A *Saccharomyces cerevisiae* Genome-Wide Mutant Screen for Altered Sensitivity to K1 Killer Toxin

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ABSTRACT

Using the set of *Saccharomyces cerevisiae* mutants individually deleted for 5718 yeast genes, we screened for altered sensitivity to the antifungal protein, K1 killer toxin, that binds to a cell wall β-glucan receptor and subsequently forms lethal pores in the plasma membrane. Mutations in 268 genes, including 42 in genes of unknown function, had a phenotype, often mild, with 186 showing resistance and 82 hypersensitivity compared to wild type. Only 15 of these genes were previously known to cause a toxin phenotype when mutated. Mutants for 144 genes were analyzed for alkali-soluble β-glucan levels; 63 showed alterations. Further, mutants for 118 genes with altered toxin sensitivity were screened for SDS, hygromycin B, and calcofluor white sensitivity as indicators of cell surface defects; 88 showed some additional defect. There is a markedly nonrandom functional distribution of the mutants. Many genes affect specific areas of cellular activity, including cell wall glucan and mannoprotein synthesis, secretory pathway trafficking, lipid and sterol biosynthesis, and cell surface signal transduction, and offer new insights into these processes and their integration.

THE sequenced and analyzed *Saccharomyces cerevisiae* genome has enabled a program of precise targeted gene disruption, resulting in a collection of mutant strains deficient in each gene (Winzeler *et al.* 1999; Giaver *et al.* 2002; see also: http://sequence-www.stanford.edu/group/yeast/yeast_deletion_project/deletions3.html). Such a collection promotes the discovery of cellular roles for genes by facilitating the characterization of mutant phenotypes and allows a comprehensive examination of the genetic complexity of a phenotype. We have used the *S. cerevisiae* gene disruption set to screen for K1 killer toxin phenotypes. Toxin resistance has been extensively studied by classical genetics, and many genes have been identified. This toxin is encoded on the M1 satellite virion of the L dsRNA virus of *S. cerevisiae* (Wickner 1996). Toxin sensitivity results from binding of the protein to the cell surface and its subsequent action at the plasma membrane promoting a lethal loss of cellular ions (reviewed in Bussey 1991; Breinig *et al.* 2002). Defects in the genes involved in these processes may change cellular sensitivity to this toxin, and known resistant mutants define genes whose products are involved in cell wall synthesis and regulation (Shahinian and Bussey 2000). Here we describe the results of global screens of haploids and homozygous and heterozygous diploid mutants for altered K1 toxin sensitivity.

MATERIALS AND METHODS

Strains and media: Wild-type strains were BY4742 (*MAT*α) and BY4743 (*MAT***a**/*MAT*α; Brachmann *et al.* 1998), except where noted in Figure 1B, which also presents some results from strain SEY6210 (Robinson *et al.* 1988). Deletant strains were from the Saccharomyces Genome Deletion Consortium (Giaver *et al.* 2002) and are available at Research Genetics (http://www.resgen.com/products/YEASTD.php3; see Table 1 for complete genotype descriptions). Haploid *big1* and *pkc1* mutants were obtained by dissection of the heterozygous diploid strains on media supplemented with 0.6 M and 1.0 M sorbitol, respectively. To improve spore viability of *pkc1* tetrads, 1.0 M sorbitol was added during the zymolyase treatment of asci. Yeast were grown in standard YPD medium (Sherman 1991), unless otherwise stated. YPD/G418 medium, used to pregrow the mutants for 18 hr on 2% agar plates, is made of YPD supplemented with 200 mg/liter geneticin (GIBCO-BRL, Grand Island, NY). To test for drug sensitivity, YPD plates contained 25 or 50 μg/ml of calcofluor white, 30 or 80 μg/ml of hygromycin B, or 0.05% SDS.

K1 killer toxin assay: K1 toxin sensitivity was measured as follows (for details see Brown *et al.* 1994). Yeast mutant strains (haploid *MAT*α as well as the homozygous and heterozygous diploids) were pregrown for 18 hr at 30° on YPD/G418 in parallel with corresponding wild types pregrown on YPD. To control for variation in toxin activity between experiments, three wild-type controls were incorporated into every batch

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TABLE 1

Yeast strains

Strain	Genotype	Source
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Brachmann *et al.* (1998)
BY4743	*MAT***a***/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 MET15/met15Δ0 ura3Δ0/ura3Δ0*	Brachmann *et al.* (1998)
Haploid[a]	As BY4742, *orfΔ::kanMX4*	Winzeler *et al.* (1999)
Heterozygous[a]	As BY4743, *orfΔ::kanMX4/ORF*	Winzeler *et al.* (1999)
Homozygous[a]	As BY4743, *orfΔ::kanMX4/orfΔ::kanMX4*	Winzeler *et al.* (1999)
SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9*	Robinson *et al.* (1988)
HAB880	As SEY6210 except *mnn9::kanMX2*	Shahinian *et al.* (1998)
HAB900[b]	As SEY6210 except *fks1::GFP-HIS3*	Ketela *et al.* (1999)

[a] Indicates mutants obtained from the Saccharomyces Genome Deletion Consortium.
[b] Haploid derived from TK103 strain.

of mutants tested (100–600 mutants/batch). Approximately 1×10^6 cells were resuspended in 100 μl of sterile water, of which 5 μl was used to inoculate 5 ml of molten YPD agar medium (1% agar, 0.001% methylene blue, and 1× Halvorson buffered at pH 4.7) held at 45°. Sorbitol was supplemented for *big1* and *pkc1* mutants, and for a wild-type control, as described above. This medium was quickly poured into 60- × 15-mm petri dishes and allowed to cool for 1 hr at room temperature. Then 5 μl K1 killer toxin (1000× stock diluted 1:10; Brown *et al.* 1994) was spotted on the surface of the solidified medium. The plates were incubated overnight at 18° followed by 24 hr at 30° (48 hr for slow growth mutants). For each mutant showing a "killing" or "death" zone different from wild type, a picture comparing the mutant and appropriate control was taken with the IS-500 Digital Imaging System, version 2.02 (Alpha-Innotech). Two measurements of the killing zone were made with PhotoShop 4.0 and the average was saved in a database (FileMaker Pro 5.0) together with the picture. Mutants with a killing zone <90% or >110% were retested up to four times to confirm the observed phenotype. These percentages were determined as [(mutant killing zone diameter)/(wild-type killing zone diameter) × 100]. A subset of mutants showing killing zones <75% or >115% was selected for further characterization.

K1 toxin survival assay: To determine cell survival after toxin treatment, 200 μl of a cell culture grown to log phase in YPD pH 4.7 and adjusted to OD_{600} 0.5 was incubated with 50 μl toxin (1000× stock diluted up to 1:25) for 3 hr at 18° on a labquake. Percentage of surviving cells was calculated following plating onto YPD agar after incubation with toxin and counting colonies after 2 days at 30°.

Drug phenotype assay: Drug sensitivity was determined by spotting diluted cultures on plates containing various drugs as described (Ram *et al.* 1994; Lussier *et al.* 1997). Briefly, 5 ml of liquid YPD medium, inoculated with freshly grown cells on YPD/G418, was incubated overnight at 30°. The cell density of these exponentially growing cultures was standardized with water at an OD_{600} of between 0.485 and 0.515, and 2 μl of a set of 10-fold serial dilutions were spotted on YPD supplemented with calcofluor white, hygromycin B, or SDS (see *Strains and media* for drug concentrations). Hypersensitivity or resistance was monitored for each drug after 48 and 72 hr growth at 30°. The cells were also spotted on a control plate (YPD without drug), which allowed a comparison with the growth rate of the mutants after 24 hr growth at 30°. Pictures of all conditions tested were downloaded into a FileMaker 5.0 database (see above for details).

Cell wall composition analysis: *Total* β*-glucan analysis:* Haploid strains used for alkali-insoluble β-glucan determinations were *MATα sla1Δ* and *big1Δ*, respectively, obtained or derived from the Saccharomyces Genome Deletion Consortium (see *Strains and media* above) and compared to wild-type strain BY4742, while *mnn9Δ* (HAB880) and *fks1Δ* (HAB900) were compared to parental strain SEY6210. Crude cell walls were isolated and the levels of alkali-insoluble β-1,3-glucan and β-1,6-glucan quantified as previously described (Dijkgraaf *et al.* 2002). The *big1Δ* mutant and the corresponding wild type were grown in medium containing 0.6 m sorbitol to provide osmotic support.

Alkali-soluble β*-1,6- and* β*-1,3-glucan analysis:* Alkali-soluble β-1,6- and β-1,3-glucan immunodetection was performed as described by Lussier *et al.* (1998) and summarized here. Yeast were pregrown on YPD/G418 for 18 hr at 30°, grown for 24 hr at 30° in 10 ml YPD liquid, and harvested by a 10-min centrifugation at 1860 × *g*. Cell pellets were washed with 5 ml of water and resuspended in 100 μl of water plus 100 μl of glass beads. The cells were then subjected to five cycles of vortexing for 30 sec, interspersed with 30-sec incubations on ice. Total cellular protein of the lysate was determined with the Bradford assay (Bradford 1976; Bio-Rad, Mississauga, ON, Canada) prior to alkali extraction (1.5 n NaOH, 1 hr, 75°). A set of 1:2 serial dilutions of the alkali-soluble fractions were then spotted on Hybond-C nitrocellulose membrane (Amersham, Oakville, ON, Canada). The immunoblotting was performed in Tris-buffered saline Tween containing 5% nonfat dried milk powder using either a 2000-fold dilution of affinity-purified rabbit anti-β-1,6-glucan primary antibody (Lussier *et al.* 1998) or a 1000-fold dilution of anti-β-1,3-glucan primary antibody (Biosupplies Australia Pty, Victoria, Australia), both with a 2000-fold dilution of horseradish peroxidase goat anti-rabbit secondary antibody (Amersham). The membranes were developed with a chemiluminescence detection kit (Amersham). Dot blots were scanned with a UMAX Astra 1220s scanner and signals were quantitated with Adobe Photoshop software, using the histogram function. The level of β-1,6- and β-1,3-glucan for each mutant was estimated by a comparison with a wild-type dilution series, with mutants classified by ranges of 20–35% (see footnote in Table 5).

