate genes. Rph1 seemed to be the most interesting. Rph1 is a JmjC domain-containing histone demethylase which can specifically demethylate H3K36 tri-and dimethyl modification states (Klose *et al.*, 2007). It is a transcriptional repressor of *RPH1* and is localized in the nucleus.

Histone modification is a very important posttranslational modification that contributes to chromatin-based processes including transcriptional regulation, DNA repair and epigenetic inheritance (Martin & Zhang, 2005). In yeast *Saccharomyces cerevisiae*, histone lysine methylation occurs on histone H3 lysines 4, 36 and 79. This modification can be dynamically regulated through histone demethylase enzymes. H3K36 methylation is tightly coupled to the process of active transcriptional elongation.

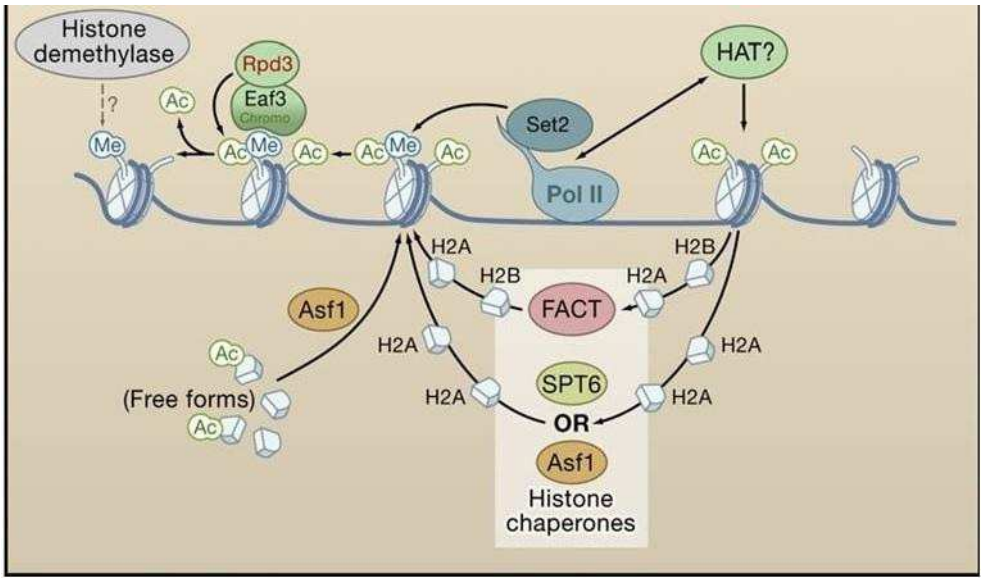


Figure D2.1: "Maintenance of nucleosomal stability during transcription. When Pol II migrates into promoter-distal regions, where the influence of activator-dependent HATs is diminishing, Pol II requires other HATs (elongators or those associated with Pol II) to acetylate the nucleosome in front of elongation machinery. The passage of Pol II causes histone displacement. Subsequently, these histones are redeposited onto the DNA behind Pol II via concerted actions of histone chaperones. Alternatively, the free forms of histones in the nucleus are also available for reassembly. These newly deposited nucleosomes are somehow hyperacetylated and are immediately methylated by Set2. Methylation of H3K36 is then recognized by the chromodomain of Eaf3, which in turn recruits the Rpd3S deacetylase complex. Rpd3S removes the acetyl marks and leaves the

nucleosome in a stable state. Methylation of H3K36 is eventually eliminated by a histone demethylase when the gene turns off "(Li *et al.*, 2007).

Beside Jhd1, Rph1 is the second budding yeast JmjC-domain-containing protein which demethylates H3K36. Interestingly, overexpression of Rph1 results in a severe inhibition of cell growth, suggesting that elevated levels of Rph1 have a detrimental effect on cell function (Klose *et al.*, 2007).

Rph1 has also been previously (Jang *et al.*, 1999) been shown to function as a transcriptional repressor, suggesting that the growth defect may be related to the silencing of genes involved in cell division or other growth related pathways. Deletion of the ZF domain completely abrogates the growth defect and indicates that the DNA binding ZF is important for growth suppression, perhaps functioning as a targeting mechanism for Rph1-mediated repression (Jang *et al.*, 1999).

According to our preliminary results, Rph1 seems to be a cell cycle regulator that functions downstream of Msn2p.

Shg1, Yox1, Vps3, Msb4 and Mrpl9 are also candidates resulting from our β-Estradiol screen but we did not find a plausible relationship to Msn2 and these candidates in the literature. The next step, to find out if the growth effect of those candidates is dependent on the yeast strain BY7092 or not, would be to create KanMX deletion strain of these candidates in an independent strain (for example W303). That approach would show if the observed growth effect of those candidates is indeed related to these genes or if they are false positives.

4. Valinomycin - Chemogenomics

4.1. Aim of this work (Valinomycin)

Among the membrane-bounded organelles of the cell, mitochondria represent ubiquitous structures enclosed by outer and folded inner membrane. While the mitochondrial outer membrane limits the organelle spatially, the mitochondrial inner membrane containing the ATP-generating machinery represents the main permeability barrier to ions and most metabolites (Saraste, 1999).

Mitochondria regulate many cell functions. Mitochondria control several fundamental cellular processes like ATP synthesis, regulation of intermediate metabolism, generation of reactive oxygen species and volume homeostasis.

Valinomycin is a member of the group of natural neutral ionophores.

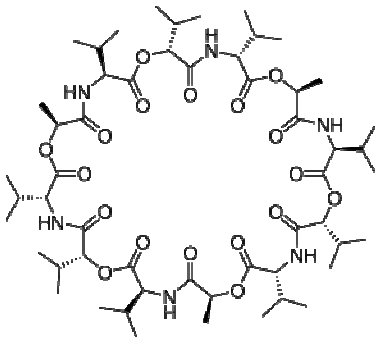


Figure A3.1: Valinomycin

Valinomycin is a dodecadepsipeptid that is made of twelve alternating amino acids and esters to form a macrocyclic molecule. The K^+ ionophore valinomycin allows a rapid electrophoretic uniport of K^+ ions across the mitochondrial inner membrane (Petrezselyova *et al.*, 2008).

Aim of this approach was to identify genes that are induced by treatment with valinomycin in *Saccharomyces cerevisiae*. For that chemo genomic screen we used the Euroscarf Deletion library and the RoTor yeast robot for the pinning steps.

4.2. Results Valinomycin

Previous microarrays analysis (Hosiner unpublished data) showed that stress-induced genes like *DDR2*, *HSP12* and *HSP26* were expressed by treatment with valinomycin.

ORF	NAME	Description	log_Av_MR	log_Av_MR_15
YMR175W	SIP18	Protein of unknown function whos	2,250	2,347
YNL160W	YGP1	Cell wall-related secretory glyc	1,904	1,409
YOL052C-A	DDR2	Multistress response protein, ex	1,818	1,252
YLR355C	ILV5	Acetohydroxyacid reductoisomeras	1,771	0,617
YDR033W	MRH1	Protein that localizes primarily	1,726	0,998
YBR054W	YRO2	Putative protein of unknown func	1,608	1,665
YOR120W	GCY1	Putative NADP(+) coupled glycero	1,436	0,617
YLR164W		Mitochondrial inner membrane of	1,368	1,743
YKL197C	PEX1	AAA-peroxin that heterodimerizes	1,367	0,138
YCR022C		Dubious open reading frame unlik	1,326	0,826
YHR090C	YNG2	Subunit of the NuA4 histone acet	1,298	0,291
YGR236C	SPG1	Protein required for survival at	1,240	2,367
YFL014W	HSP12	Plasma membrane localized protei	1,228	1,980
YBR072W	HSP26	Small heat shock protein (sHSP)	1,226	1,789
YDR342C	HXT7	High-affinity glucose transporte	1,203	0,238
YPR149W	NCE102	Protein of unknown function; con	1,197	1,238
YOR075W	UFE1	t-SNARE required for ER membrane	1,166	0,291
YML052W	SUR7	Putative integral membrane prote	1,158	0,262
YOR119C	RIO1	Essential serine kinase involved	1,141	0,359
YIL130W	ASG1	Zinc cluster protein proposed to	1,131	0,662
YNL260C		Putative protein of unknown func	1,119	0,431
YOR303W	CPA1	Small subunit of carbamoyl phosp	1,108	1,054
YGR290W		Dubious open reading frame unlik	1,085	0,393
YGR216C	GPI1	Membrane protein involved in the	1,053	0,506
YDR070C	FMP16	Putative protein of unknown func	1,048	1,941
YPR010C	RPA135	RNA polymerase I subunit A135	1,045	1,145
YJL170C	ASG7	Protein that regulates signaling	1,026	0,309
YNL272C	SEC2	Guanyl-nucleotide exchange facto	1,019	0,332
YMR196W		Putative protein of unknown func	0,998	0,685
YGL047W	ALG13	Catalytic component of UDP-GlcNA	0,998	0,199
YOR121C		Dubious open reading frame unlik	0,976	0,275
YMR107W	SPG4	Protein required for survival at	0,971	1,701
YMR296C	LCB1	Component of serine palmitoyltra	0,966	0,620
YFL012W-A		Dubious open reading frame unlik	0,965	2,005
YDR533C	HSP31	Possible chaperone and cysteine	0,950	0,581
YMR103C		Dubious open reading frame unlik	0,949	0,518
YML091C	RPM2	Protein subunit of mitochondrial	0,948	0,221

YBR065C	ECM2	Pre-mRNA splicing factor, facili	0,909	0,311
YER175C	TMT1	Trans-aconitate methyltransferas	0,903	0,559
YDL204W	RTN2	Protein of unknown function; has	0,902	1,173
YPR026W	ATH1	Acid trehalase required for util	0,900	0,433

Table R3.1: Microarray analysis with stress genes highlighted in red (Dagmar Hosiner and Christoph Schüller unpublished Data)

Comparing these genes, shown in Table R3.1, with the Msn2A1-6 induced genes it is interesting to see that several heat shock proteins such as *HSP12* and *HSP31* and also *SIP18*, *RTN2* and *DDR2* are induced in both microarray approaches. That indicates that in both approaches stress genes were induced.

