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Genome-wide identification of genes that play a role in boron stress response in yeast

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ABSTRACT

Boron is an essential micronutrient for plants and it is either necessary or beneficial for animals. Studies identified only few genes related to boron metabolism thus far and details of how boron is imported into cells and used in cell metabolism are largely unknown. In order to identify genes that play roles in boron metabolism, we screened the entire set of yeast haploid deletion mutants and identified 6 mutants that were resistant to toxic levels of boron, and 21 mutants that were highly sensitive to boron treatment. Furthermore, we performed a proteomic approach to identify additional proteins that are significantly up-regulated by boron treatment. Our results revealed many genes and pathways related to boron stress response and suggest a possible link between boron toxicity and translational control.

1. Introduction

Boron is an important micronutrient in plants and animals. In plants, boron functions as a cross-linker for rhamnogalacturonan-II in the cell wall [1–3] and also as a structural component in cytoskeleton assembly [4]. There are several genes associated with boron transport and tolerance in plants [5–7]. *A. thaliana BOR1* is a boron efflux transporter for xylem loading and protects shoots from boron deficiency [8]. Homologs of *BOR1* were found in many organisms including yeast, plants and human [5,6,9].

The yeast *Saccharomyces cerevisiae* has been used as a model organism for the characterization of plant boron tolerance genes [5,6,10,11]. Yeast can grow at high concentrations of boron and is considered as a high boron-tolerant organism [12,13].

Bor1 was the first yeast protein shown to play a role in boron tolerance. It is localized to the plasma membrane [14] and functions as a boric acid efflux transporter across the cell membrane [11]. In addition to Bor1, two other yeast transporters, Dur3 and Fps1, were suggested to be playing role in boron tolerance [12], but the function of these transporters in boron detoxification is not clear. Elimination of none of these genes results in boron sensitivity and their expression levels are not responsive to boron treatment [13].

Recently, *ATR1* has been identified as a boron tolerance gene by screening a yeast DNA expression library. High copy expression of *ATR1* conferred resistance to boron treatment and reduced the level of intracellular boron, whereas cells lacking the *ATR1* gene was sensitive to boron and contained a higher intracellular level of boron. In addition to these, expression of *ATR1* was shown to be regulated by boron treatment [13].

In order to find additional genes related to boron metabolism, we screened the collection of haploid yeast deletion mutants for resistance and sensitivity to boron. We also utilized a proteomic approach to identify proteins that are significantly up-regulated or synthesized in the presence of boron. Our analyses uncovered many non-essential genes whose products might play a role in boron tolerance and toxicity in yeast.

2. Results and discussion

2.1. Identification of boron resistant yeast deletion mutants

Boron tolerance level of yeast has been previously established [11–13] and wild type cells were shown to tolerate up to 80 mM of boron (Fig. 1A). To identify the genes whose deficiency confer boron resistance to higher concentrations, we first tested all viable mutants of a yeast haploid deletion set (approximately 4700 mutants) on solid rich media containing 100 mM boric acid and identified 30 resistant mutants (see supplementary data). Subsequent testing of these resistant mutants on higher concentrations of boron confirmed 6 mutants (*ncs2*Δ, *ncs6*Δ, *kti12*Δ, *elp1*Δ, *elp3*Δ, *elp6*Δ) that could grow in the presence of up to 150 mM of boron which is a 2-fold higher concentration than the lethal dose (Fig. 1B). Since the yeast deletion

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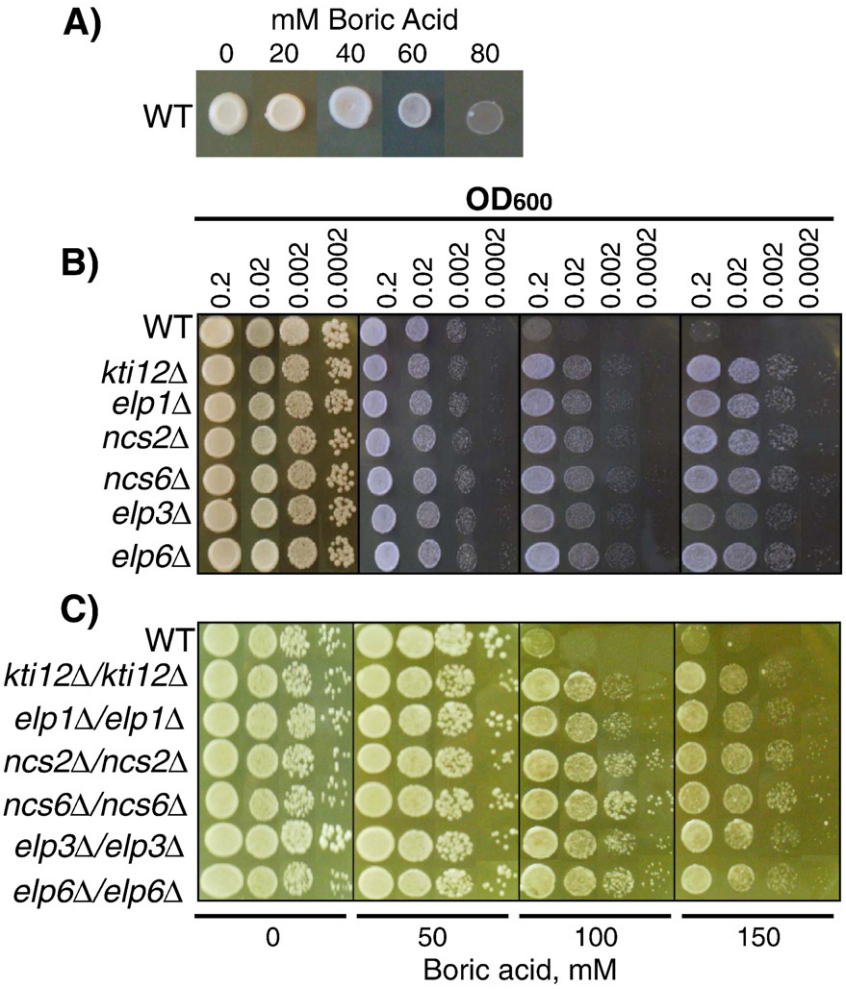

