Analysis of β-1,3-Glucan Assembly in *Saccharomyces cerevisiae* Using a Synthetic Interaction Network and Altered Sensitivity to Caspofungin

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ABSTRACT

Large-scale screening of genetic and chemical-genetic interactions was used to examine the assembly and regulation of β-1,3-glucan in *Saccharomyces cerevisiae*. Using the set of deletion mutants in ~4600 nonessential genes, we scored synthetic interactions with genes encoding subunits of the β-1,3-glucan synthase (*FKS1*, *FKS2*), the glucan synthesis regulator (*SMI1/KNR4*), and a β-1,3-glucanosyltransferase (*GAS1*). In the resulting network, *FKS1*, *FKS2*, *GAS1*, and *SMI1* are connected to 135 genes in 195 interactions, with 26 of these genes also interacting with *CHS3* encoding chitin synthase III. A network core of 51 genes is multiply connected with 112 interactions. Thirty-two of these core genes are known to be involved in cell wall assembly and polarized growth, and 8 genes of unknown function are candidates for involvement in these processes. In parallel, we screened the yeast deletion mutant collection for altered sensitivity to the glucan synthase inhibitor, caspofungin. Deletions in 52 genes led to caspofungin hypersensitivity and those in 39 genes to resistance. Integration of the glucan interaction network with the caspofungin data indicates an overlapping set of genes involved in *FKS2* regulation, compensatory chitin synthesis, protein mannosylation, and the *PKC1*-dependent cell integrity pathway.

THE cell wall is a major organelle that surrounds cells, is responsible for cell shape and osmotic stability, and acts as a filter for large molecules. The cell wall is composed mainly of β-1,3 and β-1,6-glucans, mannoproteins, and chitin, with the relative proportions of these constituents varying with growth conditions and the cellular developmental program. β-1,3-Glucan is the principal cell wall component, to which the other components are crosslinked (Smits *et al.* 1999; Klis *et al.* 2002). Synthesis of β-1,3-glucan occurs at the plasma membrane. Glucan synthase is thought to contain a catalytic subunit, encoded by the two homologous genes *FKS1* and *FKS2/GSC2* (Mazur *et al.* 1995), and a regulatory subunit, the small GTPase Rho1p (Drgonova *et al.* 1996; Mazur and Baginsky 1996; Qadota *et al.* 1996). *FKS1* and *FKS2* encode a pair of integral membrane proteins with 16 predicted transmembrane domains that share 88% identity. Deletion of *FKS1* leads to a decrease in β-glucan and an increase in chitin and mannoprotein levels in the cell wall. The deletion of *FKS2* causes no obvious cell wall defect, but a *fks1*Δ *fks2*Δ double mutant is inviable (Mazur *et al.* 1995). The yeast genome contains a third gene, *FKS3*, whose product is 72% identical to Fks1p and Fks2p. The role of *FKS3* remains unknown, but a *fks3*Δ mutant has no apparent cell wall defects or genetic interactions with *FKS1* or *FKS2* (Dijkgraaf *et al.* 2002). In addition to the Rho1p regulatory subunit, other proteins are required for normal levels of β-1,3-glucan. The *SMI1/KNR4* gene was cloned by complementation of a *Hansenula mrakii* K9 killer toxin (a glucan synthase inhibitor) resistant mutant (Hong *et al.* 1994). The *smi1*Δ mutant has a highly permeable cell wall and shows both decreased glucan synthase activity and cell wall β-1,3-glucan content (Hong *et al.* 1994; Martin *et al.* 1999). Genetic and biochemical evidence suggests that Smi1p acts in the *PKC1-SLT2* signaling cascade by modulating the kinase activity of Slt2p (Martin-Yken *et al.* 2002, 2003).

Cell wall composition changes during growth, budding, mating, and sporulation, and these dynamic processes require remodeling of the crosslinking of β-1,3- and β-1,6-glucans to themselves and to other cell wall components. Gas1p, a GPI-anchored protein localized to the extracellular face of the plasma membrane, has β-1,3-glucanosyltransferase activity and is involved in this remodeling (Mouyna *et al.* 2000). A null *gas1* mutant releases β-glucosylated proteins into the medium and shows increased chitin and mannoprotein levels (Ram *et al.* 1998). Such increased levels of cell wall components can compensate for a defect in a specific polymer: for instance, a decrease in β-1,3-glucan is buffered by an increase in chitin made by chitin synthase III (Valdivieso *et al.* 2000; Garcia-Rodriguez *et al.* 2000b; Carotti *et al.* 2002). *CHS3* encodes chitin synthase III, and Chs3p is responsible for synthesis of the chitin in a ring at the bud neck, in the lateral wall, and in response to external stress (Roncero 2002).