We used YPD plates as control plates and YPD plates containing valinomycin (15mg/l media) to compare the growth of each deletion strain. We increased the density of the cells from format 96 to 384 and pinned them onto these two types of cells.

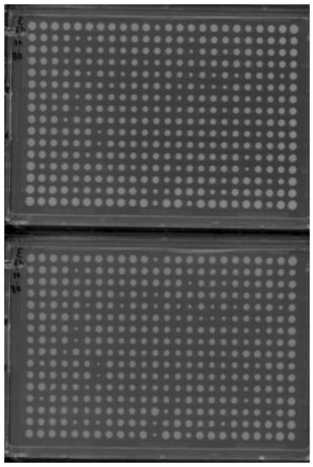


Figure R3.2: Yeast strains pinned on YPD plates (upper half) and YPD plates+ valinomycin (lower part)

The next step was to scan all plates for the next 5 days and analyze the growth of all yeast deletion strains with a special program, called Gene pix pro, which measures the diameter of each spot. We developed a formula for analyzing all of the 4950 Euroscarf deletion strains.

Fitness score (FS) = $\{(Day1+2)/(Day4+5)\}_{with}$ / $\{(Day1+2)/(Day4+5)\}_{without}$ valinomycin
Average fitness score (AFS) = $(1/3)\times\Sigma\ FS_{(1-3)}$
AFS = >1 Growth effect
AFS = <1 no Growth effect
LogAFS (LAFS) = $\log_2$ AFS

We compared the genes affected by Valinomycin with their expression profile by cluster analysis. This data is shown in the Appendix part in detail.
The next step was to analyze the 365 genes with the "GO term finder" program (Saccharomyces Gemome Database; www.yeastgenome.org)

GO_term	Cluster frequency
mitochondrion organization	55 out of 365 genes, 15.1%
generation of precursor metabolites and energy	33 out of 365 genes, 9.0%
mitochondrial translation	24 out of 365 genes, 6.6%
energy derivation by oxidation of organic compounds	26 out of 365 genes, 7.1%
aerobic respiration	17 out of 365 genes, 4.7%
mitochondrial respiratory chain complex assembly	9 out of 365 genes, 2.5%
cellular respiration	18 out of 365 genes, 4.9%
cation transport	17 out of 365 genes, 4.7%
protein complex assembly	21 out of 365 genes, 5.8%
protein complex biogenesis	21 out of 365 genes, 5.8%
mitochondrial respiratory chain complex IV assembly	6 out of 365 genes, 1.6%
respiratory chain complex IV assembly	6 out of 365 genes, 1.6%
organelle organization	84 out of 365 genes, 23.0%
ion transport	18 out of 365 genes, 4.9%
cellular component organization	113 out of 365 genes, 31.0%
oxidative phosphorylation	10 out of 365 genes, 2.7%

Table R3.4: GO term results of the first 365 induced genes

GO term	Frequency
transport	61 out of 365 genes, 16.7%
mitochondrion organization	55 out of 365 genes, 15.1%
translation	44 out of 365 genes, 12.1%
protein modification process	39 out of 365 genes, 10.7%
response to stress	35 out of 365 genes, 9.6%
RNA metabolic process	34 out of 365 genes, 9.3%
generation of precursor metabolites and energy	33 out of 365 genes, 9.0%
response to chemical stimulus	26 out of 365 genes, 7.1%
cell cycle	24 out of 365 genes, 6.6%
transcription	23 out of 365 genes, 6.3%
vesicle-mediated transport	23 out of 365 genes, 6.3%
cellular carbohydrate metabolic process	21 out of 365 genes, 5.8%
membrane organization	21 out of 365 genes, 5.8%
protein complex biogenesis	21 out of 365 genes, 5.8%
DNA metabolic process	21 out of 365 genes, 5.8%
cellular lipid metabolic process	19 out of 365 genes, 5.2%
signal transduction	19 out of 365 genes, 5.2%
cellular respiration	18 out of 365 genes, 4.9%
cellular component morphogenesis	18 out of 365 genes, 4.9%
cofactor metabolic process	17 out of 365 genes, 4.7%
heterocycle metabolic process	16 out of 365 genes, 4.4%
cellular amino acid and derivative metabolic process	15 out of 365 genes, 4.1%

Table R3.5: GO term results of the first 365 induced genes

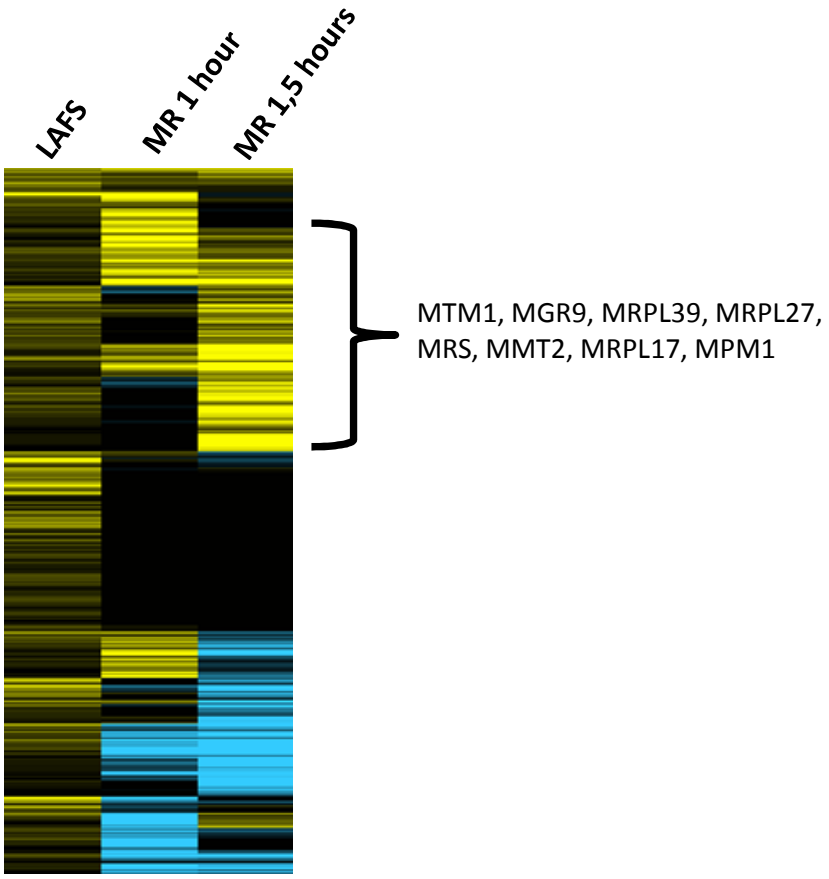


Figure R3.6: Cluster of genes induced and repressed after treatment with valinomycin. The left column shows logarithmic Average fitness score, the middle and right column show microarray results after 1hour and two hours of treatment with valinomycin. Induced genes are shown in yellow and repressed genes are shown in blue.
MTM1, *MGR9*, *MRPL39*, *MRPL27*, *MRS*, *MMT2*, *MRPL17* and *MPM1* (to name some) represent genes, induced after treatment with valinomycin and required for mitochondrial organization.

All genes that are induced and repressed after treatment with valinomycin are shown in the Appendix part.

4.3. Discussion

Valinomycin and nigericin are examples of mobile carrier ionophores and act selectively on the mitochondrial inner membrane of *Saccharomyces cerevisiae*. The K^+ ionophore valinomycin allows a rapid electrophoretic uniport of K^+ ions across the mitochondrial inner membrane.

Aim of this chemo genomic approach was to identify genes that are important for valinomycin action in *Saccharomyces cerevisiae*. We used the Euroscarf Deletion library. Previous microarray data (Hosiner/Schüller unpublished data) showed that several stress genes were induced by treatment with valinomycin, so we used a chemo genomic screen to identify those genes.

The GO term analysis showed that 55 out of the 365 genes (15,1%) are involved in the mitochondrial organization, like *MDM10*, *MRP21*, *MRPL11* and *MRP1* by treatment with valinomycin. This result confirms that mitochondria are primary targets of valinomycin. This observation overlaps with earlier results (Petrezselyova et al., 2008). Interestingly, 35 out of 365 genes refer to response to stress, like *TPS1*, *RPH1* (one of the candidates in our SGA screen) and *PHO4*. *PHO4* is an interesting gene because it is also induced in our Msn2A1-6 microarray. Pho4 is a basic leucin zipper transcription factor of the yAP-1 family and mediates pleiotropic drug resistance and salt tolerance. Pho4 is localized nuclear under oxidative stress (Saccharomyces Genome Database). 17 out of 365 genes refers to the cation transport such as *ATP5*, *ATP17*, *ADY2* and *MTM1* to name some, that reflects that K^+ ionophore valinomycin allows a rapid electrophoretic uniport of K^+ ions across the mitochondrial inner membrane. 23,0% of the 365 induced genes refers to the organization of the organelles such as *SPO7*, *MDM10*, *GEM1*, *BUD14* and *MRP21* to name some.

The antibiotic valinomycin passes probably through the plasma membrane and subsequently affects the permeability of the mitochondrial inner membrane. Through that screen we could identify the different clusters of the genes induced in cells by treatment with valinomycin.

An interesting approach for the future to find out more about valinomycin could be to perform a modified screen with the Euroscarf deletion library.

The PDR pathway in *Saccharomyces cerevisiae* is constituted by three major gene networks involved in drug resistance. The first, including *SNQ2*, *PDR5*, and *YOR1* (Sipos & Kuchler, 2006) encodes the ATP-binding cassette (ABC) transporters; the second including *ATR1* and *SGE1* (Prasad *et al.*, 2002), codes for the major facilitators superfamily (MFS) proteins. The third class of genes encodes the transcriptional

activators Pdr1 and Pdr3, which regulate the PDR network (Katzmann *et al.*, 1996) (Petrezselyova et al., 2008).

The improved query strain could be the yeast strain BY7092 lacking the plasma membrane ATP-binding cassette (ABC) transporter genes *PDR5* and *SNQ2*. Using this mutant we expect more specific results from a SGA screen with valinomycin.