Fig. 1. Spotting assay showing boron tolerance level of wild type and mutant yeast cells. (A) Wild type cells (BY4741) were grown to logarithmic phase in rich (YPD) medium, diluted to OD_{600} of 0.2, and 5 μl of cell suspension was spotted onto plates containing the indicated amounts of boric acid. The plates were incubated at 30 °C for 3 days and then photographed. (B) Haploid boron resistant mutants (BY4741 background) identified by genetic screenings were grown to logarithmic phase in rich (YPD) medium, diluted to OD_{600} of 0.2, 0.02, 0.002 and 0.0002. A 5 μl of cell suspension from each samples was spotted onto plates containing the indicated amounts of boric acid. The plates were incubated at 30 °C for 3 days and then photographed. (C) Boron resistant homozygous diploid mutants (BY4743 background) identified by genetic screenings were grown to logarithmic phase in rich (YPD) medium, diluted to OD_{600} of 0.2, 0.02, 0.002 and 0.0002. A 5 μl of cell suspension from each sample was spotted onto plates containing the indicated amounts of boric acid. The plates were incubated at 30 °C for 3 days and then photographed.

collection contains many strains with suppressor mutations or aneuploidy, we thought it would be reassuring to verify these mutants in a different background. As seen in Fig. 1C, homozygous diploid cells lacking the same genes were also boron resistant.

Interestingly, all of these boron resistant cells were lacking genes whose product play a role in the modification of the uridines at the wobble position of tRNAs. Previously, mutations in these genes were reported to play a role in the resistance to the killer toxin of *Kluyveromyces lactis*, zymocin, and these mutants were classified as class II mutants which resist to both endogenous and exogenous sources of zymocin [15–17]. Elp1, Elp3 and Elp6 are subunits of the six membered Elongator complex (Elp1–Elp6) and play a role in the formation of the 5-methoxycarbonylmethyl (mcm^5) and 5-carbamoylmethyl (ncm^5) groups on wobble uridines in several tRNAs [17,18], in transcriptional elongation by RNA Pol-II and in polarized exocytosis [19,20]. Similarly, Kti12 plays a role in the modification of the wobble base together with the Elongator complex [18]. Cells lacking *NCS2* and *NCS6* genes were shown to be lacking the activity to form 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2) and 2-thiouridine (s^2) [17,20]. The formation of modified nucleosides in the wobble position of tRNAs seem to be the most important and physiologically relevant function of the Elongator complex, because overexpression of hypomodified $tRNA^{Lys}s^2UUU$ and $tRNA^{Gln}s^2UUG$ counteracted the deficiency in exocytosis and transcriptional activation [20]. Thus, resistance of *ncs2*Δ, *ncs6*Δ, *kti12*Δ, *elp1*Δ, *elp3*Δ and *elp6*Δ mutants to boron could be related to the absence of uridine modifications at the wobble base of tRNAs which are needed for efficient decoding of lysine and glutamine codons in mRNAs during translation. The absence of these uridine modifications, preserve the tRNAs from degradation by zymocin and provide resistance to this toxin [17], however mechanisms on how the absence of tRNA modifications could cause boron resistance need to be further investigated.

2.2. Identification of boron sensitive mutants

Genetic studies in boron metabolism have been usually designed for the identification of resistance genes, however it is likely that absence of some genes may lead to hypersensitivity to this element. To find genes absence of which sensitizes cells for boron, we screened the entire set of yeast haploid deletions for the mutants that cannot tolerate the levels of boron that is not toxic to wild type cells. Through this screening, we first identified 357 mutants that cannot grow in the presence of more than 60 mM of boron (see supplementary data). When we gradually decreased boron to 40 mM, which is the half concentration of the lethal dose, only 21 strains showed sensitivity (Fig. 2A). Absence of the same