RESULTS

K1 toxin sensitivity of deletion mutants: The toxin sensitivities of deletion mutants for 5718 genes were

compared to those of the parental strain. The screen was performed on haploid (*MAT*α) and homozygous diploid mutants, with toxin sensitivity being almost identical in both backgrounds. The heterozygous diploid collection was also tested, with the finding that 42 genes have a haploinsufficient toxin phenotype. The individual deletion of most genes has no effect on toxin sensitivity. These aphenotypic mutations include genes with a wide range of other phenotypes, such as slow growth and respiratory deficiency, and provide an important control for trivial cellular alterations that might affect the killing zone phenotype. For mutants in almost all genes, despite some affliction, the killer phenotype is wild type. Mutants in 268 genes (4.7%) have a phenotype distinct from wild-type toxin sensitivity, with 15 of these genes previously known to have such a phenotype (Shahinian and Bussey 2000; de Groot *et al.* 2001). Tables 2–4 list these mutants in functional groupings. A given gene is listed just once although some could be included in more than one category. Although the phenotypes are significant and reproducible, most null mutants have partial phenotypes. For example, among 155 haploid-resistant mutants, only 30 are fully resistant at the toxin concentration used (Table 2). Toxin sensitivity can be suppressed or enhanced in mutants, leading to resistance or hypersensitivity. Toxin resistance, which was always found as a recessive phenotype, is likely caused by a loss of function of some component needed for toxin action. In hypersensitive mutants the mutation synthetically enhances toxin lethality and can be functionally informative. Among the mutations resulting in a toxin phenotype, 42 were in uncharacterized open reading frames (ORFs) of unknown function. Of these, 3 were given a *KRE* (*k*iller toxin *re*sistant) number, and 8 genes with hypersensitive mutants were called *FYV* (*f*unction required for *y*east *v*iability upon toxin exposure) and given a number (Tables 2–4).

Mutants for 118 genes with toxin phenotypes were examined for altered sensitivity to SDS, calcofluor white, and hygromycin B as hypersensitivity or resistance to these compounds is indicative of cell surface defects (Lussier *et al.* 1997; Ross-Macdonald *et al.* 1999). Mutants in 88 of these genes showed some additional phenotype (Tables 2–4), independently suggesting that they have some cell surface perturbation. As β-1,6-glucan is the primary component of the cell wall receptor for the toxin, mutants in 144 genes with toxin phenotypes were examined for alkali-soluble β-1,6- and β-1,3-glucan levels (Table 5) with 63 showing an altered level of one or both polymers. Genes previously identified as killer resistant provide positive controls for this global screen (Tables 2 and 4). A number of characterized genes not known to have altered toxin sensitivity were found, suggesting that they have roles in cell wall or surface organization. Most mutants fall into a limited set of functional classes and define specific areas of cellular biology, some of which are described below (see also Tables 2 and 3).

Glucan synthesis: The yeast cell wall is made principally of four components: mannoproteins, chitin, β-1,3-glucan, and β-1,6-glucan (Orlean 1997; Lipke and Ovalle 1998). Protein mannosylation and β-1,6-glucan synthesis defects are known to lead to toxin resistance by altering the cell wall receptor for the toxin (see Shahinian and Bussey 2000 for a review; Breinig *et al.* 2002). Many mutations resulting in resistance to the K1 toxin have a reduced amount of β-1,6-glucan in the cell wall and show slow growth or inviability depending on the severity of the defect, and we anticipated finding new genes affecting these processes. A complex pattern of glucan phenotypes was found among the mutants examined for alkali-extractable β-1,6- and β-1,3-glucan levels, with reduced or elevated amounts of one or both polymers found (Table 5). Of mutants in 63 genes with glucan phenotypes, 55 had effects on β-1,6-glucan levels, with the remaining 8 having β-1,3-glucan-specific alterations. Of the 55 with β-1,6-glucan phenotypes, 40 also had some β-1,3-glucan phenotype, with 15 showing a β-1,6-glucan-specific phenotype. Principal findings are outlined below.

β-1,6-Glucan reduced: Mutants for five genes showing partial toxin resistance had specific but partial alkali-soluble β-1,6-glucan reductions. Among these was the β-1,3-glucan synthesis-associated gene *FKS1*, and this mutant also had reduced levels of alkali-insoluble β-1,3-glucan (Figure 1B; Table 5). The involvement of Fks1p in both β-1,3- and β-1,6-glucan biogenesis has been studied further (Dijkgraaf *et al.* 2002). Mutants in *CNE1* encoding yeast calnexin have less β-1,6-glucan (Shahinian *et al.* 1998), and this is also a mutant phenotype of the uncharacterized gene *YKL037W* encoding a small integral membrane protein.

β-1,6-Glucan reduced with altered β-1,3-glucan: *big1* mutants had greatly reduced levels of β-1,6-glucan and an increase in β-1,3-glucan. *BIG1* is a conditional essential gene retaining partial viability on medium with osmotic support (Bickle *et al.* 1998). Heterozygous *big1/BIG1* diploids showed haploinsufficient toxin resistance (Table 4), and haploid mutant cells grew very slowly on medium containing 0.6 M sorbitol and were toxin resistant (Figure 1A). Determination of the amount of alkali-insoluble glucan in the cell wall of a *big1* mutant showed that the β-1,6-glucan was 5% of wild-type levels (Figure 1B). The amount of β-1,3-glucan in *big1* mutants increased, possibly through some wall compensatory mechanism. Elsewhere, we have extended work on the role of Big1p in β-1,6-glucan biogenesis (Azuma *et al.* 2002).

Mutants for 13 genes had reductions in both alkali-soluble β-1,6- and β-1,3-glucan (Table 5), and three are described briefly below. *smi1/knr4* mutants are resistant both to the K1 toxin and to the K9 toxin from *Hansenula mrakii* and have wall glucan defects and a reduced *in vitro* glucan synthase activity (Hong *et al.* 1994a,b). Smi1p localized to cytoplasmic patches near the presumptive

TABLE 2

Genes whose deletion causes resistance to the K1 killer toxin (haploid or homozygous diploid death zone <75% of the wild type)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		1. Kinases, phosphatases, signal transduction (16 genes)						
PHO80	YOL001W	Cyclin that interacts with Pho85p protein kinase	48	85	63	S	S	wt
PHO85	YPL031C	Cyclin-dependent protein kinase	56	wt	37	R	S	S
PTP3	YER075C	Protein tyrosine phosphatase	65	wt	63	R	wt	wt
CKA2	YOR061W	Casein kinase II α′ chain	65	wt	63	R	wt	wt
SAC7[a]	YDR389W	GTPase-activating protein for Rho1p	67	wt	85	S	wt	wt
ELM1	YKL048C	ser/thr-specific kinase	69	wt	58	S	wt	S
PTC1	YDL006W	Protein serine/threonine phosphatase 2c	71	wt	64			
KIN3	YAR018C	ser/thr protein kinase	72	wt	70			
SAP155	YFR040W	Sit4p-associated protein	73	wt	62			
SAP190	YKR028W	Sit4p-associated protein	74	wt	75			
GPA2[a]	YER020W	Guanine nucleotide-binding regulatory protein	74	wt	83	wt	wt	wt
RRD1	YIL153W	Phosphotyrosyl phosphatase activator	75	wt	69			
MKS1	YNL076W	Negative regulator of Ras-cAMP pathway	82	wt	83			
GLC8	YMR311C	Regulatory subunit for ser/thr phosphatase Glc7p	83	wt	69			
PHO86	YJL117W	Inorganic phosphate transporter	83	wt	70			
SAL6	YPL179W	Phosphoprotein phosphatase	85	wt	67			
		2. Transcription (21 genes)						
SSN6	YBR112C	General repressor of transcription	0	81	0			
TUP1	YCR084C	General transcription repressor	0	83	0			
CTK3	YML112W	CTD kinase, gamma subunit; RNA Pol II regulation	0	wt	0			
SNF12	YNR023W	Component of SWI/SNF global transcription activator complex	0	wt	NA			
CCR4	YAL021C	Transcriptional regulator	42	wt	57	R	wt	wt
POP2	YNR052C	Required for glucose derepression	60	wt	70			
ELP4	YPL101W	Subunit of RNA Pol II elongator complex	68	wt	76	S	wt	wt
ADA2	YDR448W	General transcriptional adaptor or coactivator	69	wt	63			
CTK1	YKL139W	CTD kinase, alpha subunit; RNA Pol II regulation	70	wt	58			
ACE2	YLR131C	Metallothionein expression activator	71	wt	75	R	R	wt
SIR4	YDR227W	Silencing regulatory and DNA-repair protein	72	wt	NA	wt	wt	wt
IK13	YLR384C	Elongator complex; RNA Pol II-associated protein	73	wt	75			
SSN3	YPL042C	Cyclin-dependent CTD kinase	73	wt	78			
SIR1	YKR101W	Silencing regulatory protein	73	wt	80			
FUN30	YAL019W	Member of the Snf2p family of ATP-dependent DNA helicases	76	wt	72			
EPL6	YMR312W	Subunit of RNA Pol II elongator complex	79	wt	58			
ELP3	YPL086C	Histone and other protein acetyltransferase	80	wt	65			
SPT10	YJL127C	Transcription regulatory protein	80	wt	70			
SAP30	YMR263W	Component of the Rpd3p-Sin3p histone deacetylase complex	82	wt	65			
SIG1	YER068W	Transcriptional repressor	83	wt	32	S	S	S
TAF14	YPL129W	TFIIF subunit (transcription initiation factor), 30 kD	85	wt	56	S	S	S

(*continued*)