5. Materials and Methods

Yeast strains used in this study

Strains	Genotype	Source
BY-4741	*MAT***a** *ura3-_0 his3-_1 leu2-_0 met15-_0*	EUROSCARF Collection
BY-7092	*MAT can1*::P_{STE2}-*Sp_HIS5 lyp1 his31 leu20 ura30 met150 LYS2*$^{+}$	Boone C.
BY-Deletions	*GEN_kanMX4* deletions of 4950 genes	EUROSCARF Collection

Table M1: Yeast strains used in this work

Oligos and plasmids used in this study

Plasmids	References
pRS316	Sikorsky et al. 1989
YCplac111	Gietz et al. 1988
YCplac111 Adh1-Msn2-GFP (AMG)	Görner et al. 1998

Table M2: Plasmids used in this work

Oligos	Sequence
Msn2 for	TCATGCTTTCTATGGGGAAT
M13 univ for	TGTAAAACGACGGCCAGT
M13 univ rev	CAGGAAACAGCTATGACC
Pho13 for	GCAACTACCCGACGTCATTGG
Pho13 rev	CCCCAACAAGACCGAATTGG

Table M3: Oligos used in this work

Methods

5.1. SGA:

SGA is an abbreviation for *synthetic genetic array analysis*. It is very efficient for assessing the biological roles of genes *in vivo* and it is a powerful tool for identifying components of specific pathways and for ordering the function of gene products within a pathway. For the budding yeast *Saccharomyces cerevisiae*, 1000 essential genes and about 5000 non essential genes were identified. In our case the Euroscarf deletion library was used, which contains 4940 kanMX deletion strains (xxxΔ::kanMX) (Tong & Boone, 2006).

SGA procedure:

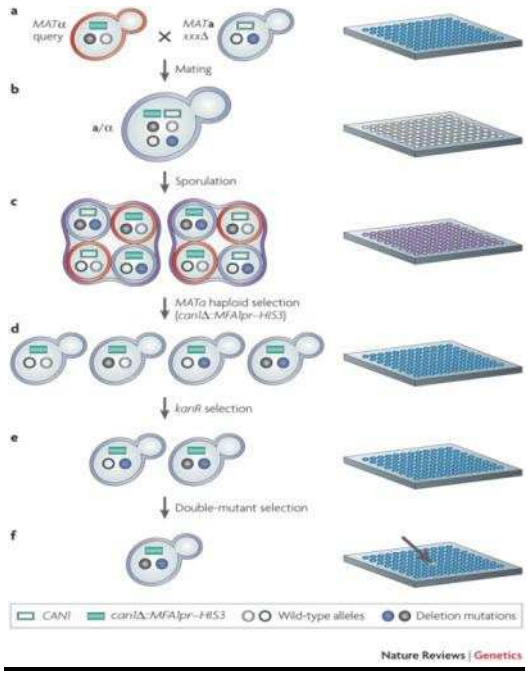


Figure M1: The synthetic genetic array (SGA) methodology (Boone *et al.*, 2007)

The "query" strain BY7092 has the genotype MATα *can1Δ::MFA1pr-HIS3* in which *MFA1pr-HIS3* is integrated into the genome so that it deletes the ORF of the *CAN1* gene, which normally confers resistance to canavanine. Afterwards the query strain is crossed to the ordered Euroscarf deletion library strains (MAT a xxxΔ::kanMX). In each of these strains one gene is disrupted by a dominant selectable marker (kanMX). The heterozygous diploid cells are then pinned to a solid media with reduced carbon and nitrogen source to induce sporulation and the formation of haploid meiotic spore progeny. These spores are then transferred to a media that lacks histidine, which allows for selective germination of *MATα* meiotic progeny because these cells express the *MFA1pr-HIS3* gene. The last step is to pin the *MATα* meiotic progeny on media containing histidine and kanamycin, which selects for haploid deletion mutants.

5.2. Northern Blot:

RNA Extraction (PCI Extraction)

Yeast cultures were grown from OD_{600}=0,3 untill logarithmic phase (OD_{600}=1,0) and stressed. After that cells were harvested for 2 minutes at 2500rpm. The pellets were frozen in liquid nitrogen and stored at -80°C. Frozen cell samples were thawn and resuspended in 200µl of RNA-extraction buffer. The samples were vortexed briefly and glass beads (2/3 of total volume) were added. After vortexing briefly, the samples were mixed with 200µl of phenol-chloroform-isoamylalcohol (PCI) solution.

The yeast cells were broken using a Thermo Savant "FastPrep" machine, two times for 15 seconds, at speed 6 and afterwards centrifuged for 20 minutes, at 13k rpm at 4°C in a microcentrifuge. The upper phase was taken off (180-190µl) and an equal amount of chloroformisoamylalcohol (CI) solution was added. The two phases were mixed by inverting briefly and then centrifuged for 15 minutes at 4°C. The procedure was repeated (130-140µl of upper layer, equal amount of CI) then 120µl were taken off and 1/20 volume of 4M Sodium Acetate and two volumes of absolute ethanol were added. For precipitation the samples were put on -20°C for one to two hours. Afterwards the tubes were centrifuged for 10 minutes at full speed in cold room and after removing the supernatant the RNA pellets were washed with 70% ethanol (2 min. full speed, RT).The pellets were incubated at 37°C for 30 minutes and then resuspended in 50µl DEPC water.
The concentration was determined as follows: RNA solutions were diluted 1:400 in water and measured at OD260/280 in spectrophotometer. The amount of RNA was calculated by the formula: OD1 at 260= 40µg/ml. 15µg- 20µg were loaded on 1% agarose gels.

Sample preparation and Gel electrophoresis:

Sample preparation:

RNA and DEPC- water	4.5µl (containing 15-20µg total RNA)
5x FGRB	2 µl
Formaldehyde (37%)	3.5µl
Formamide	10µl
Sum	20µl

The samples were incubated on 62°C for 20 minutes followed by quick spin. 2µl of DEPC-treated Loading buffer were added to each sample. The gels were run limited at 65V for six hours.

Capillary Blotting:

Three Whatman papers soaked with 20×SSC were put into a blotting apparatur filled with 20x SSC. The agarose gel was put on the Whatman papers and the transfer membrane (HybondTM, Amersham) was added on the top. The membrane was fixed with short parafilm strips. And 3 Whatman papers soaked in 20X SSC were put on the sandwich followed by a pack of paper towels and glass plates. Some weights were used to start the procedure.

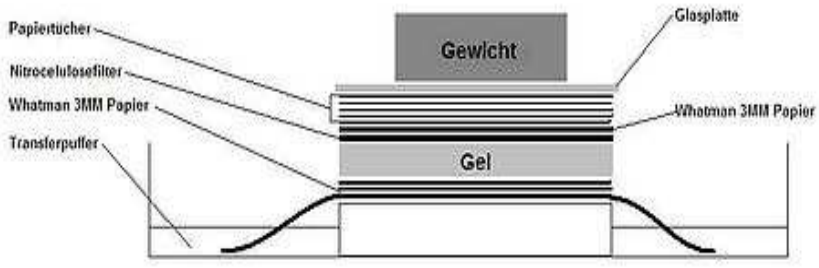


Figure M2: Composition of capillary blotting equipment

Crosslinking, Dying and Prehybridisation

The Nylon membranes were crosslinked with 150kJ on both sides and then incubated with 10% Acetic Acid for 15-25 minutes. After removing a 5% Acetic Acid solution with Methylen Blue was added to dye ribosomal RNA and check for possible RNA degradation. The dye was removed with water for two hours. Afterwards the membranes were put into glass bottles filled with 25ml of prehybridisation buffer and incubated on 63°C (in a hybridisation oven) over night (12-15 hours).

Preparation of the Probes and Hybridisation

Amplification of Northern Blot Probes by PCR:

PCR- Mix:	Taq- Polymerase	2µl
	10x Buffer (Invitrogen)	5µl
	50mM MgSO4	1.5µl
	2mM dNTPs	5µl
	Primer for (10pMol/µl)	2µl
	Primer rev (10pMol/µl)	2µl
	Genomic DNA	2µl
	H2O	30.5µl
	Sum	50µl

Program: Robocycler (Stratagene) **45 cycles**

93°C **93°C 54°C 72°C** 93°C

5min **1min 1min 1min** 5min

PCR- products were checked on a 1% Agarose gel (Running Buffer 1xTAE) at 120mV.
Correct bands were cut out and eluted from the gel by using Elution kit from QiagenTM.

Labeling of the probes with alpha^{32}PdATPs using Prime-it 2 labeling Kit (Stratagene):

17.5µl of water together with the template (400ng) were mixed with 7µl of random primer 9mer and incubated on 95°C for five minutes. After quick spin 7µl of 5xdATP mix, 1µl Klenow polymerase and 2.5µl of alphaP32dATPs were added. This mixture was mixed by flicking (~5 times) and incubated for one h at 37°C. After 60µl of 1xTE were added the samples were loaded on a self-made sephadex column (G25, 6ml/g) to remove unincorporated nucleotides followed by centrifugation (2200rpm, 25sec).

Hybridisation, Washing and Detection:

The labelled probes were denaturated at 95°C for 3 minutes, quickly spinned down with the centrifuge and the desired volume was added to the membranes. Hybridization was performed over night (12-15 hours).

After removal of the hybridization buffer containing the radioactive probes the membranes were washed f or five times: Two times at room temperature for 15 minutes with washing solution and two times for fifteen minutes on 63°C. The washed membranes were wrapped into plastic bags and the signals were detected by X-ray at

room temperature for a few hours or over night depending on strength of the signal. Furthermore the membranes were also put into a Phospho-Imager cassette for quantification with a Typhoon scanner.

5.3. Microarrays:

RNA Isolation
RNA was isolated as described for Northern Blotting

Direct labelling protocoll from the University Health Network Microarray Centre

Reverse Transcription
Reaction Mix:

5× First Strand reaction buffer	8µl
AncT primer (5' T20VN, 100pmol/µl)	1.5µl
dNTP- dCTP (6.67mM each of dATP, dTTP, dGTP)	3µl
2mM dCTP	1µl
1mM Cyanine 3 or Cyanine 5-dCTP (NEN)	1µl
0.1M DTT	4µl
RNA (10-20µg total RNA)	
Nuclease– free water	to40 µl

The two labelling reactions were incubated for 5min. at 65°C, spinned down shortly and incubated for another 7 minutes on 42°C (saved from light!). Then 2 µl of Reverse Transcriptase (SuperScriptIII, Invitrogen) were added and the mixtures were incubated for two hours at 42°C (protected from light).
The reaction was centrifuged shortly and placed on ice. Then the reaction was stopped by adding 4µl 50mM EDTA (pH8) and 2µl 10N NaOH. An incubation step on 65°C for 20min followed.
Afterwards 4.5µl of 5M Acetic acid was added and the pH-value was calibrated to pH 7. Labelled cDNA was purified using CyScribe TM GFXTM (Amersham) Purification columns. The resulting 60 µl volume was reduced to 5µl by using Speed Vac.