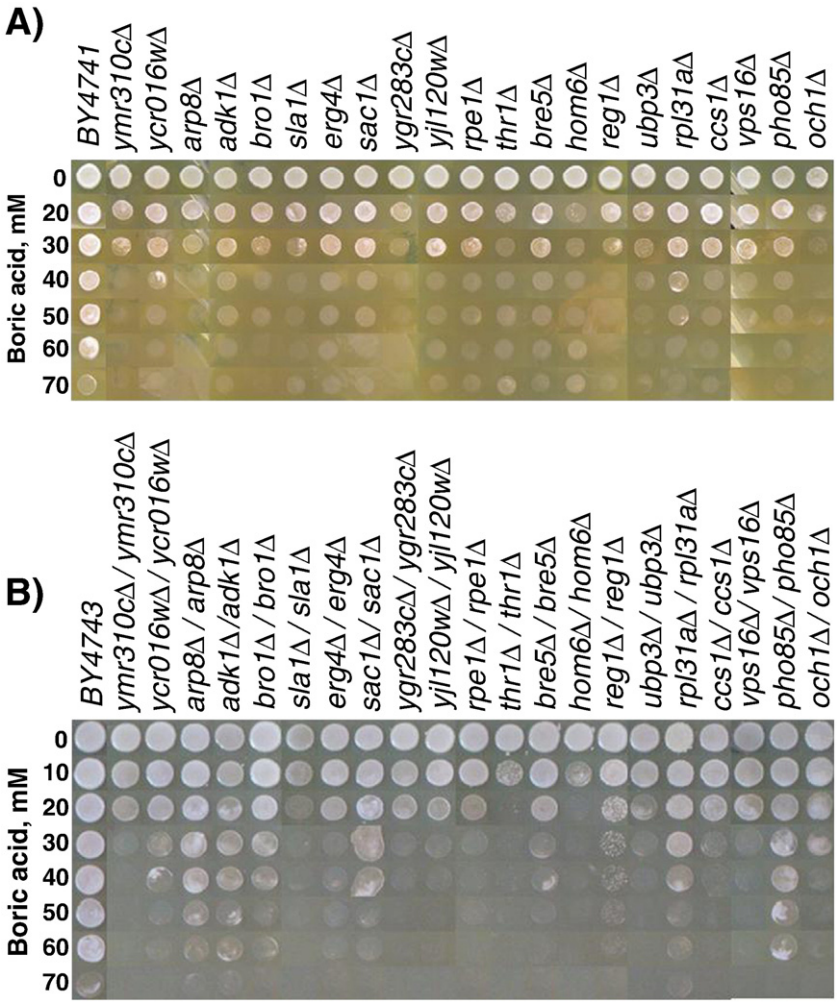

Fig. 2. Spotting assays showing boron tolerance level of boron sensitive mutants identified by genetic screenings. (A) Haploid mutants (BY4741 background) were grown to logarithmic phase in rich (YPD) medium, diluted to OD_{600} of 0.2, and 5 μl of cell suspension was spotted onto plates containing the indicated amounts of boric acid. Plates were incubated at 30 °C for 3 days and photographed. (B) Homozygous diploid mutants (BY4743 background) were grown to logarithmic phase in rich (YPD) medium, diluted to OD_{600} of 0.2, and 5 μl of cell suspension was spotted onto plates containing the indicated amounts of boric acid. The plates were incubated at 30 °C for 3 days and photographed.

genes in homozygous diploid background also caused sensitivity to boron in a similar manner (Fig. 2B) which shows that boron sensitivity was not strain dependent.

As seen in the Table 1, these boron sensitive mutants were lacking genes that are involved in many different metabolic pathways as discussed below. Interestingly, genes that were previously implicated in boron tolerance, *ATR1* and *BOR1*, were not included in Fig. 2 because the selection criteria allowed us to identify mutants that were sensitive to boron concentrations lower than 40 mM and deletion of both *ATR1* and *BOR1* genes do not create such sensitivity [13].

2.2.1. Amino acid metabolism

Apparently amino acid metabolism and boron toxicity is closely linked. In a previous study to observe the global expression response to boron, the most significantly up-regulated groups of genes were found to be amino acid biosynthesis genes which suggest that boron stress induces amino acid synthesis or activates the general amino acid control mechanism [13]. Here, the absence of two genes (*THR1* and *HOM6*) related to amino acid metabolism resulted in boron sensitivity. *THR1* is homoserine kinase which is implicated in threonine, serine, methionine and isoleucine metabolism, and regulated by the general amino acid control mechanism [21]. *HOM6* encodes for homoserine dehydrogenase which plays role in threonine, serine, methionine and lysine metabolism. The link between the

Table 1
List of the mutants that show boron sensitivity.

Systematic name	Common name	Function
YBL007C	SLA1	Cytoskeletal protein binding protein
YCR016W		Putative protein of unknown function
YDL075W	RPL31A	Protein component of the large (60S) ribosomal subunit
YDR028C	REG1	Regulatory subunit of type 1 protein phosphatase Glc7p
YDR226W	ADK1	Adenylate kinase
YER151C	UBP3	Ubiquitin-specific protease
YGL012W	ERG4	C–24(28) sterol reductase
YGL038C	OCH1	Mannosyltransferase of the *cis*-Golgi apparatus
YGR283C		Protein of unknown function
YHR025W	THR1	Homoserine kinase
YJL120W		Dubious open reading frame
YJL121C	RPE1	D-ribulose-5-phosphate 3-epimerase
YJR139C	HOM6	Homoserine dehydrogenase
YKL212W	SAC1	Phosphatidylinositol phosphate (PtdInsP) phosphatase
YMR038C	CCS1	Copper chaperone for superoxide dismutase Sod1p
YMR310C		Putative protein of unknown function
YNR051C	BRE5	Ubiquitin protease cofactor
YOR141C	ARP8	Nuclear actin-related protein
YPL031C	PHO85	Cyclin-dependent kinase
YPL045W	VPS16	Subunit of the vacuole fusion and protein sorting HOPS complex and the CORVET tethering complex
YPL084W	BRO1	Cytoplasmic class E vacuolar protein sorting (VPS) factor

Gene annotations were obtained from the Yeast Genome Database (www.yeastgenome.org).

amino acid metabolism and boron stress remains ambiguous at this time and it is being investigated by our group.