TABLE 2

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		3. Actin organization (7 genes)						
ARC18	YLR370C	Subunit of the Arp2/3 complex	0	wt	0			
VRP1[a]	YLR337C	Verprolin, involved in cytoskeletal organization	0	wt	0	R	S	S
LAS17[a]	YOR181W	Component of actin cortical patches	0	wt	NA	S	S	S
SAC6	YDR129C	Actin filament bundling protein, fimbrin	49	wt	57	S	S	wt
VIP1	YLR410W	Involved in cortical actin function	62	wt	72			
NBP2	YDR162C	Nap1p-binding protein	70	85	73			
SLA2	YNL243W	Cytoskeleton assembly control protein	71	wt	NA	S	S	S
		4. Lipid/sterol synthesis (9 genes)						
PLC1	YPL268W	Lipid, fatty-acid, and sterol metabolism and signal transduction	0	wt	0			
ERG2	YMR202W	C-8 sterol isomerase	55	wt	51	S	S	S
ERG28	YER044C	Protein involved in synthesis of ergosterol	57	wt	52	S	S	S
IPK1[a]	YDR315C	IP5 kinase	66	wt	79			
ERG4[a]	YGL012W	Sterol C-24 reductase	73	wt	70	S	S	S
DEP1	YAL013W	Regulator of phospholipid metabolism	73	wt	72			
OSH1[a]	YAR044W	Similarity to human oxysterol binding protein (OSBP)	77	wt	72			
SUR4[a]	YLR372W	Sterol isomerase, fatty acid elongase	83	wt	74			
LCB83	YJL134W	Sphingoid base-phosphate phosphatase, putative regulator	84	wt	84			
		5. Secretion/endocytosis (23 genes)						
ERV41[a]	YML067C	COPII ER-Golgi vesicle protein	0	wt	0			
CLC1	YGR167W	Clathrin light chain	0	wt	0	S	S	S
KRE11[a]	YGR166W	TRAPPII Golgi vesicular transport protein	0	wt	0	S	wt	S
LUV1	YDR027C	Involved in protein sorting in the late Golgi	0	wt	0			
SAC1[a]	YKL212W	Role in Golgi function and actin cytoskeleton organization	0	wt	0	S	S	S
SHE4[a]	YOR035C	Required for mother-cell-specific gene expression and for endocytosis	0	wt	0	S	S	wt
SWA2	YDR320C	Clathrin-binding protein required for normal clathrin function	0	wt	0	S	S	S
RCY1	YJL204C	F-box protein involved in endocytic membrane traffic	0	wt	0	S	S	S
SAC2	YDR484W	Suppressor of actin mutation, involved in vesicular transport	0	wt	NA			
ERV46	YAL042W	Vesicular transport between Golgi and ER	38	wt	42	wt	wt	wt
VPS74	YDR372C	Vacuolar protein sorting	53	wt	70	S	S	S
VAC8[a]	YEL013W	Required for vacuole inheritance and vacuole targeting	55	79	65	wt	wt	wt
CHC1	YGL206C	Clathrin heavy chain	59	wt	0			
COD3[a]	YGL223C	Complex with Sec34p-Sec35p involved in vesicle transport to the Golgi	59	wt	69	S	S	S
YPT6	YLR262C	GTP-binding protein of the rab family	69	wt	73			
ERV14[a]	YGL054C	Strong similarity to *D. melanogaster* cni protein	71	wt	76			
COD2	YNL041C	Complex with Sec34p-Sec35p involved in vesicle transport to the Golgi	73	wt	73			
ARL3	YPL051W	Required for transport from ER to Golgi and Golgi to vacuoles	73	wt	80			

(*continued*)

TABLE 2

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
VPS61[a]	YDR136C	Vacuolar protein sorting	78	wt	67			
VPS67[a]	YKR020W	Vacuolar protein sorting	78	wt	68			
VPS1	YKR001C	Member of the dynamin family of GTPases; vacuolar sorting protein	79	wt	68			
PEP3[a]	YLR148W	Vacuolar protein sorting	84	wt	57	S	S	S
END3[a]	YNL084C	Required for endocytosis and cytoskeletal organization	85	wt	70			
		6. Protein glycosylation (13 genes)						
CWH41	YGL027C	ER glucosidase I	0	wt	0	S	S	wt
GDA1	YEL042W	Guanosine diphosphatase	0	wt	0	S	S	S
ROT2[a]	YBR229C	ER glucosidase II, catalytic subunit	0	wt	0	S	S	S
YUR1[a]	YJL139C	Mannosyltransferase	0	wt	0			
OST3	YOR085W	Oligosaccharyltransferase gamma subunit	0	92	33	S	S	S
PMT1	YDL095W	Mannosyltransferase	0	wt	68			
KRE2	YDR483W	α-1,2-Mannosyltransferase	0	wt	NA			
CWH8	YGR036C	Dolichol-P-phosphatase	0	wt	75	S	S	S
ALG6	YOR002W	Glucosyltransferase	53	wt	72	S	wt	S
ALG8	YOR067C	Glucosyltransferase	61	wt	79	S	wt	S
MNN5[a]	YJL186W	Putative mannosyltransferase	64	wt	81			
ALG5	YPL227C	Dolichol-P-glucose synthetase	70	wt	79	S	wt	S
PMT2[a]	YAL023C	Mannosyltransferase	77	wt	56	S	wt	S
		7. Protein modification or degradation (6 genes)						
MAK10	YEL053C	Subunit of Mak3p-10p-31p N-terminal acetyltransferase	56	wt	73	R	wt	S
KEX1[a]	YGL203C	Carboxypeptidase (YSC-α)	57	wt	70	S	R	S
DOC1	YGL240W	Component of the anaphase-promoting complex	67	wt	80			
BTS1[a]	YPL069C	Geranylgeranyl diphosphate synthase	72	wt	65			
VPS27[a]	YNR006W	Vacuolar protein sorting-associated protein	80	wt	67			
YPS7	YDR349C	GPI-anchored aspartic protease	83	wt	58	S	S	S
		8. Cell wall organization (8 genes)						
KRE1[a]	YNL322C	GPI-anchored plasma membrane protein	0	62	0	S	S	S
KRE6[a]	YPR159W	*cis*-Golgi glucanase-like protein	0	72	0	S	S	S
FKS1[a]	YLR342W	1,3-β-D-Glucan synthase-associated protein	0	85	0	S	S	wt
CNE1[a]	YAL058W	Calnexin, regulation of secretion and cell wall organization	0	wt	0	S	S	S
SMI1[a]	YGR229C	β-1,3-Glucan synthesis protein	0	wt	0	S	S	S
SBE22[a]	YHR103W	Similarity to budding protein Sbe2p	77	91	80			
SCW4	YGR279C	Glucanase gene family member	84	wt	72			
ECM30	YLR436C	Involved in cell wall biogenesis and architecture	84	wt	73			

(*continued*)

TABLE 2

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		9. Mitochondrial, respiratory, and ATP metabolism (17 genes)						
ILM1	YJR118C	Possibly involved in mitochondrial DNA maintenance	0	wt	0			
ATP2	YJR121W	F1F0-ATPase complex, F_1 β-subunit	0	wt	65			
IMG2	YCR071C	Required for integrity of mitochodrial genome	0	wt	75			
NAM2	YLR382C	Leucine-tRNA ligase precursor, mitochondrial	54	78	79			
OCT1	YKL134C	Mitochondrial intermediate peptidase	60	wt	79			
OXA1	YER154W	Cytochrome oxidase biogenesis protein	65	wt	83			
DIA4	YHR011W	May be involved in mitochondrial function	70	wt	60			
MRPL8	YJL063C	Ribosomal protein L17, mitochondrial	70	wt	73			
MGM101	YJR144W	Mitochondrial genome maintenance protein	70	wt	76			
MRPL38	YKL170W	Ribosomal protein of the large subunit, mitochondrial	71	wt	81			
MRPL33	YMR286W	Ribosomal protein of the large subunit, mitochondrial	72	wt	77			
MMM1	YLL006W	Required for mitochondrial shape and structure	73	wt	66			
ISA2	YPR067W	Mitochondrial protein required for iron metabolism	73	wt	72			
MRPL27	YBR282W	Ribosomal protein YmL27 precursor, mitochondrial	74	wt	74			
IMG1	YCR046C	Ribosomal protein, mitochondrial	81	wt	73			
ATP15	YPL271W	F1F0-ATPase complex, F_1 epsilon subunit	84	wt	55	wt	S	S
BCS1	YDR375C	Mitochondrial protein, involved in the assembly of cytb cl complex	85	wt	81			
		10. Ungrouped genes (16 genes)						
SOD1	YJR104C	Copper-zinc superoxide dismutase	0	wt	0			
UTH1[a]	YKR042W	Involved in the aging process	0	wt	0	wt	wt	wt
DRS2	YAL026C	P-type calcium-ATPase	0	wt	56	wt	S	S
NPL3	YDR432W	Nucleolar protein	31	wt	0	S	S	S
LEM3	YNL323W	Protein with similarity to Ycx1p, mutant is sensitive to brefeldin A	44	wt	45	S	wt	S
GLY1[a]	YEL046C	L-threonine aldolase, low specificity	45	wt	55	S	S	S
ATS1	YAL020C	α-Tubulin suppressor	71	wt	78			
YTA7	YGR270W	26S proteasome subunit	74	wt	80			
DFG5	YMR238W	Required for filamentous growth, cell polarity, and cellular elongation	74	wt	80			
THP1[a]	YOL072W	Hypothetical protein	76	70	0	S	S	S
CDC50	YCR094W	Cell division cycle mutant	77	wt	62			
NEW1	YPL226W	Member of the nontransporter group of the ABC superfamily	77	wt	66			
LYS7	YMR038C	Copper chaperone for superoxide dismutase Sod1p	78	wt	62			
FPS1[a]	YLL043W	Glycerol channel protein	79	wt	65	S	S	S
UTR1	YJR049C	Associated with ferric reductase activity	84	wt	70			
PEX12	YMR026C	Required for biogenesis of peroxisomes—peroxin	84	wt	72			

(*continued*)