Hybridization:
To each 100µl DIG Easy Hyb solution (Roche) 10µl of transfer DNA (10mg/ml) were added and the mixture was incubated on 65°C for 3 minutes and then cooled to room temperature. The hybridization solution was mixed with the labelled cDNA and incubated at 65°C for 2minutes and cooled down to RT. Hybridized coverslips were put into hybridization chambers containing a small amount of DIG Easy Hyb solution at the bottom. The lid was placed onto the box and the box was wrapped with plastic wrap following incubation at 37°C over night (12-15 minutes).

Washing:
The coverslip was removed by incubating the array in 1xSSC. The slide was placed into a staining rack and placed into a staining dish with fresh 1xSSC. Each slide was washed for 20 minutes at 50°C in clean slide staining boxes containing pre-warmed (50°C) 1 x SSC/

0.1% SDS. After washing was completed the slides were rinsed twice in RT 1xSSC and then in 0.1x SSC. Spin slides dry at 600rpm for 5 minutes in a Falcon tube, 500rpm, 5 min.

Detection and Analyzation:
Axon 4000B scanner (Molecular Devices), and analyzed and normalized by using Gene Pix Pro4.1 software (Axon).

5.4. Catalase measurement:

Growth of the cells:

Cells were grown from an over night culture in rich medium for 5 hours until they reached OD600=1. The first sample was taken as a control without stress. The rest of the cells were treated with different concentrations of β-estradiol, and aliquots were taken 15´-30´-60´ minutes.

Harvesting the cells:

25ml of cells were collected in a greiner at room temperature. The pellet was spinned down for 1 minute 4000rpm at RT. The supernatant was poured off and the pellet was resuspended in the remaining liquid and transferred to a fresh eppendorf tube. The pellets were frozen in liquid N_2, used immediately for the assay.

Catalase assay:

200µl of "Z buffer" and 1/3 of glass beads were added to the frozen pellets. This mixture was vortexed for 20 minutes at highest speed (4°C) and spinned down for 20 minutes at 14000rpm (4°C).

Catalase measurements: measure breakdown of H_2O_2:

The spectrophotometer was started and the CAT90 program was chosen. In both quartz cuvettes "Z buffer" was filled and afterwards 20µl of the sample were added. The spectrophotometer measures the absorbance at 240nm over a period of 90 seconds.

Protein measurements:

10µl of the sample were added to 990µl of ddH2O and OD280nm was measured.
mg protein= (OD_{280nm}/(µl protein×0,007))
Result analysis:

Cat U/ (ml×min) = (1000/43,6)×(ΔA/min)×((3000+µl extract)/µl extract)
Cat U/min/mg protein = first result/mg protein

5.5. High efficiency yeast transformation

An over night culture of *Saccharomyces cerevisiae* was grown to exponential phase and spinned down at 2500rpm, 2 minutes. The culture was washed with 25ml of dH2O (another centrifugation step) and the pellet was resuspended in 1ml of dH2O and transferred to a fresh Eppendorf tube. After spinning for 5 seconds, the supernatant was removed and the pellet was resuspended in ddH_2O (400µl). The suspension was vortexed and the 50µl samples were pipette into new eppendorf tubes. The samples were spinned down again and the rest of the Lithium Acetate was removed. Every sample was resuspended in the

Transformation Mix:

- 240µl of Polyethylenglycol (50%)
- 36µl of 1M Lithium Acetate 50µl of ss-DNA (10mg/ml); boiled for 5 min. and then immediately put on ice
- 25µl of disruption cassette (or 5µl of plasmid DNA and 20µl of water)

The pellet was vortexed vigorously for about one minute and then the cells were put on 30°C for 30 min. (recovery) followed by 20 min. of heat shock on 42°C. The transformants were spinned down, resuspended in 200-400µl of dH_2O and plated on selective medium.

5.6. Rapid PCI isolation for genomic DNA

A yeast strain was inoculated in 10ml YPD for an over night culture. On the next day cells were spinned for 3 minutes at 2200rpm and cell pellet was resuspended in 500µl ddH2O and transferred to a new Eppendorf tube. The supernatant was removed and the pellet was vortexed in the rest of the liquid. 200µl of lysis buffer, 200µl of PCI solution (25:24:1) and glass beads were added. After that we put the mixtures on the vibrax for 5 minutes at 4°C and then spinned down for 5 minutes at full speed. The aequous supernatant was transferred in a new Eppendorf tube and 500µl of EtOH abs. were added. This mixture was spinned down for 2 minutes at full speed and supernatant was removed. The DNA was resuspended in 500µl of 1×TE and 4µl of RNase A were added and incubated on 37°C for 15 minutes.
To this mixture 1ml of EtOH (70%) and 40µl of 3M NaAc pH5,0 were added and spinned down for 2 minutes at full speed. The pellet was resuspended in 50µl of 1×TE and checked on an agarose gel (0,7%).

5.7. Plasmid minipreparation

E.coli was inoculated in 1,5ml of LB-Amp and incubated at 37°C over night (12-15 hours). On the next day the grown bacteria cells were harvested for 2 minutes at 13000rpm. The supernatant was removed and the pellet was resuspended in 100µl of buffer1. Afterwards 200µl of buffer2 were added and the tubes were inverted gently for 5 to 7 times. 300µl of buffer3 were added to that solution and inverted again. After adding

600µl of 5M LiCl the cells were spinned for 10 minutes at 13000rpm. The supernatant (~1ml) was added to 600µl isopropanol. This mixture was vortexed gently and spinned down for 10 minutes at 13000rpm. Afterwards the pellet was washed with 500µl of 70% EtOH and spinned for 5 minutes at 13000rpm. The cell pellet was dried for 15-20 minutes at room temperature and resuspended in 50µl ddH_2O.

6. Media and Buffers:

YPD plates (per liter): 20g Peptone
10g yeast extract
20g glucose
20g agar
Autoclave

YP liquid media: 10g yeast extract
20g meat peptone

YPD + G418: cool YPD medium to ~65°C, add ml of the G418 stock, mix thoroughly and poor plates.

G418: 20mg/ml

G418 stock solution: 1g G418/50ml ddH2O

SC (synthetic complete) media (per liter): 6,7g YNB without aminoacids
20g glucose
30g agar
+ 55mg uracil
+ 55mg tyrosin
+55mg Adenin
Autoclave
+10ml aminoacide mixture (100× stock)
+10ml histidine (100× stock)
+10ml tryptophan (100× stock)
+10ml leucine (100× stock)

Glucose solution: Prepare 40% solution, autoclave and store at room temperature

Enriched sporulation plates (per liter): 10g kalium acetate
1g yeast extract
0,5g glucose
0,1g amino-acids supplement powder mixture
20g yeast bacto agar
Autoclave
+2,5ml G418 of a 20mg/ml stock

Amino acids supplement powder mixture:

3g adenine	2g uracil	2g inositol	0,2g para-aminobenzoic acid
2g alanine	2g arginine	2g asparagines	2g aspartic acid
2g cysteine	2g glutamic acid	2g glutamine	2g glycine
2g histidine	2g isoleucine	10g leucine	2g lysine
2g methionine	2g phenylalanine	2g proline	2g serine
2g threonine	2g tryptophan	2g tyrosine	2g valine
3g adenine	2g uracil	2g inositol	0,2g para-aminobenzoic acid

Northern Blot:

RNA Extraction Buffer: 50mM Tris- HCl pH 7-7.4
130mM NaCl
5mM EDTA
5% SDS

Phenol Chloroform Isoamylalcohol (PCI): 25:24:1 (Roti)
Chloroform Isoamylalcohol (CI): 24:1
DEPC- water: 0.1% Diethylpyrocarbonate (autoclaved)

Northern Blot Prehybridisation Buffer: 0.5M Sodium phosphate buffer pH7.2
7% SDS
1mM EDTA pH8

Washing Buffer: 0.5x SSC
0.1%SDS

Stripping Buffer: 1mM Tris-Cl pH8
1mM EDTA pH8
0.1x Denhardt`s reagent
Dying solution: 5% Acetic Acid
Methylenblue

Catalase assay:

"Z-buffer": $Na_2HPO_4.7H_2O$: 16,1g/l
$NaH_2PO_4.H_2O$: 5,5g/l
KCl: 0,75g/l
$MgSO_4.7H_2O$: 0,246g/l
Adjust to pH7 and autoclave- store at room temperature

H2O2 buffer:
0,1M phosphate buffer pH7: 96ml
0,3% w/v triton X100: 3,6ml
30% H_2O_2: 0,16ml

High efficiency yeast transformantion:

1M Lithium-Acetate
50% 3350 Polyethylenglycole

ssCarrier DNA: 10mg/ml

Plasmid minipreparation

Buffer1: 50mM Tris, pH8,0; 10mM EDTA

Buffer2: 0,2M NaOH; 1% SDS

Buffer3: 3M KAc, pH5,5

SGA (plates and accessories)

Singer Rotor HAD bench top robot

The Singer RoToR HDA is a very small benchtop robot for easy ultra-fast manipulation of high density arrays of yeast or bacteria. It allows reagents sets such as deletion mutant collection and the complete set of cloned yeast genes to be utilized for large-scale two hybrid, synthetic genetic array, phenotypic and chemical-genetic analysis. (Singer Instruments; www.singerinst.co.uk)

Plus plates for pouring plates

96er re-pads (from agar to agar)

96er re-pads (from liquid to agar or agar to liquid media)