2.2.2. Vesicular and vacuolar transport system components

Several deletion strains that are deficient in vesicular and vacuolar transport systems were found to be sensitive to boron (Table 1). These cells were lacking *VPS16*, *BRE5*, *UBP3*, *BRO1* and *SAC1* genes. Products of these genes function in many diverse processes such as autophagy, cell wall maintenance, secretion, vacuole biogenesis, vacuolar protein sorting, antegrade or retrograde transport between endoplasmic reticulum and Golgi compartments [22–25]. Sac1 shows phosphatase activity and is localized to Golgi and endoplasmic reticulum and regulates the levels of phosphatidylinositol phosphates [26]. Bro1 is an important player in multivesicular body (MBV) pathway [27] and it functions in coordinating the deubiquitination process of cargo molecules. Two other genes that play a role in deubiquitination are *UBP3* and *BRE5*. They work together to regulate two-way trafficking between Golgi and ER [24]. Vacuolar sequestration or secretion of toxic substances are known to play a role in detoxification processes, however the relationship between these paths and boron toxicity is not clear. Ablation of secretory paths indeed results in sensitivity to many different stress conditions in yeast [28]. Recent studies also highlighted the importance of these pathways in detoxification of nickel and arsenic compounds [29,30].

2.2.3. Other boron sensitive mutants

One of the mutants that were hypersensitive to boron was lacking the *PHO85* gene which encodes for a multifunctional cyclin-dependent kinase (CDK). Depending on the cyclin partner, this CDK plays a role in cellular responses to nutrients, environmental stressors, and also regulates transcription during the cell cycle [31,32].

CCS1 is considered as an antioxidant protein since it plays a role as a copper chaperone for superoxide dismutases I (Sod1) [33] and its null mutant is also sensitive to boron stress. Cells lacking the *CCS1* gene also are sensitive to oxidative stress generating drugs because of a deficiency in Sod1 activity [33,34].

There were sensitive mutants deficient in carbohydrate metabolism (*rpe1*Δ, *reg1*Δ, *och1*Δ). Rpe1 is an epimerase in the pentose phosphate pathway and its absence limits the generation of NADPH and renders cells sensitive to oxidative stress [35]. Reg1 is a regulatory protein in negative control of glucose repression [36]. Och1 is localized to Golgi and plays a role in the mannosylation of proteins [37,38]. In plants, boron plays a structural role by cross linking structural carbohydrates [1], however it is not clear how these metabolic genes are related to boron metabolism in yeast.

Apart from these, the absence of one gene in lipid metabolism (*ERG4*) and one in purine metabolism (*ADK1*) also provided boron sensitivity. Erg4 is sterol reductase which plays a role in ergosterol synthesis [39], and the absence of this enzyme causes pleiotropic effects and cells become sensitive to many chemicals and divalent cations [39]. We also detected several genes with unknown functions the absence of which also rendered cells sensitive to boron (Table 1).

2.3. Proteomic analyses of boron stress response

In order to identify additional proteins that may play a role in boron stress response, proteins from untreated and treated cells were separated by a 2D-SDS PAGE and protein spots that were newly formed upon boron treatment were detected and analyzed by mass spectrometry. For mass analyses, we picked 11 spots that were easily seen by the naked eye on the gel run for boron treated samples. As seen in Table 2, three different proteins that play a role in translation were found to be overproduced by boron which were; cytoplasmic lysyl-tRNA synthase that is responsible for the acylation of $tRNA^{Lys}$ (*KRS1*), a large subunit of ribosomal protein L5 (*RPL5*) and translational elongation factor EF-3 (*HEF3*). Among these, lysyl-tRNA synthase has shown to be playing roles in the general amino acid control pathway as a regulator and expression of itself is regulated by general amino acid control [40]. A diminished level of lysyl-tRNA, which is the product of this enzyme, was shown to be activating general amino acid control pathways to expand amino acid pools [40,41].

Table 2
Proteins that are overexpressed in response to boron treatment.