TABLE 2

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		11. Genes of currently unknown function or poorly characterized (19 genes)						
BUD14	YAR014C	Protein of unknown function	54	wt	47	S	S	S
	YFR043C	Hypothetical protein	55	wt	70	wt	wt	wt
[a]	YKL037W	Weak similarity to *Caenorhabditis elegans* ubc-2 protein	59	wt	67	S	S	S
[a]	YGL007W	Questionable ORF	63	wt	70			
[a]	YNL213C	Hypothetical protein	67	wt	NA	R	S	wt
PIN4[a]	YBL051C	Similarity to *Schizosaccharomyces pombe* Z66568_C protein	70	wt	70			
	YOR154W	Similarity to hypothetical *Arabidopsis thaliana* proteins	71	wt	84			
	YLR270W	Strong similarity to YOR173w	71	wt	NA	R	wt	wt
	YNL063W	Similarity to S-adenosyl methionine-dependent methyl-transferase	73	wt	76			
MON2	YNL297C	Unknown function, sensitive to monensin and brefeldin A	80	wt	70			
TOS1	YBR162C	Protein with similarity to Aga1p	81	wt	66			
	YER140W	Hypothetical protein	82	wt	66			
SYS1[a]	YJL004C	Multicopy suppressor of ypt6	82	wt	74			
	YLL007C	Hypothetical protein	83	wt	67			
EAF6[a]	YJR082C	Hypothetical protein	83	wt	76			
	YDR126W	Similarity to hypothetical protein YLR246w and YOL003c	84	wt	66			
	YGR263C	Weak similarity to *Escherichia coli* lipase like enzyme	84	wt	68			
KRE27	YIL027C	K1 toxin resistance phenotype; has a hydrophobic domain	85	80	54			
	YBR284W	Similarity to AMP deaminase	85	wt	70			

Mutants not listed are those with a wild-type phenotype in haploid or homozygous diploid background and those that are hypersensitive (Table 3) or haploinsufficient (Table 4). R, resistant; S, hypersensitive; wt, wild type to Calcofluor white, Hygromycin B, and SDS. NA, not available.

[a] Indicates mutants with a β-glucan phenotype (see Table 5).

TABLE 3

Genes whose deletion causes hypersensitivity to the K1 killer toxin (haploid or homozygous diploid death zone >115% of the wild type)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		1. Kinases, phosphatases, signal transduction (8 genes)						
HOG1	YLR113W	ser/thr protein kinase of MAPK family	196	wt	160	wt	wt	S
PBS2	YJL128C	Tyrosine protein kinase of the MAP kinase kinase family	193	wt	179			
SPS1	YDR523C	ser/thr protein kinase	152	wt	NA			
STE11	YLR362W	ser/thr protein kinase of the MEKK family	139	wt	NA	wt	wt	S
SSK1	YLR006C	Two-component signal transducer	135	wt	111	R	wt	S
SSK2	YNR031C	MAP kinase kinase kinase of the HOG pathway	130	wt	114			
TPD3	YAL016W	ser/thr protein phosphatase 2A, regulatory chain A	122	wt	NA			
DIG1	YPL049C	Downregulator of invasive growth and mating	113	wt	118	R	wt	wt
		2. Transcription (7 genes)						
MED2	YDL005C	Transcriptional regulation mediator	166	wt	NA			
GAL11	YOL051W	RNA Pol II holoenzyme (SRB) subcomplex subunit	158	wt	162	S	S	S
SRB5	YGR104C	RNA Pol II holoenzyme (SRB) subcomplex subunit	131	wt	124	S	S	S
SRB2	YHR041C	RNA Pol II holoenzyme (SRB) subcomplex subunit	124	wt	128			
ITC1	YGL133W	Subunit of Isw2 chromatin remodeling complex	121	wt	125			
UME6	YDR207C	Negative transcriptional regulator	118	wt	139	S	wt	S
SW16	YLR182W	Transcription factor	118	wt	119	S	S	S
		3. RNA processing (6 genes)						
PRP18	YGR006W	U5 snRNA-associated protein	148	wt	103	wt	wt	wt
CBC2	YPL178W	Small subunit of the nuclear cap-binding protein complex CBC	139	wt	119	R	wt	wt
CDC40	YDR364C	Required for mRNA splicing	137	wt	NA	S	S	S
NSR1	YGR159C	Nuclear localization sequence binding protein	122	wt	116	S	wt	wt
BRR1	YPR057W	Involved in snRNP biogenesis	120	wt	116	wt	wt	wt
STO1	YMR125W	Large subunit of the nuclear cap-binding protein complex CBC	119	wt	124			
		4. Ribosomal and translation initiation proteins (18 + 2 genes)						
RPS16B	YDL083C	Ribosomal protein S16.e	157	wt	116			
RPS19B	YNL302C	40S small subunit ribosomal protein S19.e	149	wt	NA			
RPS24B	YIL069C	40S small subunit ribosomal protein S24.e	149	wt	144			
RPS24A	YER074W	40S small subunit ribosomal protein S24.e	129	wt	142	wt	wt	wt
RPS10A	YOR293W	Ribosomal protein S10.e	128	112	118	R	wt	wt
ASC1	YMR116C	40S small subunit ribosomal protein	127	wt	130			
RPS17A	YML024W	Ribosomal protein S17.e.A	127	wt	128	wt	wt	wt
RPS11A	YDR025W	Ribosomal protein S11.e	126	wt	139			
RPS23B	YPR132W	40S small subunit ribosomal protein S23.e	126	wt	123			

(*continued*)

TABLE 3

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
RPL13B	YMR142C	60S large subunit ribosomal protein	124	wt	130			
RPS16A	YMR143W	Ribosomal protein S16.e	121	wt	135	wt	wt	wt
RPS0B	YLR048W	40S ribosomal protein p40 homolog B	120	wt	125			
RPS30B	YOR182C	Similarity to human ubiquitin-like protein/ribosomal protein S30	120	wt	120			
RPL2B	YIL018W	60S large subunit ribosomal protein L8.e	118	111	136			
RPS4B	YHR203C	Ribosomal protein S4.e.c8	117	wt	114			
RPS30A	YLR287C-A	40S small subunit ribosomal protein	116	wt	114			
RPL14A	YKL006W	Ribosomal protein	115	wt	145	wt	wt	wt
RPS6B	YBR181C	Ribosomal protein S6.e	114	115	126			
TIF3	YPR163C	Translation initiation factor eIF4B	154	wt	139	wt	wt	S
YIF2	YAL035W	General translation factor eIF2 homolog	122	wt	NA			
		5. Protein modification or *N*-glycosylation (5 genes)						
MNN9[a]	YPL050C	Required for complex *N*-glycosylation	152	wt	146	S	S	S
ANP1[a]	YEL036C	Required for protein glycosylation in the Golgi	135	wt	129	S	S	S
MAP1[a]	YLR244C	Methionine aminopeptidase, isoform 1	134	wt	130	S	S	S
MNN10[a]	YDR245W	Subunit of α-1,6-mannosyl transferase complex	133	wt	136	S	S	S
LAS21[a]	YJL062W	Required for side-chain addition to GPI	114	wt	119			
		6. Cellular polarity (5 genes)						
BUD30[a]	YDL151C	K1 toxin hypersensitivity phenotype	134	wt	107			
BUD22	YMR014W	Protein with possible role in bud site polarity	128	wt	144	S	S	wt
BUD25	YER014C-A	Protein involved in bipolar budding	123	wt	115	S	S	S
BEM1	YBR200W	Bud emergence mediator	121	wt	114	S	S	S
BUD27[a]	YFL023W	K1 toxin hypersensitivity phenotype	115	wt	157			
		7. New *FYV* genes (8 genes)						
FYV1[a]	YDR024W	K1 toxin hypersensitivity phenotype	141	wt	138			
FYV4	YHR059W	K1 toxin hypersensitivity phenotype	127	wt	116			
FYV5[a]	YCL058C	K1 toxin hypersensitivity phenotype	125	wt	119			
FYV6[a]	YNL133C	K1 toxin hypersensitivity phenotype	123	wt	112			
FYV7[a]	YLR068W	K1 toxin hypersensitivity phenotype	120	wt	116	wt	wt	wt
FYV8	YGR196C	K1 toxin hypersensitivity phenotype; similarity to Arp1p	119	wt	121	S	S	S
FYV10[a]	YIL097W	K1 toxin hypersensitivity phenotype	116	wt	115	wt	S	S
FYV12[a]	YOR183W	K1 toxin hypersensitivity phenotype	114	wt	123	wt	S	wt

(*continued*)

TABLE 3

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone (%)			Calcofluor white	Hygromycin B	SDS
			Haploid	Heterozygous	Homozygous			
		8. Ungrouped or poorly characterized genes (12 genes)						
CSF1[a]	YLR087C	Required for normal growth rate and resistance to NaCl and H_2O_2	140	wt	125	S	S	S
VID21[a]	YDR359C	Mutant impaired in fructose-1,6-bisphosphatase degradation	139	wt	136	S	S	S
ARV1[a]	YLR242C	Lipid and sterol metabolism	137	wt	123	S	S	S
SEC66[a]	YBR171W	ER protein-translocation complex subunit	133	wt	144			
PFD1	YJL179W	Prefoldin subunit 1	133	wt	117	S	S	S
CTF4	YPR135W	DNA-directed polymerase α-binding protein	127	wt	112	wt	wt	wt
SYG1	YIL047C	Member of the major facilitator superfamily	126	wt	145			
IWR1	YDL115C	Hypothetical protein	126	wt	110			
YDJ1	YNL064C	Mitochondrial and ER import protein	121	wt	130			
APL4	YPR029C	Gamma-adaptin of clathrin-associated AP-1 complex	116	wt	117	wt	S	wt
HMO1	YDR174W	Nonhistone protein	115	wt	109			
ADK1	YDR226W	Adenylate kinase, cytosolic	112	wt	127	S	S	S

Mutants with a wild-type phenotype in haploid or homozygous diploid background and those with a resistant phenotype in these backgrounds are not listed. NA, not available.

[a] Mutants with a β-glucan phenotype (see Table 5).

bud site in unbudded cells and at the site of bud emergence (Martin *et al.* 1999) and may act in the polarization of glucan synthetic components. *CSF1 (YLR087C)* encodes an integral membrane protein that may be a plasma membrane carrier. The null mutant is hypersensitive to K1 toxin, calcofluor white, SDS, and hygromycin; Tokai *et al.* (2000) showed the mutant to be salt and hydrogen peroxide sensitive with low temperature defects in growth and the uptake of glucose and leucine. *LAS21 (YJL062W)* participates in glycosylphosphatidylinositol (GPI) synthesis, adding an ethanolamine phosphate to the α-1,6-linked mannose of the GPI mannose core (Benachour *et al.* 1999). As this mannose core is the site of attachment of the β-1,6-glucan moiety to GPI-linked cell wall proteins, altered levels of β-1,6-glucan might be expected, although the basis of neither the β-1,3-glucan defect nor the mutant hypersensitivity to K1 toxin is evident, indicating a need for further work.

