Analysis of Cell wall stress in yeast

This section contains the additional material for the analysis of the zymolyase signature.

a) Analysis of the yeast transcriptional response to zymolyase in a wild-type strain:

The list of genes included in the transcriptional profile of BY4741 cells treated with 0.8 U/ml of zymolyase for 3 hours (1) was analyzed using MARQ. Data from this analysis is summarized in Table 3.

Table 3.. MARQ analysis of the yeast transcriptional profile to zymolyase cell wall stress in a wild-type strain.

MARQ position	GEO ref	Experiment	Condition	Score	p-value	Reference
1	GSE966	exposure to zymolyase	2h wt zym	1.9	<0.001	
2	GSE961	exposure to zymolyase	2h wt rc	1.71	<0.001	
3 4 5 6 8 9 12 13 15		O_RourkeData: Hyper osmotic shock	WT 1M KCL 120 m WT 0.5M KCL 40 m WT 1M KCL 90 m WT 1M KCL 180 m WT 1M sorbitol 30 m WT 0.25M KCL 30 m WT 1M sorbitol 20 m WT 1M sorbitol 30 m ssk1Δ 0.5M KCL 40 m	1.69 1.67 1.67 1.67 1.64 1.63 1.61 1.61 1.59	<0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001	(2)
7 17	GDS113	Dithiothrietol exposure		1.65 1.58	<0.001 <0.001	
10		RobertsData: Overexpression of elemens of MAPK pathways		1.63	<0.001	(3)
11	GDS750	HAC1 transcription and unfolded protein response		1.62	<0.001	
14		RobertsData: Mating response		1.6	<0.001	(3)
16	GDS20	Hyper-osmotic shock		1.59	<0.001	
-9 -2	GDS113	Dithiothrietol exposure		-1.47 -1.55	<0.001 <0.001	
-7 -5 -1	GDS33	Hypo-osmotic shock		-1.51 -1.53 -1.65	<0.001 <0.001 <0.001	

b) Identification of a common signature of over-expressed genes among different cell wall stress conditions:

Four of the more relevant signatures identified above (MARQ positions 1, 2, 7 and 10) were used determine common over-expressed genes using the "signature comparison" tool. Those genes from this analysis with p values ≤ 0.001 are included in the Table 4 together with the description of their function according to SGD and PROTEOME databases. Using this approach we were able to identify the common cell wall compensatory "signature" developed by the yeast in response to cell wall stress. As shown in Table 4, four main functional groups were found. The main group is related to cell wall. Cell wall damage needs to be compensated by cell wall remodeling processes. So, as previously reported (4), genes encoding cell wall-remodeling enzymes like *CRH1* or *BGL2* and other cell wall proteins, like *YLR1194C,*

SED1, CWP1, PST1, PIR3 or *YPS3* are the main components included in the common response. In agreement with an increase in the chitin content, as part of this compensatory response, genes like *GFA1*, encoding for a protein involved in the biosynthesis of chitin, are included in the common over-expressed signature (4). Also in agreement with our previous findings (4), genes related to metabolism, stress and signaling were also identified. The last group is particularly interesting because two of the genes included in this group include *SLT2* and *MLP1*, both of them being signaling components of the CWI pathway, the main pathway involved in the regulation of the transcriptional responses to cell wall stress.