No	Gene name	Description	Matched peptides
1	RPL5/ YPL131W	Large subunit ribosomal protein L5e	IAALAGQQ
2	KRS1/ YDR037W	Lysyl-tRNA synthetase	RFLDQR
3	SSE1/ YPL106C	ATPase, component of Hsp90	LVAETEDR
4	PGK1/ YCR012W	3-phosphoglycerate kinase	IQLIDNLLDK
5	CDC19/ YAL038w	Pyruvate kinase	GDLGIEIPAPEVLAVQK
6	ADE13/ YLR359W	Adenylosuccinate lyase	VTELLGFDK
7	HEF3/ YNL014W	Translational elongation factor EF-3	MTPSGHNWVSGQGAGPR
8	PRE9/ YGR135W	Alpha 3 subunit of the 20S proteosome	IHAQNYLK
9	TDH3/ YGR192C	G-3-phosphate dehydrogenase	LVSWYDNEYGYSTR
10	TSA1/ YML028	Thioredoxin peroxidase	DYGVLIEEEGVALR
11	FBA1/ YKL060C	Fructose 1,6-bisphosphate aldolase	FAIPAINVTSSSTAVAALEAAR

Protein samples from boron treated and control cells were separated by 2D-SDS PAGE, analyzed by mass spectra and identified by Mascot search (see Materials and methods).

Four of the spots contained proteins that play a role in glycolysis reactions (pyruvate kinase, 3-phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, and fructose 1,6-biphosphate aldolase). These proteins were also observed to be overproduced by different stress conditions such as osmotic stress, oxidative stress and drugs [42,43], thus overproduction of glycolysis enzymes in response to boron treatment may not be a specific response. Tsa1 is a well known antioxidant protein that acts as a thioredoxin-dependent peroxidase [44]. Ade13 is an adenylosuccinate lyase that plays a role in purine biosynthesis [45]. Apart from these, Pre9 which plays role in the structure of 20S proteosome, and Sse1 which is the ATPase subunit of Hsp90 were also identified as overproduced proteins in the presence of boron. Even though these proteins are significantly up-regulated by boron treatment, their roles in preserving cell functions in response to boron are not clear.

2.4. Boron stress versus other stress conditions: Comparison to other genome-wide screens

We found many genes that seem to play roles in boron stress response, however it is not clear whether these genes are specific to boron stress or they participate in other stress response pathways. To clarify their specificity we analyzed several genome-wide studies including nickel [46], arsenic [29,30], cadmium [30], alcohol [47] and oxalate tolerance [48], and compared our data with these studies. As seen in Table S.1 (see supplementary data), many boron sensitive mutants were also sensitive to arsenic, cadmium, nickel, ethanol and 1-pentanol. Among the boron resistant mutants, *kti12*Δ and *elp6*Δ cells were found to be ethanol and propanol sensitive. Thus, approximately half of the genes that play a role in boron stress response have roles in other stress tolerance mechanisms and they are not strictly boron specific genes.

We also analyzed the expression levels of genes the absence of which provides sensitivity or resistance to boron by using the genomic expression data from Kaya et al. [13]. Surprisingly, none of these genes

were shown to be up-regulated by boron (Fig. S.1, see supplementary data) and their expression levels were either not affected or down-regulated by boron treatment.

3. Conclusion

Previously, we performed genome-wide analyses such as global gene expression profiling and genomic DNA library screening to detect boron metabolism genes in yeast, and identified Atr1 as a boron efflux pump [13]. Here, we screened the entire set of yeast deletion mutants to find boron resistant and sensitive mutants. Furthermore, to find additional genes or proteins that play a role in response to boron, we analyzed the proteomic changes in yeast. The results suggest the possibility of enrolment of many distinct genes and pathways in boron stress response. Nevertheless, our data suggest a possible link between the general amino acid control mechanism, protein synthesis and boron toxicity. This idea is based on the findings that boron treatment induces the expression of amino acid biosynthesis genes which are under the regulation of general amino acid control [13]. Furthermore, boron resistant mutants identified in this study were all lacking the genes that play roles in the wobble base modifications of tRNAs during translation. Additionally, lysyl-tRNA synthase, ribosomal protein L5 (*RPL5*) and translational elongation factor EF-3 were found to be overproduced in response to boron treatment. However, the details of this putative mechanism among boron toxicity, general amino acid control and tRNA metabolism are not clear and needs to be further investigated.

Apart from the genes that are enrolled in translation, boron stress is conducted by many other genes whose protein products play a role in diverse biochemical paths. Some of these genes were shown to be involved in different stress conditions, which suggest that boron stress may share some common mechanisms with other stress conditions such as nickel, arsenic and cadmium stresses.

Most of the genes that we identified in this study have homologs in animals and plants, and we hope that this study will facilitate the elucidation of boron metabolism and toxicity in higher organisms.

4. Materials and methods

4.1. Yeast growth and media

WT strain BY4741 (*MATa* his3 leu2 met15 ura3) and its isogenic deletion mutants (4700 mutants) were obtained from the yeast deletion library (Invitrogen) and diploid strain BY4743 and its isogenic deletion mutants were obtained from EUROSCARF. YPD medium (2% glucose, 2% peptone, 1% yeast extract and 2% agar for solid media) was used for cell growth. For spotting assays, overnight cultures were diluted to an optical density of 0.2 at 600 nm initially and to 0.2, 0.02, 0.0002 by serial dilution. 5 μl of each dilution was spotted on YPD agar plates containing different amounts of boric acid. Plates were incubated 3 days at 30 °C and photographed.