β-1,6-Glucan elevated: Killer mutants in 33 genes had elevated levels of β-1,6-glucan (Table 5). A group of β-1,6-glucan overproducers are mutant in genes involved in assembly of the outer fungal-specific α-1,6-glucan chain of *N*-glycosyl chains (*mnn9*, *mnn10*, and *anp1*; see Table 5 and Figure 2). Mutants in these genes are hypersensitive to killer toxin and are described further in *N-glycosylation* below. A contrasting group of resistant mutants overproducing β-1,6-glucan (and to a lesser extent, β-1,3-glucan) are in a subgroup of genes involved in cortical actin assembly and endocytosis (Table 2 and *sla1* mutant in Figure 1). Our results are consistent with work reporting thickened cell walls in some of these mutants (for a review see Pruyne and Bretscher 2000). Cell wall synthesis is normally restricted to the growing bud, but in these mutants new material is added inappropriately to the mother cell, resulting in a thickened wall (Li *et al.* 2002). It is surprising that cells with thickened cell walls and more β-1,6-glucan can be killer toxin resistant, since resistance typically arises through loss of cell wall β-1,6-glucan and less binding of the toxin. One explanation is that more toxin is bound to the walls, reducing its effective concentration, a resistance mechanism proposed for the SMKT toxin of *Pichia farinosa* (Suzuki and Shimma 1999). A second explanation is that the thickened cell wall blocks toxin access to the plasma membrane.

Mutants for other genes that specifically overproduce alkali-soluble β-1,6-glucan have broadly acting gene products, with mutants expected to be pleiotropic and their effects indirect. These include *MAP1* encoding one of an essential pair of methionine aminopeptidases; this mutant is killer toxin, calcofluor white, hygromycin, and SDS hypersensitive (Table 3) and has a random budding pattern (Ni and Snyder 2001). *ERG4* encodes an oxidoreductase required for ergosterol synthesis. This mutant is partially toxin resistant, hypersensitive to calcofluor white, hygromycin, and SDS (Table 2),

TABLE 4

Genes whose deletion results in a K1 killer toxin haploinsufficiency phenotype

Gene name	ORF	Description of gene product	K1 killer toxin death zone % heterozygous	Calcofluor white	Hygromycin B	SDS
		1. Resistant (death zone <90% of the wild type; 28 + 3 genes)				
RSP5	YER125W	Ubiquitin-protein ligase	59			
GLC7	YER133W	ser/thr phosphoprotein phosphatase 1, catalytic chain	69			
PUP2	YGR253C	20S proteasome subunit(α5)	70			
CCT4	YDL143W	Component of chaperonin-containing T-complex	72			
ARC35	YNR035C	Subunit of the Arp2/3 complex	73			
CCT7	YJL111W	Component of chaperonin-containing T-complex	78			
SMD3	YLR147C	Strong similarity to small nuclear ribonucleoprotein D3	80	wt	wt	wt
NSA2	YER126C	K1 toxin resistance phenotype; nuclear protein	81			
ARP3	YJR065C	Actin-related protein	82			
ARC15	YIL062C	Subunit of the Arp2/3 complex	83			
SFH1	YLR321C	Subunit of the RSC complex	83	wt	wt	wt
CCT2	YIL142W	Chaperonin of the TCP1 ring complex, cytosolic	84			
CCT5	YJR064W	T-complex protein 1, epsilon subunit	84			
TAD3	YLR316C	Subunit of tRNA-specific adenosine-34 deaminase	85[b]			
BIG1[a]	YHR101C	Big cells phenotype	85	wt	wt	S
GCD7	YLR291C	Translation initiation factor eIF2b, 43-kD subunit	85	wt	wt	wt
SEC53	YFL045C	Phosphomannomutase	86			
SSL2	YIL143C	DNA helicase	86			
ACC1[a]	YNR016C	Acetyl-CoA carboxylase	87			
CDC25[a]	YLR310C	GDP/GTP exchange factor for Ras1p and Ras2p	87	wt	wt	wt
GAA1	YLR088W	Required for attachment of GPI anchor onto proteins	87	wt	wt	wt
TAF3	YPL011C	Component of the TBP-associated protein complex	87	wt	wt	wt
TIP20	YGL145W	Required for ER-to-Golgi transport	87			
TRS120	YDR407C	Weak similarity to Myo1p	87	wt	wt	wt
CEG1	YGL130W	mRNA guanylyltransferase (mRNA capping enzyme, α-subunit)	88			
MEX67	YPL169C	Factor for nuclear mRNA export	88	wt	wt	wt
LSG1	YGL099W	Required for normal growth, morphology, mating, sporulation	89			
ERG27	YLR100W	3-Keto sterol reductase, required for ergosterol biosynthesis	89	wt	wt	wt
CDC3	YLR314C	Cell division control protein	89	wt	wt	wt
New KRE genes						
KRE29	YER038C	Two-hybrid interaction with Ym1023p and Lys14p	76			
KRE33[a]	YNL132W	Unknown function, Amp1p-interaction complex (20 members)	83			

(*continued*)

TABLE 4

(Continued)

Gene name	ORF	Description of gene product	K1 killer toxin death zone % heterozygous	Calcofluor white	Hygromycin B	SDS
		2. Hypersensitive (death zone >110% of the wild type; 11 genes)				
TUB1	YML085C	α-1 tubulin	135			
RPB8	YOR224C	DNA-directed RNA polymerase I, II, III 16 kD subunit	128	S	wt	wt
RPS13	YDR064W	Ribosomal protein	122	wt	wt	wt
RPS15	YOL040C	40S small subunit ribosomal protein	121	wt	wt	wt
RPB3	YIL021W	DNA-directed RNA-polymerase II, 45 kD	118	wt	wt	wt
RPS3	YNL178W	Ribosomal protein S3.e	118	wt	wt	wt
RPB7	YDR404C	DNA-directed RNA polymerase II, 19-kD subunit	117	wt	wt	wt
AUT2	YNL223W	Essential for autophagy	116	S	wt	S
RPO26	YPR187W	DNA-directed RNA polymerase I, II, III 18-kD subunit	116	wt	wt	wt
TSC10	YBR265W	3-ketosphinganine reductase	115	S	wt	S
PKC1	YBL105C	Regulates MAP kinase cascade involved in regulating cell wall metabolism	wt[c]			

These gene deletion mutants are available only as heterozygotes and are usually essential.
[a] Mutants with a β-glucan phenotype (see Table 5).
[b] Under normal conditions this gene is essential, but haploid mutants can grow on sorbitol and are toxin resistant.
[c] Under normal conditions this gene is essential, but haploid mutants can grow on sorbitol and are toxin hypersensitive.

and has a random budding pattern (Ni and Snyder 2001). *ERV14 (YGL054C)* and *ERV41 (YNL067C)* encode COPII vesicle coat proteins involved in endoplasmic reticulum (ER)-to-Golgi trafficking (Otte *et al.* 2001), and both show toxin resistance. Mutants in four genes of unknown function also overproduce alkali-soluble β-1,6-glucan (Table 5). Two of these genes, *BUD27 (YFL023W)* and *BUD30 (YDL151C)*, have random budding patterns when mutated (Ni and Snyder 2001), and both are hypersensitive to killer toxin. *FYV5 (YCL058C)* encodes a predicted small integral membrane protein, with the mutant sensitive to sorbitol and low temperature (Bianchi *et al.* 1999) and K1 toxin hypersensitive. Finally, the null mutant of *YGL007C* has partial killer toxin resistance (Table 2).

***N*-glycosylation:** Defects in *N*-glucosylation and its processing can lead to partial toxin resistance and reduced levels of β-1,6-glucan (Romero *et al.* 1997; Shahinian *et al.* 1998). Our results extend this finding to many other genes whose products are involved in the biosynthesis and elaboration of the $Glc_3Man_9GlcNAc_2$ oligosaccharide precursor of *N*-glycoproteins (Tables 2 and 3; Figure 2). If Golgi synthesis of the fungal-specific α-1,6-mannose outer arm of the *N*-chain is blocked by mutation in *OCH1* or in *MNN9*, *MNN10*, or *ANP1* of the mannan polymerase complex, toxin hypersensitivity results, concomitant with higher levels of β-1,6-glucan in the cell wall (Tables 3 and 5; Figures 1 and 2; and see Magnelli *et al.* 2002 for *mnn9*). The glucan levels observed in an *och1* mutant were similar to those obtained in a *mnn9* mutant (not shown). To explore this further we determined the alkali-soluble glucan levels for other mutants in the mannan polymerase complex and the outer chain α-mannosyltransferases (Figure 2), irrespective of toxin phenotype. A mutant in *mnn11*, part of the α-1,6-mannose-synthesizing mannan polymerase complex, also showed elevated glucan levels, as did *mnn2* encoding the major α-1,2-mannosyltransferase that initiates mannose branching from the α-1,6-glucan backbone. However, a mutant in *mnn5*, whose gene product extends the α-1,2-mannose branches from the α-1,6-glucan backbone, had reduced levels of both β-glucans. Previous work showed that a small amount of glucan is attached to the *N*-chain structure (Tkacz 1984; van Rinsum *et al.* 1991; Kollar *et al.* 1997), and a genetic study by Shahinian *et al.* (1998) also suggested this possibility. Our results show that core *N*-chain processing is required for wild-type β-1,6-glucan levels, while absence of the outer α-1,6-linked mannose side chain or its first α-1,2-mannose branch can result in an increase in cell wall β-1,6-glucan. However, mutants in later mannosylation steps in elaborating branches from the outer α-1,6-linked mannose side chain have no effect or lead to reduced β-glucan levels.