384er re-pads

7. Appendix

Table A1: Microarray results Msn2A1-6 induced with 3nM of β-Estradiol

ORF	Name	Av	Function	27_F635 Mean - B635	27_F532 Mean - B532
YMR169C	ALD3	3,77	Cytoplasmic aldehyde dehydrogenase,	11727	1631
YGR088W	CTT1	3,17	Cytosolic catalase T, has a role in	4384	621
YFL014W	HSP12	2,77	Plasma membrane localized protein t	21906	2642
YDL204W	RTN2	2,71	Protein of unknown function; has si	6512	911
YFL012W-A		2,63	Dubious open reading frame unlikely	4801	593
YEL039C	CYC7	2,61	Cytochrome c isoform 2, expressed u	1730	242
YJR096W		2,54	Putative xylose and arabinose reduc	3625	564
YBR116C		2,37	Dubious open reading frame unlikely	1821	617
YER150W	SPI1	2,24	GPI-anchored cell wall protein invo	6257	1296
YPL186C	UIP4	2,23	Protein that interacts with Ulp1p,	1356	435
YML054C	CYB2	2,19	Cytochrome b2 (L-lactate cytochrome	659	246
YKL151C		2,18	Putative protein of unknown functio	3027	907
YOR173W	DCS2	2,18	Non-essential, stress induced regul	1029	265
YKL026C	GPX1	2,06	Phospholipid hydroperoxide glutathi	884	184
YML128C	MSC1	2,00	Protein of unknown function; mutant	3570	1262
YGR008C	STF2	1,90	Protein involved in regulation of t	13714	2634
YIL136W	OM45	1,89	Protein of unknown function, major	2851	1163
YHR087W	RTC3	1,88	Protein of unknown function involve	3073	845
YDL222C	FMP45	1,86	Integral membrane protein localized	1400	383
YGR248W	SOL4	1,85	6-phosphogluconolactonase with simi	3141	1092
YNL194C		1,85	Integral membrane protein required	687	204
YDR453C	TSA2	1,76	Stress inducible cytoplasmic thiore	9343	2627
YMR175W	SIP18	1,71	Protein of unknown function whose e	1345	297
YMR107W	SPG4	1,69	Protein required for survival at hi	577	150
YDR074W	TPS2	1,68	Phosphatase subunit of the trehalos	2418	1044
YGR043C	NQM1	1,66	Transaldolase of unknown function;	2465	833
YPL230W	USV1	1,62	Putative transcription factor conta	701	252
YGR061C	ADE6	1,59	Formylglycinamidine-ribonucleotide	648	154
YNL195C		1,58	Putative protein of unknown functio	225	75
YNL160W	YGP1	1,57	Cell wall-related secretory glycopr	8071	3391
YCR021C	HSP30	1,53	Hydrophobic plasma membrane localiz	2005	794
YLR177W		1,52	Putative protein of unknown functio	2877	644
YDR070C	FMP16	1,47	Putative protein of unknown functio	741	221
YKL091C		1,47	Putative homolog of Sec14p, which i	1454	506
YKL150W	MCR1	1,46	Mitochondrial NADH-cytochrome b5 re	5248	1895
YFL029C	CAK1	1,45	Cyclin-dependent kinase-activating	3005	1197
YJL015C		1,44	Dubious open reading frame unlikely	944	411
YLR306W	UBC12	1,42	Enzyme that mediates the conjugatio	2528	767
YFL021W	GAT1	1,41	Transcriptional activator of genes	10026	8663
YHL021C	AIM17	1,41	Putative protein of unknown functio	3172	1350
YFL050C	ALR2	1,40	Probable Mg(2+) transporter; overex	2206	634
YPR127W		1,39	Protein of unknown function, differ	1864	598
YJR066W	TOR1	1,37	PIK-related protein kinase and rapa	3295	1574

YJL142C	IRC9	1,36	Dubious open reading frame unlikely	2261	929
YER054C	GIP2	1,36	Putative regulatory subunit of the	641	266
YNL200C		1,33	Putative protein of unknown functio	815	380
YML004C	GLO1	1,33	Monomeric glyoxalase I, catalyzes t	4778	1871
YDL124W		1,32	NADPH-dependent alpha-keto amide re	7317	3529
YMR090W		1,28	Putative protein of unknown functio	1350	584
YBR230C	OM14	1,27	Integral mitochondrial outer membra	2389	862
YFL030W	AGX1	1,19	Alanine:glyoxylate aminotransferase	450	204
YNL015W	PBI2	1,19	Cytosolic inhibitor of vacuolar pro	2376	651
YCL035C	GRX1	1,18	Hydroperoxide and superoxide-radica	4226	1387
YMR105C	PGM2	1,18	Phosphoglucomutase, catalyzes the c	2911	2289
YML005W	TRM12	1,18	S-adenosylmethionine-dependent meth	2478	1143
YHR104W	GRE3	1,18	Aldose reductase involved in methyl	1713	863
YJR048W	CYC1	1,17	Cytochrome c, isoform 1; electron c	4870	1923
YFL051C		1,15	Putative protein of unknown functio	403	213
YER053C	PIC2	1,15	Mitochondrial phosphate carrier, im	2286	1098
YNL045W	LAP2	1,14	Leucyl aminopeptidase yscIV (leukot	2818	1382
YLR222C	UTP13	1,13	Nucleolar protein, component of the	5899	5417
YML070W	DAK1	1,13	Dihydroxyacetone kinase, required f	2159	1944
YFR017C		1,12	Putative protein of unknown functio	1462	529
YAL067C	SEO1	1,11	Putative permease, member of the al	573	308
YDR227W	SIR4	1,10	Silent information regulator that,	3829	4147
YPL247C		1,08	Putative protein of unknown functio	434	309
YER067W		1,07	Putative protein of unknown functio	1386	496
YLR178C	TFS1	1,06	Carboxypeptidase Y inhibitor, has a	4707	1765
YHR138C		1,05	Putative protein of unknown functio	1099	528
YBL015W	ACH1	1,03	Acetyl-coA hydrolase, primarily loc	485	346
YLL023C		1,01	Protein of unknown function; highly	1547	794
YOR092W	ECM3	1,00	Non-essential protein of unknown fu	1720	976
YML100W	TSL1	1,00	Large subunit of trehalose 6-phosph	216	134
YNL144C		1,00	Putative protein of unknown functio	358	292
YJL161W	FMP33	1,00	Putative protein of unknown functio	453	237
YPR160W	GPH1	0,99	Non-essential glycogen phosphorylas	196	138
YLL026W	HSP104	0,99	Heat shock protein that cooperates	2256	1960
YPL185W		0,98	Dubious open reading frame unlikely	366	193
YJL141C	YAK1	0,96	Serine-threonine protein kinase tha	495	261
YDR017C	KCS1	0,96	Inositol hexakisphosphate (IP6) and	281	210
YLR277C	YSH1	0,95	Putative endoribonuclease, subunit	1831	1382
YIL160C	POT1	0,95	3-ketoacyl-CoA thiolase with broad	239	211
YIL033C	BCY1	0,95	Regulatory subunit of the cyclic AM	2427	1600
YBR117C	TKL2	0,95	Transketolase, similar to Tkl1p; ca	371	271
YDL205C	HEM3	0,92	Porphobilinogen deaminase, catalyze	694	321
YMR291W		0,91	Putative kinase of unknown function	361	257
YLR206W	ENT2	0,90	Epsin-like protein required for end	2985	3310
YMR173W-A		0,90	Dubious open reading frame unlikely	4684	2983
YNL134C		0,89	Putative protein of unknown functio	2001	1501
YML042W	CAT2	0,89	Carnitine acetyl-CoA transferase pr	261	136
YLR425W	TUS1	0,88	Guanine nucleotide exchange factor	8154	7429
YLR190W	MMR1	0,88	Phosphorylated protein of the mitoc	5310	5174

YIL045W	PIG2	0,88	Putative type-1 protein phosphatase	475	290
YJL016W		0,87	Putative protein of unknown functio	505	424
YGR104C	SRB5	0,87	Subunit of the RNA polymerase II me	1471	1024
YDR533C	HSP31	0,86	Possible chaperone and cysteine pro	1100	504
YIL155C	GUT2	0,86	Mitochondrial glycerol-3-phosphate	293	255
YPL223C	GRE1	0,85	Hydrophilin of unknown function; st	739	456
YPL093W	NOG1	0,84	Putative GTPase that associates wit	3970	3020
YDR346C	SVF1	0,84	Protein with a potential role in ce	2020	1668
YFR053C	HXK1	0,84	Hexokinase isoenzyme 1, a cytosolic	9140	8171
YML037C		0,83	Putative protein of unknown functio	469	333
YLR252W		0,83	Dubious open reading frame unlikely	562	337
YNL273W	TOF1	0,82	Subunit of a replication-pausing ch	685	772
YPL014W		0,82	Putative protein of unknown functio	1303	588
YMR181C		0,81	Protein of unknown function; mRNA t	1394	628
YKL216W	URA1	0,81	Dihydroorotate dehydrogenase, catal	3398	2745
YFL012W		0,81	Putative protein of unknown functio	127	81
YJR031C	GEA1	0,80	Guanine nucleotide exchange factor	733	356
YMR173W	DDR48	0,79	DNA damage-responsive protein, expr	2608	1515
YJR125C	ENT3	0,79	Protein containing an N-terminal ep	1904	841
YFL059W	SNZ3	0,79	Member of a stationary phase-induce	396	258
YPL004C	LSP1	0,78	Primary component of eisosomes, whi	13227	10114
YAL048C	GEM1	0,78	Evolutionarily-conserved tail-ancho	2059	2363
YFL042C		0,77	Putative protein of unknown functio	2355	1418
YDR125C	ECM18	0,76	Protein of unknown function, simila	1068	620
YDL022W	GPD1	0,76	NAD-dependent glycerol-3-phosphate	2741	2189
YHR077C	NMD2	0,76	Protein involved in the nonsense-me	296	245
YOL071W	EMI5	0,76	Non-essential protein of unknown fu	877	536
YDL023C		0,76	Dubious open reading frame, unlikel	1927	1868
YOR031W	CRS5	0,76	Copper-binding metallothionein, req	417	190
YDL174C	DLD1	0,75	D-lactate dehydrogenase, oxidizes D	358	383
YLR311C		0,75	Dubious open reading frame unlikely	206	190
YIL113W	SDP1	0,75	Stress-inducible dual-specificity M	350	170
YJL164C	TPK1	0,75	cAMP-dependent protein kinase catal	668	906
YHR097C		0,75	Putative protein of unknown functio	899	655
YPL018W	CTF19	0,75	Outer kinetochore protein, required	278	238
YGR174C	CBP4	0,75	Mitochondrial protein required for	595	328
YDR513W	GRX2	0,74	Cytoplasmic glutaredoxin, thioltran	6325	2842
YOR389W		0,74	Putative protein of unknown functio	5152	5223
YKR049C	FMP46	0,74	Putative redox protein containing a	696	417
YIL111W	COX5B	0,74	Subunit Vb of cytochrome c oxidase,	1477	885
YLR210W	CLB4	0,74	B-type cyclin involved in cell cycl	513	568
YLR327C	TMA10	0,74	Protein of unknown function that as	928	409
YKL134C	OCT1	0,73	Mitochondrial intermediate peptidas	360	198
YLR151C	PCD1	0,73	Peroxisomal nudix pyrophosphatase w	379	221
YGR213C	RTA1	0,73	Protein involved in 7-aminocholeste	78	160
YAL034C	FUN19	0,72	Non-essential protein of unknown fu	795	579
YFR034C	PHO4	0,72	Basic helix-loop-helix (bHLH) trans	879	683
YLR213C	CRR1	0,72	Putative glycoside hydrolase of the	1575	1757
YMR086W		0,71	Protein of unknown function that ma	1081	845
YOR374W	ALD4	0,71	Mitochondrial aldehyde dehydrogenas	1836	1551
YDL019C	OSH2	0,70	Member of an oxysterol-binding prot	1585	1255