Table 4. Common over-expressed genes among different cell wall stress conditions

Gene	Group	Description
MSC1	cell cycle	Protein that affects recombination
YLR194C	cell wall	GPI-anchored protein
YPS3	cell wall	GPI-anchored aspartyl protease
YLR042C	cell wall	Putative GPI-anchored protein
YLR040C	cell wall	Weak similarity to mannoproteins PAU family
PIR3	cell wall	Similarity to members of the Pir1p/Hsp150p/Pir3p family
GFA1	cell wall	Catalyzes first step in chitin biosynthesis pathway
CWP1	cell wall	Mannoprotein , member of the PAU family
YJL171C	cell wall	Putative GPI-protein
HSP150	cell wall	Member of Pir1/Hsp150p/Pir3 family
CIS3	cell wall	Similarity to members of the Pir1p/Hsp150p/Pir3p family
YPS6	cell wall	GPI-anchored aspartyl protease
BGL2	cell wall	Endo-beta-1,3-glucanase of the cell wall
CRH1	cell wall	Cell wall protein with putative transglycosidase activity
SED1	cell wall	May contribute to cell wall integrity
PST1	cell wall	Plasma membrane protein that acts in cell wall
YPL088W	metabolism	Putative aryl alcohol dehydrogenase
YMR315W	metabolism	Member of the oxidoreductase family NAD-binding
YHR209W	metabolism	Low similarity to trans-aconitate methyltransferase
YGL157W	metabolism	Similarity to plant reductases
HOR2	metabolism	Functions in glycerol-3-phosphate metabolism
PRB1	metabolism	Mediates maturation of vacuolar phosphatases
GPD1	metabolism	Glycerol-3-phosphate dehydrogenase 1
DFG5	morphogenesis	Required for cell polarity and cellular elongation
PTP2	signal transduction	Hog1p and Fus3p MAP kinases phosphatase
MLP1	signal transduction	Protein kinase with similarity to MAP kinases
SLT2	signal transduction	MAP kinase involved in the cell wall integrity pathway
HAL1	stress	Involved in ion homeostasis
DIA1	stress	Involved in invasive growth
DDR48	stress	Induced by several stresses
PRM5	stress	Regulated by cell stress
CTT1	stress	Catalase T 1
HSP12	stress	Induced by several stresses
YNL208W	unknown	Protein of unknown function
YNL058C	unknown	Hyphothetical protein
YMR090W	unknown	Hyphothetical protein
YLR414C	unknown	Hyphothetical protein
PRY2	unknown	Protein expressed under starvation conditions
FMP33	unknown	Hyphothetical protein
PRM10	unknown	Protein regulated by alpha factor
YJL107C	unknown	Weak similarity to a region mouse Dspp
YHR087W	unknown	Hyphothetical protein
YGR043C	unknown	High similarity to transaldolase Tal1p

c) Identification of connections between different signaling pathways in yeast:

The transcriptional profile of a mutant strain deleted in *HOG1*, encoding the MAPK of the yeast HOG pathway, challenged with zymolyase, was analyzed with MARQ. As shown in Table 5, the signatures ranked in the top of the list where those related to the "mating" response. Moreover, the "Annotations menu" tool clearly identified

those GO functional groups related to mating as the ones with the lowest p-values (Table 6). It has been previously shown that under hyper-osmotic stress, the Pheromone pathway is inappropriately activated in cells lacking a functional HOG pathway (2,5) In agreement, the profiles of *hog1* mutants stressed with KCl 0.5M (2) also appeared in the top of the list (Table 5). Therefore, this crosstalk between HOG and Pheromone/Mating pathways is also operating under cell wall stress conditions mediated by zymolyase, as deduced from our MARQ analysis.

Table 5. MARQ analysis of the yeast transcriptional profile to zymolyase cell wall stress in a *hog1* mutant strain (The 10 top-ranked positions with the highest positive scores are included in the table).

MARQ position	GEO ref	Experiment	Condition	Score	p-value	Reference
1		RobertsData:	fus3 50 nM aF-30 m	0.926	<0.001	(3)
2		Mating response	far1 50 nM aF-30 m	0.912	<0.001	
3			rst1 rst2 wt	0.908	<0.001	
4			fus3-K42 50 nM aF-30 m	0.902	<0.001	
7			fus3 50 nM aF-30 m	0.899	<0.001	
8			rst1 rst2 tec1	0.899	<0.001	
5		O_RourkeData:	hog1 0.5M KCl 90 m	0.899	<0.001	(2)
6		Mating response	hog1 0.5M KCl 120 m	0.899	<0.001	
10			hog1 0.5M KCl 60 m	0.893	<0.001	
9	GSE8982	Mating response	wt aF-600nM	0.898	<0.001	

Table 6. Most significant GO terms enriched in signatures positively correlated with the query.

Term	P-Value
cell wall organization	<0.001
sexual reproduction	<0.001
biopolymer biosynthetic process	<0.001
conjugation	<0.001
response to pheromone	<0.001
reproduction	<0.001
pheromone-dependent signal transduction involved in conjugation with cellular fusion	<0.001
conjugation with cellular fusion	<0.001
transposition, RNA-mediated	<0.001
transposition	<0.001
viral procapsid maturation	<0.001
DNA integration	<0.001
proteolysis	<0.001
karyogamy during conjugation with cellular fusion	<0.001
DNA recombination	<0.001
filamentous growth	<0.001
regulation of cell shape	<0.001
signal transduction	<0.001
cytokinesis, completion of separation	<0.001
karyogamy	<0.001
adaptation to pheromone during conjugation with cellular fusion	<0.001
invasive growth in response to glucose limitation	<0.001
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	<0.001
positive regulation of transcription from RNA polymerase II promoter	<0.001
actin cytoskeleton organization	<0.001
establishment of cell polarity	<0.001
G1 phase of mitotic cell cycle	<0.001
G-protein coupled receptor protein signaling pathway	<0.001
cell surface receptor linked signal transduction	<0.001

References

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