4.2. Screening of yeast deletion collection set

To identify mutants that were boron resistant or sensitive, haploid yeast deletion collection mutants were inoculated into 200 μl of liquid YPD media in microtiter plates (approximately 70 plates) using a 96 pin-replicator to create master plates. Using the same replicator, 5 μl cell culture from each mutant was transferred to agar plates containing 0, 10, 20, 30, 40, 50, 60, 70, 100, 125, 150, 200 mM boric acid. Strains were grown for 3 days, and sensitive and resistant strains were identified by visual inspection of the cell growth. Strains were labeled sensitive to boric acid if the boron treatment inhibited cell growth below 40 mM which is half of the value that wild type cells can tolerate. Strains were labeled resistant if boron did not inhibit cell growth over 150 mM boron which is a 2-fold higher dose than wild type cells can tolerate. The screening assay was repeated four times. Growth scores for the resistant and sensitive cells were listed in Tables S.2 and S.3, respectively. Cells that were found to be boron sensitive or resistant were picked and analyzed further for confirmation purposes by spotting assays. Each spotting assay was repeated 4 times. Additionally, homozygous diploid deletion mutants for boron sensitive and resistant phenotypes were similarly analyzed by spotting assays to confirm that observed phenotypes are not strain dependent.

4.3. Proteomic analyses

Logarithmically growing wild type cells were split into two cultures and one of them was added boric acid to 50 mM, and the other one was used as control. After 1 h of treatment, cells were harvested by centrifugation and washed with distilled water two times and once more with TE-PMSF containing 1 mM EDTA, 0.1 M Tris, 14 μM PMSF pH 7,5 and protease inhibitor cocktail (Sigma P8215). Cells were extracted as described previously [49]. The protein samples (440 μg) were diluted in a final volume of 330 μl of rehydration buffer and 300 μl of which was applied onto IPG strips (pH 3–10 non-linear, 17 cm). Rehydration for transferring proteins into the strips was done actively at 50 V for 15 h in Biorad IEF system (Biorad Protean IEF Cell) with subsequent isoelectric focusing at 20 °C under mineral oil for 56 kVh (0–500 V 2000 Vh, 500 V 2000 Vh, 500–3500 V 12 kVh, 3500 V 40 kVh). Prior to SDS polyacrylamide gel electrophoresis, strips were equilibrated in 6 M urea, 0.375 M Tris–HCl pH 8.8, 2% SDS, 20% glycerol with DTT 2% for 15 min and with iodoacetamide 2.5% for the alkylation of the reduced sulphydryl groups for another 15 min. Electrophoresis was carried out at 220 V for 6 h (Biorad Protean II xi cell). Gels were stained with colloidal Coomassie Brilliant Blue. Spots that were under the detection limit (20 ng) in no treatment gel but with enhanced intensity in boron treated sample gels were identified and subjected to mass spectrometric analysis with MALDI TOF/TOF system (Bruker autoflex III Smartbeam, Bremen, Germany). Spots were excised and subjected to in-gel digestion with trypsin overnight [50]. Samples were desalted using Ziptip (Millipore) before running. Finally, peptides that were isolated from digested proteins were identified using MS/MS spectra and Mascot search engine (Matrix Science, London, England) and NCBInr protein database (National Center for Biotechnology Information, Bethesda, USA).

Supplementary materials related to this article can be found online at doi:10.1016/j.ygeno.2010.10.006.

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References

[1] M. Kobayashi, T. Matoh, J. Azuma, Two chains of Rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls, Plant Physiol. 110 (1996) 1017–1020.
[2] M.A. O'Neill, T. Ishii, P. Albersheim, A.G. Darvill, Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide, Annu. Rev. Plant Biol. 55 (2004) 109–139.
[3] H. Iwai, A. Hokura, M. Oishi, H. Chida, T. Ishii, S. Sakai, S. Satoh, The gene responsible for borate cross-linking of pectin Rhamnogalacturonan-II is required for plant reproductive tissue development and fertilization, Proc. Natl Acad. Sci. USA 103 (2006) 16592–16597.
[4] E. Bassil, H.N. Hu, P.H. Brown, Use of phenylboronic acids to investigate boron function in plants. possible role of boron in transvacuolar cytoplasmic strands and cell-to-wall adhesion, Plant Physiol. 136 (2004) 3383–3395.
[5] T. Sutton, U. Baumann, J. Hayes, N.C. Collins, B.J. Shi, T. Schnurbusch, A. Hay, G. Mayo, M. Pallotta, M. Tester, P. Langridge, Boron-toxicity tolerance in barley arising from efflux transporter amplification, Science 318 (2007) 1446–1449.
[6] J. Takano, M. Wada, U. Ludewig, G. Schaaf, N. von Wiren, T. Fujiwara, The *Arabidopsis* major intrinsic protein NIP5;1 is essential for efficient boron uptake