YOR028C	CIN5	0,70	Basic leucine zipper (bZIP) transcr	672	433
YKL085W	MDH1	0,70	Mitochondrial malate dehydrogenase,	3815	3320
YDR018C		0,70	Probable membrane protein with thre	287	252
YCR083W	TRX3	0,70	Mitochondrial thioredoxin, highly c	773	344
YER037W	PHM8	0,70	Protein of unknown function, expres	406	368
YML035C	AMD1	0,70	AMP deaminase, tetrameric enzyme th	606	605
YIL101C	XBP1	0,70	Transcriptional repressor that bind	216	177
YCR091W	KIN82	0,70	Putative serine/threonine protein k	533	384
YDL048C	STP4	0,69	Protein containing a Kruppel-type z	1060	682
YMR106C	YKU80	0,69	Subunit of the telomeric Ku complex	327	410
YLR398C	SKI2	0,69	Ski complex component and putative	6811	8691
YKR076W	ECM4	0,69	Omega class glutathione transferase	238	203
YNR014W		0,69	Putative protein of unknown functio	963	605
YJL119C		0,69	Dubious open reading frame unlikely	121	126
YGL226W	MTC3	0,69	Protein of unknown function; green	782	855
YCR046C	IMG1	0,69	Mitochondrial ribosomal protein of	856	688
YGR130C		0,69	Putative protein of unknown functio	700	481
YDR391C		0,68	Putative protein of unknown functio	620	641
YDL240W	LRG1	0,68	Putative GTPase-activating protein	1246	1700
YDR031W	MIC14	0,68	Mitochondrial intermembrane space c	269	242
YLR219W	MSC3	0,68	Protein of unknown function, green	370	325
YHR023W	MYO1	0,68	Type II myosin heavy chain, require	301	279
YFR013W	IOC3	0,68	Member of a complex (Isw1a) with Is	1267	1016
YCL040W	GLK1	0,68	Glucokinase, catalyzes the phosphor	4327	2960
YDR171W	HSP42	0,67	Small heat shock protein (sHSP) wit	1748	1450
YKL037W	AIM26	0,67	Putative protein of unknown functio	215	240
YDR189W	SLY1	0,67	Hydrophilic protein involved in ves	928	1058
YGR259C		0,66	Dubious open reading frame unlikely	940	1176
YEL047C		0,66	Soluble fumarate reductase, require	1262	1033
YDR168W	CDC37	0,66	Essential Hsp90p co-chaperone; nece	3696	4386
YPR172W		0,66	Protein of unknown function, transc	1325	821
YOL091W	SPO21	0,65	Component of the meiotic outer plaq	323	250
YNL275W	BOR1	0,65	Boron efflux transporter of the pla	606	641
YOL036W		0,65	Protein of unknown function; potent	666	686
YDR406W	PDR15	0,65	Plasma membrane ATP binding cassett	711	375
YLR251W	SYM1	0,65	Protein required for ethanol metabo	553	411
YIL087C	AIM19	0,65	Putative protein of unknown functio	545	381
YDR273W	DON1	0,65	Meiosis-specific component of the s	148	124
YMR165C	PAH1	0,64	Mg²⁺-dependent phosphati	122	96
YKL219W	COS9	0,64	Protein of unknown function, member	2151	1698
YDR278C		0,64	Dubious open reading frame unlikely	464	317
YDR160W	SSY1	0,64	Component of the SPS plasma membran	1791	1133
YLR291C	GCD7	0,64	Beta subunit of the translation ini	1321	1784
YKL051W	SFK1	0,64	Plasma membrane protein that may ac	870	594
YMR174C	PAI3	0,63	Cytoplasmic proteinase A (Pep4p) in	1803	968
YLR120C	YPS1	0,63	Aspartic protease, attached to the	520	512
YOL118C		0,62	Dubious open reading frame unlikely	281	181
YDL220C	CDC13	0,62	Single stranded DNA-binding protein	453	516
YMR251W-A	HOR7	0,62	Protein of unknown function; overex	11298	4743

YGR205W		0,62	ATP-binding protein of unknown func	485	407
YOR052C		0,62	Nuclear protein of unknown function	2991	1513
YPR149W	NCE102	0,61	Protein of unknown function; contai	7969	4126
YBL064C	PRX1	0,61	Mitochondrial peroxiredoxin (1-Cys	1035	574
YHR099W	TRA1	0,61	Subunit of SAGA and NuA4 histone ac	388	239
YOL004W	SIN3	0,61	Component of the Sin3p-Rpd3p histon	432	427
YDR343C	HXT6	0,61	High-affinity glucose transporter o	5124	4468
YMR152W	YIM1	0,61	Protein of unknown function; null m	936	779

Table A2: Cluster of genes induced and repressed after treatment with valinomycin.

ORF	NAME	LAFS	log_Av_MR	log_Av_MR_15
YOR241W	MET7	0,24	0,32	0,32
YBR295W	PCA1	0,18	0,21	0,25
YDR067C	OCA6	0,25	0,18	0,32
YDR230W		0,11	0,07	0,15
YPL060W	LPE10	0,22	0,19	0,28
YOR196C	LIP5	0,14	0,09	0,15
YDR175C	RSM24	0,12	0,10	0,12
YBR105C	VID24	0,30	0,15	0,15
YPR093C	ASR1	0,20	0,11	0,13
YCL007C		0,17	0,06	0,05
YJL063C	MRPL8	0,08	0,03	0,03
YFR033C	QCR6	0,14	0,10	0,05
YOL148C	SPT20	0,13	0,12	0,04
YPR067W	ISA2	0,44	0,91	-0,04
YMR184W	ADD37	0,20	0,56	-0,04
YOL027C	MDM38	0,16	0,49	0,04
YGL168W	HUR1	0,11	0,35	0,07
YDR231C	COX20	0,09	0,18	0,03
YMR135W-A		0,15	0,14	0,00
YBR126C	TPS1	0,14	0,14	0,00
YHR199C	AIM46	0,03	0,04	0,00
YGR174C	CBP4	0,13	0,55	-0,03
YKL214C	YRA2	0,05	0,24	-0,03
YKL142W	MRP8	0,08	0,49	0,01
YDR506C		0,10	0,53	0,00
YNL119W	NCS2	0,05	0,26	0,00
YDL104C	QRI7	0,06	0,23	0,00
YNL091W	NST1	0,07	0,88	0,00
YFR053C	HXK1	0,05	0,60	0,00
YDR379W	RGA2	0,02	0,32	0,00
YDR116C	MRPL1	0,00	0,29	-0,02
YKL129C	MYO3	0,10	0,44	0,13
YBR147W	RTC2	0,10	0,55	0,12

YGR101W	PCP1	0,04	0,19	0,07
YJR122W	IBA57	0,04	0,19	0,08
YKL055C	OAR1	0,03	0,75	0,27
YMR161W	HLJ1	0,01	0,43	0,16
YLL009C	COX17	0,08	0,53	0,07
YFR047C	BNA6	0,06	0,25	0,03
YDL076C	RXT3	0,04	0,50	0,10
YAL009W	SPO7	0,03	0,63	0,07
YKR030W	GMH1	0,14	0,25	0,16
YBR111C	YSA1	0,13	0,26	0,17
YLR452C	SST2	0,15	0,36	0,19
YPL097W	MSY1	0,08	0,24	0,10
YDL049C	KNH1	0,07	0,24	0,12
YPL215W	CBP3	0,07	0,24	0,11
YFR022W	ROG3	0,10	0,62	0,53
YDR241W	BUD26	0,07	0,40	0,34
YJL058C	BIT61	0,06	0,21	0,19
YGR255C	COQ6	0,06	0,27	0,21
YDR197W	CBS2	0,04	0,13	0,10
YJL200C	ACO2	0,10	0,36	0,23
YDL068W		0,05	0,56	0,33
YOL151W	GRE2	0,04	0,34	0,20
YCR033W	SNT1	0,09	0,32	0,35
YPR065W	ROX1	0,04	0,15	0,21
YLL027W	ISA1	0,07	0,41	0,45
YDL107W	MSS2	0,05	0,50	0,55
YMR175W	SIP18	0,00	0,00	0,00
YDR090C		0,00	0,19	0,15
YMR152W	YIM1	0,27	-0,12	0,25
YCL044C	MGR1	0,15	-0,07	0,11
YLR242C	ARV1	0,17	-0,17	0,24
YHR154W	RTT107	0,16	-0,19	0,28
YER103W	SSA4	0,27	0,00	0,14
YJL176C	SWI3	0,17	0,00	0,06
YLR003C	CMS1	0,26	0,00	0,21
YDR456W	NHX1	0,26	0,02	0,29
YDL202W	MRPL11	0,08	0,00	0,08
YDR125C	ECM18	0,19	0,10	0,54
YML111W	BUL2	0,17	0,11	0,43
YBR268W	MRPL37	0,08	0,05	0,24
YLR337C	VRP1	0,18	0,18	0,56
YKL201C	MNN4	0,08	0,07	0,28
YKL170W	MRPL38	0,10	0,06	0,17
YGR183C	QCR9	0,08	0,04	0,15