Lipid and sterol synthesis and ion homeostasis: Mutants for 10 genes involved in the biosynthesis or regulation of lipids or sterols show partial toxin resistance

TABLE 5

Genes whose deletion results in an altered alkali-soluble β-glucan phenotype

Gene name	ORF	β-1,6-Glucan	β-1,3-Glucan
	β-1,6-Glucan only affected		
Elevated			
MAP1	YLR244C	++	wt
ANP1	YEL036C	+	wt
ERG4	YGL012W	+	wt
ERV14	YGL054C	+	wt
ERV41	YML067C	+	wt
KEX1	YGL240W	+	wt
FYV5	YCL058C	+	wt
BUD30	YDL151C	+	wt
BUD27	YFL023W	+	wt
	YGL007W	+	wt
Reduced			
	YKL037W	−−	wt
CNE1	YAL058W	−	wt
FKS1	YLR342W	−	wt
KRE11	YGR166W	−	wt
PEP3	YLR148W	(−)	wt
	β-1,3-Glucan only affected		
Elevated			
THP1	YOL072W	wt	+
	YNL213C	wt	+
BTS1	YPL069C	wt	(+)
GPA2	YER020W	wt	(+)
FYV7	YLR068C	wt	(+)
Reduced			
SEC66	YBR171W	wt	−
ACC1	YNR016C	wt	(−)
SYS1	YJL004C	wt	(−)
	β-1,6-Glucan and β-1,3-glucan affected		
Both elevated			
END3	YNL084C	+++	++
VRP1	YLR337C	+++	++
SAC7	YDR389W	+++	+
LAS17	YOR181W	+++	(+)
VAC8	YEL013W	++	+
FYV1	YDR024W	++	+
IPK1	YDR315C	++	(+)
FPS1	YLL043W	+	+
SAC1	YKL212W	+	+
VID21	YDR359C	+	+
EAF6	YJR082C	+	+
CDC25	YLR310C	+	(+)
GLY1	YEL046C	+	(+)
SUR4	YLR372W	+	(+)
VPS61	YDR136C	+	(+)
UTH1	YKR042W	(+)	+
VPS27	YNR006W	(+)	+
VPS67	YKR020W	(+)	+
FYV12	YOR183W	(++)	(++)
Both reduced			
SMI1	YGR229C	−−−	−−
PIN4	YBL051C	−−	−−
CSF1	YLR087C	−	−
LAS21	YJL062W	−	−
COD3	YGL223C	(−)	−−
PMT2	YAL023C	(−)	−
ARV1	YLR242C	(−)	(−)
MNN5	YJL186W	(−)	(−)
FYV10	YIL097W	(−)	(−)
KRE33	YNL132W	(−)	(−)
OSH1	YAR044W	(−)	(−−)
SBE22	YHR103W	(−)	(−−)
FYV6	YNL133C	(−−)	(−−)
	β-1,6-Glucan elevated and β-1,3-glucan reduced		
MNN10	YDR245W	+	−−
MNN9	YPL050C	+	−
YUR1	YJL139C	(+)	(−)
SHE4	YOR035C	(+)	(−−)
	β-1,6-Glucan reduced and β-1,3-glucan elevated		
BIG1	YHR101C	−−−	(+)
KRE1	YNL322C	−−	(++)
KRE6	YPR159W	−−	(++)
ROT2	YBR229C	(−)	(++)

Increase (I): +++, $I > 100\%$; ++, $65 < I < 100$; +, $45 < I < 65$; (+), $25 < I < 45$; (++), $I < 25\%$. Decrease (D): −−−, $85 < D < 100$; −−, $65 < D < 85$; −, $45 < D < 65$; (−), $25 < D < 45$; (−−), $D < 25\%$.

(*continued*)

(Table 2). These mutants have defects in membrane structure, possibly affecting the efficiency of insertion of the toxin into the plasma membrane or altering the cellular membrane potential leading to reduced toxin-induced ion permeability. Pertinently, defects in the ATP-dependent Drs2p and Atp2p membrane channels involved in cation and proton pumping confer toxin resistance. The altered membrane composition in lipid or sterol mutants could also affect secretory pathway function, possibly linking their partial toxin resistance phenotypes to those found in protein trafficking and secretion (Table 2). For example, *KES1* is implicated in ergosterol biology and can partially suppress the toxin resistance of a *kre11-1* mutant, with Kre11p being involved in Golgi vesicular transport as a subunit of the TRAPP II complex (Jiang *et al.* 1994; Sacher *et al.* 2001).

High-osmolarity and stress response pathways: To survive hyperosmotic conditions, *S. cerevisiae* increases cellular glycerol levels by activation of the high-osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway. Such activation leads to elevated transcription of genes required to cope with stress conditions, including the synthesis of glycerol with a resultant increase in internal osmolarity (Posas *et al.* 1998; Rep *et al.* 2000). Mutants with an inactive HOG pathway are toxin hypersensitive, while deletion of protein phosphatases, such as *PTP3*, *PTC1*, or *PTC3*, which act negatively on the

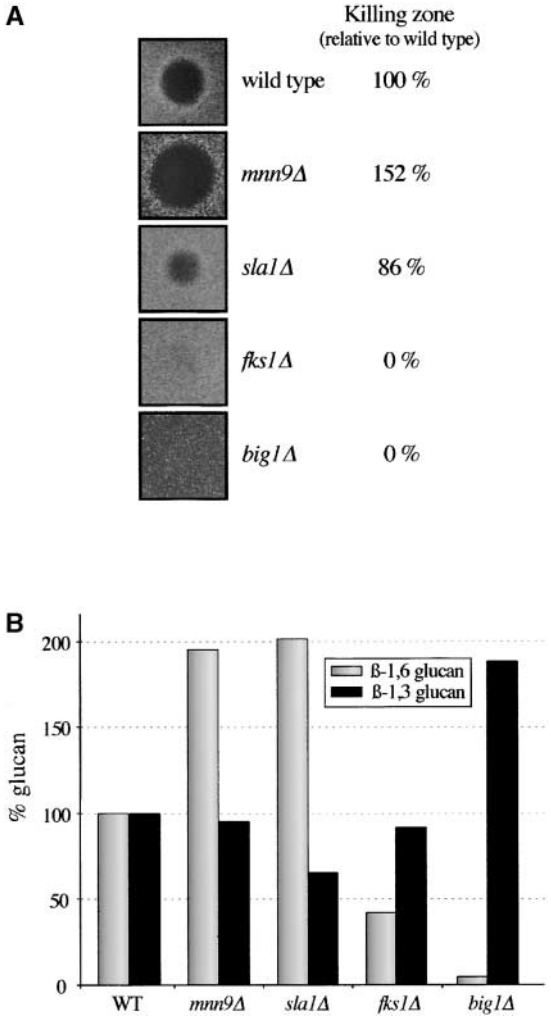

FIGURE 1.—Killer toxin sensitivity and quantification of major cell wall polymers of different strains. (A) A total of 5 μl of toxin was spotted onto agar seeded with a fresh culture of each strain (see MATERIALS AND METHODS). The mutant "killing zone" diameter was compared to the corresponding wild type and expressed as a percentage (see MATERIALS AND METHODS). (B) Measurement of cell wall β-1,6- and β-1,3-glucan levels was performed by extraction and fractionation of these polymers from cell wall preparations, followed by quantification of the alkali-insoluble fractions. The haploid mutants were from the Saccharomyces Genome Deletion Consortium (*sla1*Δ and *big1*Δ) or from strains HAB880 and HAB900, respectively, for *mnn9*Δ and *fks1*Δ mutants (see Table 4). To facilitate comparison, the values of alkali-insoluble glucans were expressed as percentages of the corresponding wild-type level. The data represent averages of at least three independent experiments with standard deviations not exceeding 10%.

pathway, lead to resistance (Tables 3 and 2, respectively; Figure 3B). Deletion of *HOG1* resulted in a killing zone diameter almost twice that of the wild type. For such large killing zones, the diameter is limited by the diffusion rate of the protein toxin and greatly underestimates increased mutant sensitivity. To quantify sensitivity in a *hog1* mutant, toxin-induced cell mortality was measured using a cell survival assay (see MATERIALS AND METHODS). A 10,000-fold reduction in cell viability was found when compared to the wild type. Previous estimates indicate that $\sim 3 \times 10^4$ molecules of toxin are required to kill a wild-type cell (BUSSEY *et al.* 1979). We compared the sensitivity of the *hog1* parental wild type from the deletion collection (strain BY4742) with strain S14a, on which the original lethal dose estimate was made, and found the strains to be of similar sensitivity (data not shown). Thus, just a few toxin molecules per cell are required to kill a *hog1* mutant, indicating that a functional HOG pathway provides cells with a powerful way to ameliorate the effects of this toxin.

The sequence of action of the K1 toxin begins with its binding to β-1,6-glucan cell wall receptors (SHAHINIAN and BUSSEY 2000). In a second step, the toxin inserts into the plasma membrane in a receptor-dependent process (BREINIG *et al.* 2002) and forms pores causing the leakage of ions and cellular metabolites, leading to cell death (MARTINAC *et al.* 1990; AHMED *et al.* 1999). To explore the defect in a *hog1* mutant we asked where it occurred in the path of action of the toxin, by examining its epistasis in double-mutant combinations of *hog1* with the toxin-resistant cell wall mutants *kre1* and *kre2*, both of which block synthesis of the cell wall receptor. A *kre1 hog1* mutant was as fully resistant as a *kre1* single mutant, and a *kre2 hog1* mutant was nearly so. Thus, defects in the cell wall receptor preventing binding of the toxin are dominant over the hypersensitivity of the *hog1* mutant. This result is consistent with hypersensitivity occurring through some downstream effect such as ion homeostasis and/or lethal pore formation. One consequence of the activation of the HOG pathway is the induced expression of the glycerol-3-phosphate dehydrogenase Gpd1p, required in glycerol biosynthesis (ALBERTYN *et al.* 1994). To test whether impaired glycerol production was the basis of the *hog1* mutant hypersensitivity, a *gpd1 gpd2* double deletion mutant was made to reduce glycerol synthesis (GARCÍA-RODRIGUEZ *et al.* 2000). This mutant had wild-type toxin sensitivity (data not shown). In further efforts to identify the downstream effectors of Hog1p responsible for the basal toxin resistance, we examined deletion mutants in the known transcription factors of the pathway, namely Msn1p, Msn2p, Msn4p, Hot1p, Sko1p, and Rck2p (PROFT and SERRANO 1999; REP *et al.* 1999, 2000; BILSLAND-MARCHESAN *et al.* 2000). All were wild type in sensitivity, as was the *msn2 msn4* double mutant.