and plant development under boron limitation, Plant Cell 18 (2006) 1498–1509.
[7] K. Miwa, J. Takano, H. Omori, M. Seki, K. Shinozaki, T. Fujiwara, Plants tolerant of high boron levels, Science 318 (2007) 1417.
[8] J. Takano, K. Noguchi, M. Yasumori, M. Kobayashi, Z. Gajdos, K. Miwa, H. Hayashi, T. Yoneyama, T. Fujiwara, *Arabidopsis* boron transporter for xylem loading, Nature 420 (2002) 337–340.
[9] M. Park, Q. Li, N. Shcheynikov, W. Zeng, S. Muallem, NaBC1 is a ubiquitous electrogenic Na+-coupled borate transporter essential for cellular boron homeostasis and cell growth and proliferation, Mol. Cell 16 (2004) 331–341.
[10] A. Nozawa, K. Miwa, M. Kobayashi, T. Fujiwara, Isolation of *Arabidopsis thaliana* cDNAs that confer yeast boric acid tolerance, Biosci. Biotechnol. Biochem. 70 (2006) 1724–1730.
[11] J. Takano, M. Kobayashi, Y. Noda, T. Fujiwara, *Saccharomyces cerevisiae* Bor1p is a boron exporter and a key determinant of boron tolerance, FEMS Microbiol. Lett. 267 (2007) 230–235.
[12] A. Nozawa, J. Takano, M. Kobayashi, N. von Wiren, T. Fujiwara, Roles of BOR1, DUR3, and FPS1 in boron transport and tolerance in *Saccharomyces cerevisiae*, FEMS Microbiol. Lett. 262 (2006) 216–222.
[13] A. Kaya, H.C. Karakaya, D.E. Fomenko, V.N. Gladyshev, A. Koc, Identification of a novel system for boron transport: Atr1 is a main boron exporter in yeast, Mol. Cell. Biol. 29 (2009) 3665–3674.
[14] R.M. Zhao, R.A.F. Reithmeier, Expression and characterization of the anion transporter homologue YNL275w in *Saccharomyces cerevisiae*, Am. J. Physiol. Cell Physiol. 281 (2001) C33–C45.
[15] A.R. Butler, J.H. White, Y. Folawiyo, A. Edlin, D. Gardiner, M.J.R. Stark, Two *Saccharomyces cerevisiae* genes which control sensitivity to G1 arrest induced by *Kluyveromyces lactis* toxin, Mol. Cell. Biol. 14 (1994) 6306–6316.
[16] F. Frohloff, L. Fichtner, D. Jablonowski, K.D. Breunig, R. Schaffrath, *Saccharomyces cerevisiae* Elongator mutations confer resistance to the *Kluyveromyces lactis* zymocin, EMBO J. 20 (2001) 1993–2003.
[17] B. Huang, J. Lu, A.S. Bystrom, A genome-wide screen identifies genes required for formation of the wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine in *Saccharomyces cerevisiae*, RNA 14 (2008) 2183–2194.
[18] B. Huang, M.J.O. Johansson, A.S. Bystrom, An early step in wobble uridine tRNA modification requires the Elongator complex, RNA 11 (2005) 424–436.
[19] G.S. Winkler, A. Kristjuhan, H. Erdjument-Bromage, P. Tempst, J.Q. Svejstrup, Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo, Proc. Natl Acad. Sci. USA 99 (2002) 3517–3522.
[20] A. Esberg, B. Huang, M.J.O. Johansson, A.S. Bystrom, Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis, Mol. Cell 24 (2006) 139–148.
[21] G. Mannhaupt, H.D. Pohlenz, A.K. Seefluth, U. Pilz, H. Feldmann, Yeast homoserine kinase. Characteristics of the corresponding gene, THR1, and the purified enzyme, and evolutionary relationships with other enzymes of threonine metabolism, Eur. J. Biochem. 191 (1990) 115–122.
[22] J.E. Rothman, Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells, Cell 59 (1989) 591–601.
[23] A. Kihara, T. Noda, N. Ishihara, Y. Ohsumi, Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*, J. Cell Biol. 152 (2001) 519–530.
[24] M. Cohen, F. Stutz, C. Dargemont, Deubiquitination, a new player in Golgi to endoplasmic reticulum retrograde transport, J. Biol. Chem. 278 (2003) 51989–51992.
[25] K. Obara, T. Noda, K. Niimi, Y. Ohsumi, Transport of phosphatidylinositol 3-phosphate into the vacuole via autophagic membranes in *Saccharomyces cerevisiae*, Genes Cells 13 (2008) 537–547.
[26] T. Strahl, J. Thorner, Synthesis and function of membrane phosphoinositides in budding yeast, *Saccharomyces cerevisiae*, Biochim Biophys Acta Lipids Lipid Metab 1771 (2007) 353–404.
[27] G. Odorizzi, D.J. Katzmann, M. Babst, A. Audhya, S.D. Emr, Bro1 is an endosome-associated protein that functions in the MVB pathway in *Saccharomyces cerevisiae*, J. Cell Sci. 116 (2003) 1893–1903.
[28] M.E. Hillenmeyer, E. Fung, J. Wildenhain, S.E. Pierce, S. Hoon, W. Lee, M. Proctor, R.P. St Onge, M. Tyers, D. Koller, R.B. Altman, R.W. Davis, C. Nislow, G. Giaever, The chemical genomic portrait of yeast: uncovering a phenotype for all genes, Science 320 (2008) 362–365.