YDR194C	MSS116	0,15	0,00	0,25
YOL001W	PHO80	0,14	0,00	0,22
YLR004C	THI73	0,20	0,00	0,31
YOL087C		0,09	0,00	0,14
YGR102C		0,06	0,00	0,09
YLR338W	OPI9	0,13	0,00	0,19
YKL137W	CMC1	0,07	0,00	0,09
YGL237C	HAP2	0,15	0,02	0,35
YMR064W	AEP1	0,09	0,00	0,20
YKL126W	YPK1	0,18	0,00	0,37
YDR057W	YOS9	0,07	0,00	0,14
YDL225W	SHS1	0,09	0,00	0,18
YJL046W	AIM22	0,10	0,00	0,19
YNR051C	BRE5	0,10	0,00	0,18
YMR139W	RIM11	0,14	0,21	0,56
YJL003W	COX16	0,11	0,27	0,67
YBR026C	ETR1	0,08	0,13	0,64
YDL238C	GUD1	0,04	0,25	0,74
YER124C	DSE1	0,04	0,12	0,39
YPR166C	MRP2	0,04	0,11	0,42
YER154W	OXA1	0,16	0,30	0,49
YJL066C	MPM1	0,23	0,27	0,57
YGL167C	PMR1	0,04	0,06	0,11
YIL005W	EPS1	0,08	0,25	0,45
YGR112W	SHY1	0,07	0,26	0,50
YFR034C	PHO4	0,09	0,68	0,00
YMR191W	SPG5	0,02	0,28	0,54
YPL189W	GUP2	0,05	0,28	0,60
YLR193C	UPS1	0,02	0,12	0,25
YDR213W	UPC2	0,02	0,07	0,16
YDL085W	NDE2	0,02	0,18	0,27
YNL027W	CRZ1	0,11	-0,08	0,25
YER061C	CEM1	0,05	-0,04	0,15
YNL252C	MRPL17	0,07	-0,12	0,31
YJL164C	TPK1	0,04	-0,21	0,50
YPL224C	MMT2	0,02	-0,09	0,30
YBR163W	DEM1	0,14	-0,04	0,40
YOR334W	MRS2	0,11	0,00	0,35
YBR282W	MRPL27	0,11	0,00	0,31
YJL067W		0,22	0,00	0,93
YPL132W	COX11	0,09	0,00	0,36
YKL204W	EAP1	0,11	0,00	0,42
YKR029C	SET3	0,17	0,00	0,63
YLR292C	SEC72	0,08	0,00	0,29

YML009C	MRPL39	0,05	0,01	0,17
YGR041W	BUD9	0,04	0,01	0,17
YOR062C		0,16	-0,03	0,67
YGR257C	MTM1	0,10	-0,03	0,45
YDL112W	TRM3	0,07	0,00	0,41
YBL059W		0,12	0,00	0,60
YMR067C	UBX4	0,08	0,00	0,38
YGR086C	PIL1	0,07	0,00	0,36
YHR189W	PTH1	0,05	0,00	0,26
YDR477W	SNF1	0,05	0,00	0,23
YBR212W	NGR1	0,05	-0,03	0,37
YPR170C		0,05	0,00	0,33
YLR352W		0,03	0,00	0,22
YLR393W	ATP10	0,03	0,00	0,20
YFR035C		0,01	0,00	0,12
YMR115W	MGR3	0,02	0,00	0,25
YMR209C		0,01	0,00	0,14
YFL012W		0,03	0,00	0,89
YDL032W		0,03	0,00	0,65
YCL061C	MRC1	0,03	0,00	0,64
YGR111W		0,03	0,00	0,47
YMR174C	PAI3	0,04	0,00	0,00
YDR350C	ATP22	0,02	0,00	0,74
YGR109C	CLB6	0,01	0,00	0,74
YGL226W	MTC3	0,00	0,00	0,37
YAR015W	ADE1	0,00	0,03	0,32
YPL059W	GRX5	0,28	0,13	-0,22
YMR150C	IMP1	0,19	0,09	-0,11
YGR076C	MRPL25	0,08	0,04	-0,03
YJL216C		0,34	-0,06	-0,10
YEL036C	ANP1	0,65	0,04	-0,14
YNL229C	URE2	0,27	0,00	-0,08
YGR162W	TIF4631	0,11	0,00	-0,05
YDL146W	LDB17	0,10	0,00	-0,04
YBR101C	FES1	0,34	-0,03	0,07
YFL025C	BST1	0,22	-0,02	0,00
YGL038C	OCH1	0,24	0,00	0,02
YGR171C	MSM1	0,18	0,00	0,00
YGL064C	MRH4	0,15	0,00	0,00
YIR030C	DCG1	0,23	0,00	0,00
YHR168W	MTG2	0,15	0,00	0,00
YDR220C		0,27	0,00	0,00
YKR055W	RHO4	0,26	0,00	0,00
YER151C	UBP3	0,37	0,00	0,00

YER122C	GLO3	0,17	0,00	0,00
YDR300C	PRO1	0,15	0,00	0,00
YDR010C		0,13	0,00	0,00
YLR001C		0,33	0,00	0,00
YJL204C	RCY1	0,11	0,00	0,00
YMR144W		0,02	0,00	0,00
YFR055W	IRC7	0,02	0,00	0,00
YOR291W	YPK9	0,02	0,00	0,00
YMR303C	ADH2	0,19	0,00	0,00
YIL038C	NOT3	0,04	0,00	0,00
YDR347W	MRP1	0,16	0,00	0,00
YBR271W		0,05	0,00	0,00
YDR369C	XRS2	0,03	0,00	0,00
YMR151W	YIM2	0,22	0,00	0,00
YMR083W	ADH3	0,13	0,00	0,00
YDR078C	SHU2	0,10	0,00	0,00
YER155C	BEM2	0,22	0,00	0,00
YFL003C	MSH4	0,15	0,00	0,00
YOR300W		0,26	0,00	0,00
YLL047W		0,22	0,00	0,00
YCR044C	PER1	0,28	0,00	0,00
YJL199C	MBB1	0,16	0,00	0,00
YEL044W	IES6	0,06	0,00	0,00
YAR014C	BUD14	0,02	0,00	0,00
YFL055W	AGP3	0,22	0,00	0,00
YFR054C		0,07	0,00	0,00
YGR050C		0,03	0,00	0,00
YDL044C	MTF2	0,18	0,00	0,00
YDL239C	ADY3	0,11	0,00	0,00
YJL102W	MEF2	0,06	0,00	0,00
YOR333C		0,13	0,00	0,00
YJL206C		0,00	0,00	0,00
YHR011W	DIA4	0,08	0,00	0,00
YPL183W-A	RTC6	0,04	0,00	0,00
YOL023W	IFM1	0,07	0,00	0,00
YGR129W	SYF2	0,12	0,00	0,00
YLR456W		0,09	0,00	0,00
YER017C	AFG3	0,05	0,00	0,00
YDL129W		0,02	0,00	0,00
YPR133W-A	TOM5	0,09	0,00	0,00
YBL044W		0,02	0,00	0,00
YJL139C	YUR1	0,05	0,00	0,00
YLR280C		0,03	0,00	0,00
YDR058C	TGL2	0,04	0,00	0,00

YAL021C	CCR4	0,04	0,00	0,00
YGL177W		0,11	0,00	0,00
YKL118W		0,12	0,00	0,00
YDL062W		0,10	0,00	0,00
YER169W	RPH1	0,12	0,00	0,00
YER091C-A		0,09	0,00	0,00
YBR106W	PHO88	0,11	0,00	0,00
YOR290C	SNF2	0,07	0,00	0,00
YMR063W	RIM9	0,02	0,00	0,00
YMR307W	GAS1	0,04	0,00	0,00
YJL175W		0,08	0,00	0,00
YPL040C	ISM1	0,05	0,00	0,00
YNR059W	MNT4	0,08	0,00	0,00
YDR065W		0,10	0,00	0,00
YAR029W		0,12	0,00	0,00
YMR102C		0,10	0,00	0,00
YNR070W	PDR18	0,05	0,00	0,00
YER188W		0,09	0,00	0,00
YBR044C	TCM62	0,02	0,00	0,00
YHL023C	RMD11	0,05	0,00	0,00
YGL224C	SDT1	0,06	0,00	0,00
YKL194C	MST1	0,10	0,00	0,00
YBL090W	MRP21	0,08	0,00	0,00
YDR360W	OPI7	0,02	0,00	0,00
YBL007C	SLA1	0,07	0,00	0,00
YJL007C		0,01	0,00	0,00
YJL182C		0,04	0,00	0,00
YLR015W	BRE2	0,04	0,00	0,00
YHR060W	VMA22	0,13	0,03	0,01
YBR274W	CHK1	0,08	0,02	0,01
YAL010C	MDM10	0,04	0,01	0,00
YDR320C	SWA2	0,24	0,26	-0,19
YKL201C	MNN4	0,08	0,07	-0,06
YDR337W	MRPS28	0,12	0,10	-0,15
YKR006C	MRPL13	0,16	0,32	-0,17
YPL194W	DDC1	0,07	0,11	-0,06
YGL054C	ERV14	0,15	0,41	-0,34
YPL098C	MGR2	0,07	0,16	-0,18
YDR295C	HDA2	0,08	0,26	-0,35
YOR253W	NAT5	0,07	0,21	-0,32
YBR288C	APM3	0,05	0,09	-0,14
YLL041C	SDH2	0,02	0,36	-0,65
YKL096W-A	CWP2	0,06	0,43	-0,56
YDR380W	ARO10	0,06	0,45	-0,50