Cell integrity signaling: In response to cell wall alterations, *S. cerevisiae* stimulates the Mpk1/Slt2p MAP kinase by activation of a cell integrity signaling pathway under the control of *PKC1* (Figure 3B). Loss of function of this pathway results in deficiencies in cell wall construction and cell lysis phenotypes, which can be partially suppressed by osmotic stabilizers (LEVIN and BARTLETT-HEUBUSCH 1992; PARAVICINI *et al.* 1992; ROEMER *et al.* 1994). Consistent with playing a key role in cell surface integrity, a *pkc1* haploid mutant kept alive by osmotic support is extremely sensitive to the toxin.

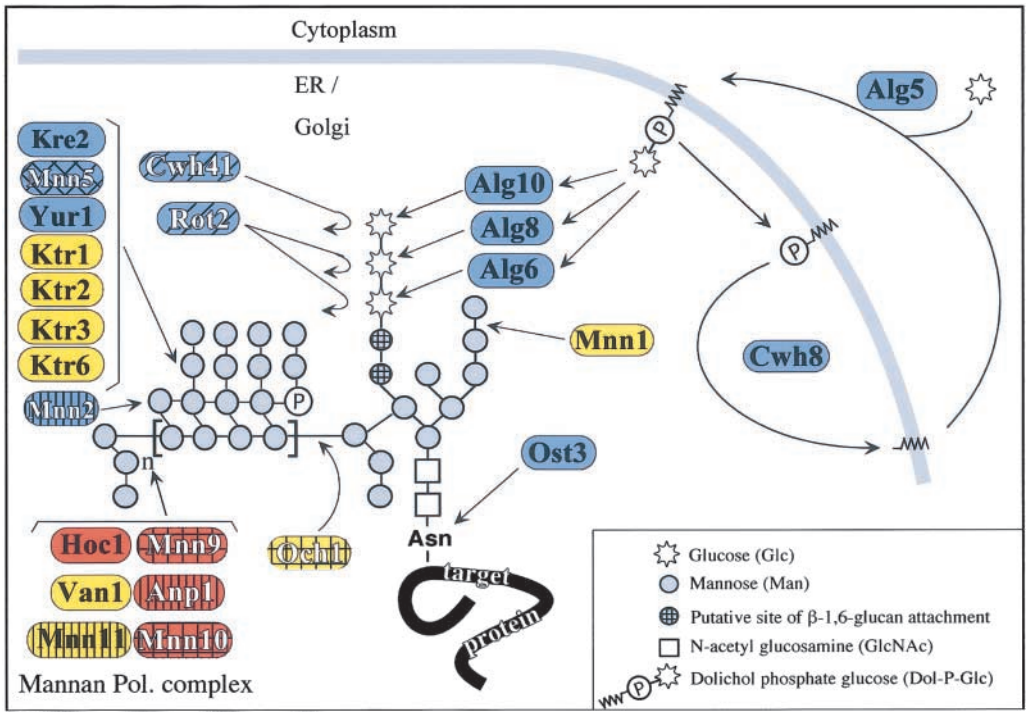

FIGURE 2.—Schematic summary of *N*-glycan biosynthesis in yeast. *N*-glycosyl precursor assembly is initiated in the endoplasmic reticulum. At the stage of $GlcNAc_2Man_9$, three glucose residues are serially transferred from the Dol-P-Glc donor to the *N*-glycan by the glucosyltransferases Alg6p, Alg8p, and Alg10p. Glucosylation is required for efficient transfer of the *N*-glycan to target proteins by a complex that includes Ost3p. The glucose residues are subsequently trimmed by the sequential action of glucosidases I and II, Cwh41p and Rot2p, respectively. *N*-linked oligosaccharides undergo further maturation in the Golgi, where addition of the fungal-specific "outer-chain" is initiated by Och1p and elaborated by various enzymes, including the mannan polymerase complex (adapted from ORLEAN 1997; SHAHINIAN and BUSSEY 2000). Arrows indicate activation and bars indicate negative effects. (*) indicates essential genes; *i.e.*, only heterozygous mutants were tested. Genes whose deletion causes toxin hypersensitivity, red; resistance, blue; no phenotype, yellow; not tested, white. β-Glucans are shown as follows: β-1,6-glucan and β-1,3-glucan both reduced; β-1,6-glucan reduced and β-1,3-glucan wild type; β-1,6-glucan and β-1,3-glucan both wild type; β-1,6-glucan elevated and β-1,3-glucan wild type; β-1,6-glucan elevated and β-1,3-glucan reduced. Mnn2p, Alg10p, and Hoc1p are not listed in Tables 2 or 3; they are resistant or hypersensitive to K1 toxin, but fall outside of the chosen ranges.

However, most of the upstream activators of Pkc1p and all known downstream MAPK signaling components of the cell integrity pathway show no toxin phenotype (see Figure 3B). The absence of phenotype for the upstream integral plasma membrane activators of the pathway may be explained by the functional redundancy of the components (VERNA *et al.* 1997; KETELA *et al.* 1999; PHILIP and LEVIN 2001). Rho1p, the GTP-binding protein involved in relaying the signal from the plasma membrane to Pkc1p, is essential and the heterozygote has a wild-type phenotype. However, in the MAP kinase cascade downstream of Pkc1p, the kinase Bck1p and the MAP kinase Mpk1p are unique and nonessential (LEVIN and ERREDE 1995). The absence of a toxin phenotype upon mutation of these components indicates that hypersensitivity of a *pkc1* mutant is not caused by the absence of activation of the *MPK1* MAP kinase pathway, but in some other way (Figure 3B).

Ribosomal subunit proteins: Defects in many ribosomal subunit proteins lead to toxin hypersensitivity. Of the 32 small ribosomal subunit genes, 8 are found as single copy and 24 are duplicated, for a total of 56 ORFs (PLANTA and MAGER 1998). Toxin hypersensitivity is observed for mutants for 21 of the duplicated genes (Tables 3 and 4). A single deletion of either copy often shows hypersensitivity. In some cases only one of the duplicated gene mutants shows the phenotype (*RPS0B*, *4B*, *10A*, *17A*, *19B*, *23B*), suggesting that they have distinct functions. Since some phenotypes were relatively weak (killing zone diameters <115% of the wild type), not all mutants are listed in Table 3. Of the 8 single-copy genes of the small ribosomal subunit, heterozygous deletions in just 2 essential genes, *RPS13* and *RPS15*, gave toxin hypersensitivity (Table 4). The toxin hypersensitivity phenotype was more prevalent among mutants in the small subunit (43%) than among those in the large (16%). A total of 46 genes encode the large ribosomal subunit proteins, among which 35 are duplicated (PLANTA and MAGER 1998); 12 of the duplicated genes show toxin hypersensitivity when mutated (Tables 3 and 4).

DISCUSSION

The ability to directly establish a phenotype-to-gene relationship is a great enabling strength of the mutant collection. Moreover, since each gene can be examined simply by testing a mutant, partial or weak phenotypes can be readily analyzed (BENNETT *et al.* 2001; NI and SNYDER 2001). The collection allows comprehensive screening and a knowledge of which genes have been examined, overcoming many of the limitations of a clas-

sical random mutant screen. Despite the extensive use of random screens for toxin resistance these failed to saturate the genome, as we have found mutants in many new genes. In addition, the mutant collection allows one to know which genes remain to be tested and, importantly, which genes do not have phenotypes. Such comprehensive testing can turn up the unexpected, as illustrated by a few examples. The extent of the relationships between cell wall polymers was unanticipated. Wall glucan work normally focuses on one or the other glucan synthetic pathway, and these are implicitly seen to be specific. Yet *fks1* mutants, defective for a component of the β-1,3-glucan synthase, are affected for both β-1,3- and β-1,6-glucan (Figure 1A), as are a large number of other mutants (Table 5). These interactions likely indicate synthetic or regulatory links between these polymers. The *mnn9* mutation, which blocks synthesis of the outer α-1,6-mannose arm of *N*-glycans, was assumed specific and has been used to simplify structural analyses of glucomannoproteins in the cell wall (Van Rinsum *et al.* 1991; Montijn *et al.* 1994). The fact that a *mnn9* mutation has other secondary effects that increase the amount of glucan in the wall is an unexpected complication, with the possibility that previous work analyzed structures absent from wild-type cells. Electrophysiological work links the Tok1p potassium channel with toxin action (Ahmed *et al.* 1999). In the deletion mutant collection used here neither the haploid *MAT***a** or *MAT*α nor the diploid heterozygous or homozygous deletion of this gene had a phenotype. Thus, in this strain background Tok1p has no detectable role in toxin action, indicating that despite the ability of the toxin to activate conductance of Tok1p, this channel protein cannot be the only target for the K1 toxin and is not a significant *in vivo* target in this sensitive strain. Having mutants in all cellular pathways allows the pursuit of phenotype through functional modules and has value in making such connections. Some specific examples are discussed below.

Functional clustering: The screen identified several examples of interactions that connect biological functions into larger cellular processes, sometimes already known in detail. For example, toxin phenotypes trace the relationship between almost every biosynthetic step of the *N*-glycosyl moiety of glycoproteins. The cytoskeletal mutants provide an example of a less well-characterized connectivity. Here a set of mutants in cytoskeletal processes has a common toxin resistance phenotype that correlates with mother cells showing abnormal wall proliferation. This wall phenotype, which is not a general one for all cytoskeletal defects, has been reported for individual genes (see Pruyne and Bretscher 2000). This functional cluster of genes, which may function in limiting wall growth to daughter cells, offers insight into a new facet of morphogenesis.