[29] X. Zhou, A. Arita, T.P. Ellen, X. Liu, J.X. Bai, J.P. Rooney, A.D. Kurtz, C.B. Klein, W. Dai, T.J. Begley, M. Costa, A genome-wide screen in *Saccharomyces cerevisiae* reveals pathways affected by arsenic toxicity, Genomics 94 (2009) 294–307.
[30] M. Thorsen, G.G. Perrone, E. Kristiansson, M. Traini, T. Ye, I.W. Dawes, O. Nerman, M.J. Tamas, Genetic basis of arsenite and cadmium tolerance in *Saccharomyces cerevisiae*, BMC Genomics 10 (2009).
[31] R. Shemer, A. Meimoun, T. Holtzman, D. Kornitzer, Regulation of the transcription factor Gcn4 by Pho85 cyclin Pc15, Mol. Cell. Biol. 22 (2002) 5395–5404.
[32] D. Huang, H. Friesen, B. Andrews, Pho85, a multifunctional cyclin-dependent protein kinase in budding yeast, Mol. Microbiol. 66 (2007) 303–314.
[33] P.J. Schmidt, M. Ramos-Gomez, V.C. Culotta, A gain of superoxide dismutase (SOD) activity obtained with CCS, the copper metallochaperone for SOD1, J. Biol. Chem. 274 (1999) 36952–36956.
[34] E.S. Unlu, A. Koc, Effects of deleting mitochondrial antioxidant genes on life span, in: S.I.S. Rattan, S. Akman (Eds.), Biogerontology: Mechanisms and Interventions, 2007, pp. 505–509.
[35] H. Juhnke, B. Krems, P. Kotter, K.D. Entian, Mutants that show increased sensitivity to hydrogen peroxide reveal an important role for the pentose phosphate pathway in protection of yeast against oxidative stress, Mol. Genet. Genomics 252 (1996) 456–464.
[36] J.L. Tu, M. Carlson, REG1 binds to protein phosphatase type 1 and regulates glucose repression in *Saccharomyces cerevisiae*, EMBO J. 14 (1995) 5939–5946.
[37] K. Nakayama, T. Nagasu, Y. Shimma, J. Kuromitsu, Y. Jigami, OCH1 encodes a novel membrane bound mannosyltransferase: outer chain elongation of asparagine-linked oligosaccharides, EMBO J. 11 (1992) 2511–2519.
[38] L. Karhinen, M. Makarow, Activity of recycling Golgi mannosyltransferases in the yeast endoplasmic reticulum, J. Cell Sci. 117 (2004) 351–358.
[39] D. Zweytick, C. Hrastnik, S.D. Kohlwein, G. Daum, Biochemical characterization and subcellular localization of the sterol C-24(28) reductase, Erg4p, from the yeast *Saccharomyces cerevisiae*, FEBS Lett. 470 (2000) 83–87.
[40] M. Mirande, J.P. Waller, The yeast lysyl-tRNA synthetase gene. Evidence for general amino acid control of its expression and domain structure of the encoded protein, J. Biol. Chem. 263 (1988) 18443–18451.
[41] M.L. Greenberg, P.L. Myers, R.C. Skvirsky, H. Greer, New positive and negative regulators for general control of amino acid biosynthesis in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 6 (1986) 1820–1829.
[42] B. Soufi, C.D. Kelstrup, G. Stoehr, F. Frohlich, T.C. Walther, J.V. Olsen, Global analysis of the yeast osmotic stress response by quantitative proteomics, Mol. Biosyst. 5 (2009) 1337–1346.
[43] P.M. Santos, T. Simoes, I. Sa-Correia, Insights into yeast adaptive response to the agricultural fungicide mancozeb: a toxicoproteomics approach, Proteomics 9 (2009) 657–670.
[44] H.Z. Chae, S.J. Chung, S.G. Rhee, Thioredoxin-dependent peroxide reductase from yeast, J. Biol. Chem. 269 (1994) 27670–27678.
[45] M.L. Guetsova, K. Lecoq, B. DaignanFornier, The isolation and characterization of *Saccharomyces cerevisiae* mutants that constitutively express purine biosynthetic genes, Genetics 147 (1997) 383–397.
[46] A. Arita, X. Zhou, T.P. Ellen, X. Liu, J.X. Bai, J.P. Rooney, A. Kurtz, C.B. Klein, W. Dai, T.J. Begley, M. Costa, A genome-wide deletion mutant screen identifies pathways affected by nickel sulfate in *Saccharomyces cerevisiae*, BMC Genomics 10 (2009).
[47] K. Fujita, A. Matsuyama, Y. Kobayashi, H. Iwahashi, The genome-wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols, FEMS Yeast Res. 6 (2006) 744–750.
[48] V. Cheng, H.U. Stotz, K. Hippchen, A.T. Bakalinsky, Genome-wide screen for oxalate-sensitive mutants of *Saccharomyces cerevisiae*, Appl. Environ. Microbiol. 73 (2007) 5919–5927.
[49] T. von der Haar, Optimized protein extraction for quantitative proteomics of yeasts, PLoS ONE 2 (2007).
[50] A. Shevchenko, M. Wilm, O. Vorm, O.N. Jensen, A.V. Podtelejnikov, G. Neubauer, P. Mortensen, M. Mann, A strategy for identifying gel-separated proteins in sequence databases by MS alone, Biochem. Soc. Trans. 24 (1996) 893–896.