YMR316C-A		0,02	0,15	-0,16
YKL138C	MRPL31	0,09	0,61	-0,11
YPR064W		0,04	0,19	-0,03
YOL050C		0,04	0,22	-0,05
YBR185C	MBA1	0,06	0,30	-0,11
YMR072W	ABF2	0,05	0,23	-0,10
YJL193W		0,02	0,17	-0,07
YHR001W-A	QCR10	0,01	0,20	-0,05
YNR036C	MRPS12	0,00	0,54	-0,17
YJL055W		0,03	0,26	-0,17
YMR173W-A		0,03	0,41	-0,24
YDL128W	VCX1	0,24	-0,04	-0,16
YKL113C	RAD27	0,33	0,00	-0,27
YPR050C		0,28	0,00	-0,33
YGL057C	GEP7	0,12	0,00	-0,13
YCR034W	FEN1	0,41	-0,25	-0,65
YLR370C	ARC18	0,25	-0,17	-0,43
YER068W	MOT2	0,22	-0,10	-0,41
YHR067W	HTD2	0,13	-0,05	-0,16
YOR040W	GLO4	0,21	0,09	-0,39
YDR484W	VPS52	0,20	0,00	-0,28
YDR245W	MNN10	0,08	0,00	-0,11
YLR011W	LOT6	0,03	0,00	-0,06
YER087W	AIM10	0,21	0,19	-0,64
YDR209C		0,03	0,07	-0,17
YLR448W	RPL6B	0,26	-0,11	0,00
YMR145C	NDE1	0,07	0,00	-0,43
YEL029C	BUD16	0,06	0,00	-0,23
YPL234C	TFP3	0,08	0,00	-0,25
YJL120W		0,06	0,00	-0,17
YER129W	SAK1	0,06	0,00	-0,15
YIL065C	FIS1	0,06	0,07	-0,59
YLR038C	COX12	0,05	0,05	-0,69
YPL271W	ATP15	0,03	0,02	-0,39
YJL189W	RPL39	0,05	0,14	-0,75
YLR372W	SUR4	0,36	-0,44	0,00
YHR026W	PPA1	0,13	-0,23	-0,65
YDR072C	IPT1	0,12	-0,16	-0,32
YBR035C	PDX3	0,06	-0,07	-0,14
YKL101W	HSL1	0,23	-0,67	0,00
YER070W	RNR1	0,08	-0,33	-0,48
YPL078C	ATP4	0,10	-0,51	-0,67
YDL189W	RBS1	0,03	-0,13	-0,17
YGL084C	GUP1	0,19	-0,75	0,00

YAL044C	GCV3	0,09	-0,29	-0,52
YLR180W	SAM1	0,12	-0,95	0,00
YDR377W	ATP17	0,06	-0,28	-0,46
YKR059W	TIF1	0,11	-0,68	0,00
YGL135W	RPL1B	0,11	-0,69	0,00
YBR179C	FZO1	0,01	-0,44	-0,82
YMR142C	RPL13B	0,08	-0,74	0,00
YMR157C	AIM36	0,02	-0,15	-0,20
YDR226W	ADK1	0,01	-0,68	0,00
YDL135C	RDI1	0,05	-0,12	-0,48
YJL192C	SOP4	0,03	-0,15	-0,57
YLR333C	RPS25B	0,04	-0,28	-0,86
YDR435C	PPM1	0,04	-0,15	-0,46
YPL045W	VPS16	0,03	-0,09	-0,30
YFR049W	YMR31	0,03	-0,13	-0,42
YHR100C	GEP4	0,03	-0,13	-0,37
YJL190C	RPS22A	0,03	-0,55	0,00
YPR020W	ATP20	0,00	-0,27	-0,86
YLR294C		0,04	-0,06	-0,39
YDR298C	ATP5	0,01	-0,15	-0,74
YOR221C	MCT1	0,07	-0,03	-0,73
YBR074W		0,04	0,00	-0,45
YOR187W	TUF1	0,03	0,00	-0,42
YBR269C	FMP21	0,02	0,00	-0,33
YCR010C	ADY2	0,01	0,00	-0,31
YEL062W	NPR2	0,01	-0,02	-0,49
YJL052W	TDH1	0,00	0,00	-0,28
YJR010W	MET3	0,00	0,00	-0,21
YMR156C	TPP1	0,00	0,00	-0,09
YKL148C	SDH1	0,43	-0,63	0,00
YLR304C	ACO1	0,19	-0,26	0,00
YMR039C	SUB1	0,33	-0,26	-0,22
YJL123C	MTC1	0,07	-0,08	-0,05
YJL201W	ECM25	0,29	-0,14	0,10
YLR233C	EST1	0,25	-0,14	0,00
YER093C-A		0,09	-0,05	0,00
YCR009C	RVS161	0,10	-0,07	0,00
YDL240W	LRG1	0,26	-0,90	0,28
YDR129C	SAC6	0,16	-0,70	0,07
YOR179C	SYC1	0,09	-0,60	0,11
YLR369W	SSQ1	0,05	-0,55	0,13
YDR221W	GTB1	0,02	-0,63	0,17
YPL031C	PHO85	0,01	-0,78	0,15
YCL046W		0,06	-0,30	0,22

YCL051W	LRE1	0,05	-0,51	0,31
YBL058W	SHP1	0,11	-0,28	-0,12
YBR127C	VMA2	0,09	-0,50	-0,20
YGR250C		0,03	-0,22	-0,07
YJL080C	SCP160	0,08	-0,34	-0,09
YDR028C	REG1	0,07	-0,32	-0,05
YBL100C		0,16	0,00	0,00
YDR444W		0,06	-0,98	0,00
YLR322W	VPS65	0,06	-0,78	0,00
YBR218C	PYC2	0,06	0,00	0,01
YJL209W	CBP1	0,01	-0,67	0,00
YDR346C	SVF1	0,00	-0,23	0,00
YAL048C	GEM1	0,04	-0,97	-0,17
YER144C	UBP5	0,00	-0,71	-0,13
YDL181W	INH1	0,10	-0,37	-0,31
YGR285C	ZUO1	0,12	-0,65	-0,49
YJL062W	LAS21	0,03	-0,24	-0,20
YGR258C	RAD2	0,05	-0,24	-0,24
YBR272C	HSM3	0,01	-0,04	-0,05
YFL018C	LPD1	0,08	-0,86	-0,63
YJL171C		0,03	-0,45	-0,31
YBR287W		0,06	-0,72	-0,43
YPR011C		0,03	-0,23	-0,15
YKL216W	URA1	0,01	-0,59	-0,40
YMR034C		0,00	-0,24	-0,19

Table A3: Primary isolated yeast strains and catalase results (1nM β-Estradiol induced expression of Msn2A1-6) (6 positive candidates highlighted in grey)

Name	U/mg Catalase	Name	U/mg Catalase
ADA2	11,69	**RTN2**	24,60
ADA2 1nM	98,42	**RTN2 1nM**	101,92
AKR1	1,76	**RTT109**	9,86
AKR1 1nM	38,73	**RTT109 1nM**	46,26
ANP1	8,33	**SHG1**	**20,81**
ANP1 1nM	16,66	**SHG1 1nM**	**97,48**
BIM1	27,09	**SIT4**	12,94
BIM1 1nM	85,56	**SIT4 1nM**	20,34
CBF1	7,67	**SRB2**	5,82
CBF1 1nM	15,34	**SRB2 1nM**	43,92
CGI121	5,82	**SSA4**	9,20

CGI121 1nM	54,70	**SSA4 1nM**	56,29
CLB1	15,59	**SSN8**	3,31
CLB1 1nM	35,79	**SSN8 1nM**	5,46
EUG1	14,21	**TPC1**	10,98
EUG1 1nM	16,95	**TPC1 1nM**	56,37
FAR7	23,68	**UGA1**	20,30
FAR7 1nM	58,40	**UGA1 1nM**	65,37
Gal11	3,53	**VPS3**	**15,59**
Gal11 1nM	4,98	**VPS3 1nM**	**38,65**
ILM1	30,20	**VPS54**	2,67
ILM1 1nM	33,71	**VPS54 1nM**	49,55
IOC3	6,04	**VPS71**	43,92
IOC3 1nM	64,14	**VPS71 1nM**	43,92
MCM22	3,43	**YBL051W**	20,45
MCM22 1nM	11,78	**YBL051W 1nM**	70,95
MEF1	12,08	**YBL053W**	14,87
MEF1 1nM	31,17	**YBL053W 1nM**	83,92
MRPL9	**13,80**	**YCR095C**	13,51
MRPL9 1nM	**50,86**	**YCR095C 1nM**	67,81
MRPS28	19,86	**YDR278C**	39,82
MRPS28 1nM	59,72	**YDR278C 1nM**	136,74
MSB4	**12,71**	**YER066W**	11,64
MSB4 1nM	**38,14**	**YER066W 1nM**	61,03
MSW1	5,77	**YGL199C**	7,99
MSW1 1nM	46,23	**YGL199C 1nM**	12,47
PCP1	8,95	**YGR001C**	8,33
PCP1 1nM	42,01	**YGR001C 1nM**	50,56
PER1	2,91	**YGR164W**	3,29
PER1 1nM	80,53	**YGR164W 1nM**	63,57
PIB1	8,33	**YLR173W**	17,89
PIB1 1nM	54,67	**YLR173W 1nM**	37,17
PRB1	9,12	**YLR278C**	6,90
PRB1 1nM	18,58	**YLR278C 1nM**	27,35
RMD9	5,72	**YML082W**	9,47
RMD9 1nM	11,78	**YML082W 1nM**	15,59
RND9	6,12	**YMR003W**	17,26
RND9 1nM	70,28	**YMR003W 1nM**	24,78

RNR1	11,24	**YOX1**	**24,16**
RNR1 1nM	52,52	**YOX1 1nM**	**50,86**
RPH1	**9,12**	**ZRT1**	11,93
RPH1 1nM	**56,84**	**ZRT1 1nM**	30,68

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09/98 – 07/03 Grammar School: BORG für Leistungssportler in St. Pölten

10/04 – 08/08 Study of Molecular Biology at the University of Vienna; Branch: Biochemistry

08/08 – 11/09: Diploma thesis in Lab of Univ Doz. Dr. Christoph Schüller at the University of Vienna

Diploma thesis: **Systematic Genetic Array (SGA) screening for stress-regulating factors in *Saccharomyces cerevisiae***

Internships and Additional Work*:*

08-09/07 Synthesis, Folding, Transport, and Degradation of proteins in the early secretory pathway (Lab Prof. Ivessa)

03-05/08 Automated Identification of Protein Fold Topology Similarity as an efficient Guiding Principle for Drug Discovery (Lab Prof. Konrat)

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