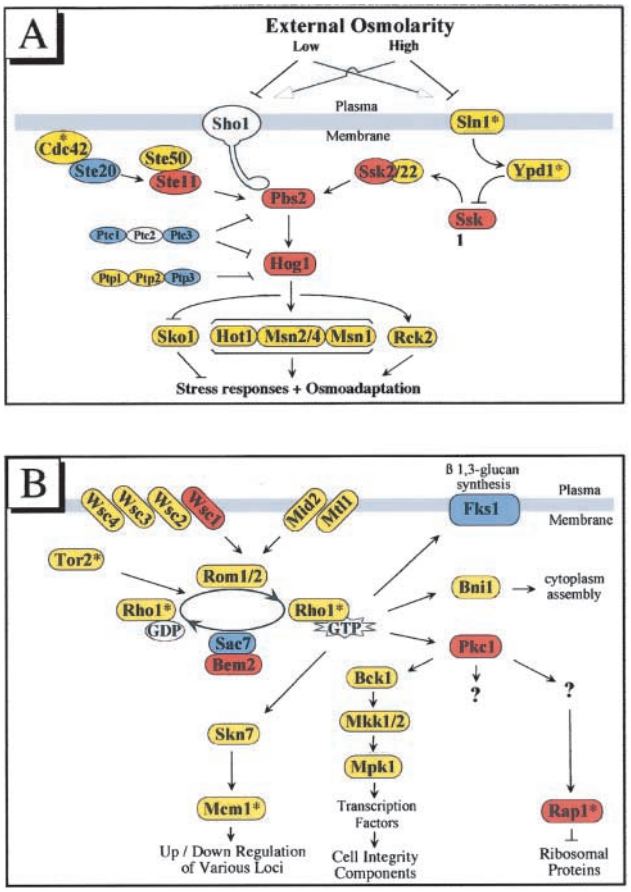

Figure 3.—Schematic summary of signal transduction pathways involved in osmoadaptive responses and cell wall synthesis in yeast. (A) Exposure to high extracellular osmolarity triggers an adaptive response mediated by two pathways that converge at Pbs2p. One arm of the pathway involves the binding of Pbs2p to plasma membrane protein Sho1p. Pbs2p is phosphorylated by the Ste11p MAPKKK, through a process requiring Cdc42p, Ste50p, and Ste20p (Desmond *et al.* 2000). A second pathway involves the two-component osmosensor module Sln1p-Ypd1p-Ssk1p, which activates Pbs2p via a pair of related MAPKKK proteins, Ssk2p and Ssk22p. Activation of this MAPK cascade culminates at Hog1p with Hog1p-dependent activation of the Rck2p protein kinase and activation and inactivation of transcription factors. The model also outlines the action of some negative regulators of the pathway (Posas *et al.* 1998; Rep *et al.* 1999, 2000; Bilsland-Marchesan *et al.* 2000; and references therein). (B) Environmental stresses cause changes in cell wall state, which are detected by the Wsc proteins and Mid2p and Mtl1p. The information is transmitted to Rho1p by the guanine nucleotide exchange factors Rom1p and Rom2p. Tor2p is also an activator of Rho1p, whereas Sac7p and Bem2p are GTPase-activating proteins for Rho1p. Activated, GTP-bound Rho1p interacts with a transcription factor (Skn7p) and regulates the activity of proteins involved in cytoskeleton assembly (Bni1p), cell wall synthesis (Fks1p), and signal transduction (Pkc1p). Pkc1p in turn activates the cell integrity MAP kinase pathway and independently on "another arm" effects Rap1p-dependent transcriptional repression of ribosomal protein genes (Li *et al.* 2000; Philip and Levin 2001; and references therein). For the color-coding scheme, see Figure 2.

The HOG pathway buffers toxin action: Mutants in *hog1* are close to being maximally sensitive to the toxin, dying at ~1 molecule/cell, while in a HOG1 strain, four orders of magnitude more toxin is needed to kill a cell. How is this *HOG1*-dependent resistance achieved? One possibility is that the HOG pathway is stress induced as the toxin causes ion loss. Activation of this signaling pathway may result in changes in membrane conductance, intracellular osmotic pressure, or some other stress response, which can act to reduce the efficiency of the toxin in promoting loss of cellular ions. Although the toxin sensitivity of a *gpd1 gpd2* double mutant is similar to wild-type cells, the possible involvement of Hog1p-dependent osmoadaptation cannot be excluded. Consistent with this scenario, García-Rodriguez *et al.* (2000) observed increased intracellular glycerol levels after treatment with the cell-wall-perturbing agent calcofluor white, independent of the action of *GPD1* and *GPD2*. An alternative explanation that there is some constitutive *HOG1*-dependent effect on cell wall synthesis seems less likely on the basis of the following observations. Epistatic tests using *kre1 hog1* and *kre2 hog1* mutants are consistent with the HOG pathway acting at the membrane or intracellularly, as cell wall mutants are epistatic to the *hog1* defect and remain toxin resistant in double mutants. Deficiencies in the HOG pathway result in extreme toxin sensitivity, and we reasoned that mutations in genes regulated by this pathway might also cause hypersensitivity. In looking for candidates, it is striking that some components specific to the RNA polymerase II complex (*e.g.*, Gal11p, Med2p, Rpb4p, Rpb3p, Rpb7p, Srb5p, and Srb2p) or components shared between RNA polymerases I, II, and III (*e.g.*, Rpb8p, Rpc10p, and Rpo26p) all display a strong toxin hypersensitivity, similar to that of HOG pathway mutants (Tables 3 and 4). Is this response specific to the HOG pathway? Among the MAPK pathways in yeast (Hunter and Plowman 1997; Gustin *et al.* 1998), only the HOG pathway exhibits toxin hypersensitivity. Mutants in *SMK1*, *MPK1*, and *YKL161c*, which encode, respectively, the MAP kinase of the sporulation pathway, the cell integrity pathway, and a putative uncharacterized pathway, are not toxin hypersensitive. Similarly, a null mutation in the MAP kinase kinase encoding gene *STE7*, which is involved in both the haploid mating and invasive pathways, has no effect on toxin sensitivity. These observations suggest a possible connection between the signaling elements of the HOG pathway and the activity of the RNA polymerase II complex. To investigate which potential target genes of Hog1p are responsible for the hypersensitivity, we looked for toxin phenotypes resulting from mutations in genes known to be induced by osmotic shock (Rep *et al.* 2000). None of these genes have an effect comparable to a *hog1* mutant. Similar results were obtained for genes whose mRNA level is affected by a mutation of *HOG1*. However, among the genes whose mRNA level is diminished after a shift to high osmolarity (Rep *et al.* 2000), *ASC1* had a significant hypersensitivity (Table 3). *ASC1* encodes a 40S small subunit ribosomal protein, one of many small ribosomal protein encoding genes that, when mutated, show toxin hypersensitivity (see Table 3 and below). Together, these observations suggest that, if the phenotype observed in a *hog1* mutant results from a defect in expression, it is not through a single gene but may originate from a combined deficiency in more than one gene.

Signaling components involved in toxin sensitivity: Although the HOG pathway is the only MAP kinase cascade showing a toxin phenotype, two upstream activators of MAPK pathways were identified in the screen: *SSK1* and *PKC1*. The toxin hypersensitivity of an *ssk1* mutant is consistent with its place upstream of the HOG signal transduction cascade. However, no toxin phenotype is found for the components of the cell integrity MAPK pathway signaling downstream of *PKC1*, namely, the sequentially acting kinases Bck1p, the redundant pair Mkk1p and Mkk2p, and the Mpk1p MAP kinase (Figure 3B). This raises the question of how Pkc1p signals in producing a normal response to the toxin. Previous genetic analysis suggested a bifurcation of the signaling downstream of *PKC1* (Errede and Levin 1993; Helliwell *et al.* 1998). Our data are consistent with such a model since some "other arm" of the PKC pathway, distinct from the Bck1p-dependent arm, is responsible for the toxin phenotype. Additional evidence for an alternative pathway comes from studies on the coordination of cell growth and ribosome synthesis, where a block in protein secretion reduces ribosomal protein gene transcription (Mizuta and Warner 1994; Nierras and Warner 1999). This mechanism is: (i) dependent on Pkc1p activity; (ii) not mediated by the cell integrity pathway MAPK cascade (*BCK1* or *MPK1*); and (iii) blocked by *rap1-17*, a silencing-defective allele of *RAP1* (Li *et al.* 2000). We found that a heterozygous *rap1* mutant exhibits haploinsufficient toxin hypersensitivity (Figure 3B), providing additional support for Rap1p being an effector of Pkc1p.

Ribosomal subunit mutants show toxin sensitivity: The coupling of protein secretion to ribosome synthesis through the PKC pathway (Nierras and Warner 1999; Li *et al.* 2000) raises the possibility of regulation operating in the reverse direction: that is, defects in protein synthesis mediated predominantly through 40S ribosomal subunit proteins might affect protein secretion and cell wall synthesis. The binding of the rough ER ribosomes to Sec61p of the signal recognition particle is through the 60S ribosomal subunit (Beckmann *et al.* 1997), and fewer mutants in 60S ribosomal proteins have toxin phenotypes, arguing that the coupling step in itself is unlikely to be the primary site of any such effect. A more mundane alternative explanation is that nonessential defects in protein synthesis through loss of redundant ribosomal proteins have nonspecific knock-on effects on protein secretion/cell wall synthesis

through failure to make enough of a component required for protein secretion.

Strength and limitations of comprehensive phenotyping with the collection: In addition to phenotypic clustering of genes, the simple discovery of biological roles for genes through phenotype remains an important part of this screen. For example, a number of mutants in poorly characterized genes have β-glucan phenotypes that warrant investigation. The yeast disruption mutant collection has limitations. Duplicated genes and gene families having synthetic phenotypes but no phenotype when individually deleted will be overlooked. Also, the 1105 essential genes representing 18.7% of the yeast genome (Giaver *et al.* 2002) cannot be screened directly. Haploinsufficiency phenotypes in heterozygotes disrupted in one copy of an essential gene provide a partial solution, as in the case of *BIG1*. In our screen such haploinsufficiency was found in the heterozygous mutants of 42 genes, but we still do not know the full extent of the involvement of essential genes in cell surface biology. A set of conditional lethal mutants in all essential genes would improve the value of the collection for screening these genes.

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