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TABLE 1

Yeast strains used

Strain	Genotype	Source
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Brachmann *et al.* (1998)
BY4743	*MAT***a**/*MAT*α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 MET15/met15Δ0 ura3Δ0/ura3Δ0 LYS2/lys2Δ0*	Brachmann *et al.* (1998)
Haploid	Same as BY4741 *orfΔ::KanMX4*	Winzeler *et al.* (1999)
Heterozygous	Same as BY4743 *ORF/orfΔ::KanMX4*	Winzeler *et al.* (1999)
HAB1122	*MAT*α *chs3Δ::NatMX4 can1Δ::MFA1-prHIS3-MFα1pr-LEU2 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
HAB1123	*MAT*α *fks1Δ::NatMX4 mfα1Δ::MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
HAB1124	*MAT*α *fks2Δ::NatMX4 mfα1Δ::MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
HAB1125	*MAT*α *fks3Δ::NatMX4 mfα1Δ::MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
HAB1126	*MAT*α *gas1Δ::NatMX4 mfα1Δ::MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
HAB1127	*MAT*α *smi1Δ::NatMX4 mfα1Δ::MFα1-prLEU2 can1Δ::MFA1-prHIS3 his3Δ leu2Δ lys2Δ met15Δ ura3Δ*	Tong *et al.* (2004)
Y3084	*MAT*α *mfα1Δ::MFα1pr-LEU2 can1Δ::MFA1pr-HIS3 his3Δ1 leu2Δ lys2Δ ura3Δ*	Tong *et al.* (2001)
Y3656	*MAT*α *can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 his3Δ1 leu2Δ lys2Δ ura3Δ*	C. Boone

To uncover the network of genes involved in β-1,3-glucan biology, we made two studies, using the collection of yeast mutants singly disrupted in each gene. As part of a larger study of the yeast genetic network (Tong *et al.* 2004), we looked for mutations leading to a growth defect when combined with a *FKS1*, *FKS2*, *GAS1*, or a *SMI1* deletion. We found that deletion of 135 genes impaired growth of *fks1*Δ, *fks2*Δ, *gas1*Δ, or *smi1*Δ mutants and we analyze these interactions here. As a complementary approach, we looked for single deletions leading to an altered sensitivity to the β-1,3-glucan synthase inhibitor, caspofungin. Caspofungin is an echinocandin-like antifungal lipopeptide that inhibits β-1,3-glucan synthesis *in vitro* by affecting the *FKS1* and *FKS2* gene products in *Saccharomyces cerevisiae* (for a review see Deresinski and Stevens 2003; Letscher-Bru and Herbrecht 2003). Mutant alleles in *FKS1* and *FKS2* that lead to echinocandin resistance in *S. cerevisiae* have been identified (Douglas *et al.* 1994; Mazur *et al.* 1995). We globally tested for altered sensitivity of ~4600 haploid deletion mutants and the ~1100 heterozygous diploids in essential genes and found 91 genes with such a phenotype.

MATERIALS AND METHODS

Strains, media, and drugs: All strains used (Table 1) are available from the deletion project consortium (Winzeler *et al.* 1999). Haploid deletion mutants were previously arrayed on 16 768-format plates using a colony picker (Tong *et al.* 2001). Three plates with 1058 diploid strains heterozygous for essential genes were also arrayed. Arrays were propagated at 30° on standard YEPD (10 g/liter yeast extract, 20 g/liter bacto-peptone, 20 g/liter glucose) or YEPD supplemented with 200 μg/ml G-418 (Invitrogen, Carlsbad, CA). Caspofungin acetate (Cancidas; Merck, Whitehouse Station, NJ) was a gift from Elitra Canada (Montreal). Nourseothricin (clonNAT) was purchased from Werner Bioagent (Jena, Germany).

SGA analysis: The synthetic genetic array (SGA) analysis procedure is fully described elsewhere (Tong *et al.* 2001, 2004) and is briefly summarized below.

Construction of query strains: Query strains HAB1122, HAB1123, HAB1124, HAB1125, HAB1126, and HAB1127 (Table 1) were obtained in four steps. First, the *KanMX4* from BY4741-derived strains (Table 1) was switched to *NatMX4* by PCR-based transformation. Second, the nourseothricin-resistant transformants derived from *chs3* or other mutants were mated to Y3656 or Y3084 (Table 1), respectively, and the *MAT***a**/α diploids were transferred to sporulation medium. *MAT*α meiotic progeny were then selected on synthetic medium lacking leucine and arginine but containing canavanine. The mating type was confirmed by PCR, according to Huxley *et al.* (1990). Third, cells were replica plated onto medium containing clonNAT to select for the deletion mutants. Fourth, cells were replica plated onto medium lacking lysine to identify *lys2*Δ derivatives.

SGA screens: A given query strain was pinned onto a fresh YEPD plate at a density of 768/plate, and then the deletion mutant array was pinned on top of the query cells. The resulting diploids were selected on medium containing G418 and clonNAT. Arrays were then pinned onto sporulation medium. After a 5-day incubation at 22° spores were pinned onto haploid selection medium to select for growth of *MAT***a** spore progeny. This step was performed twice. Then, meiotic progeny carrying the deletion mutation derived from the deletion mutant array parental strain were selected on medium containing G418. Finally, double mutants were selected on haploid-selection medium containing G418 and clonNAT for 2 days. Colony size was then scored by visual plate inspection. Each screen was done in triplicate, and putative interactions scored multiple times (~1800 interactions for the six SGA screens) were subjected to confirmation tests.

Confirmation of synthetic interactions: Spores were germinated

for 2 days at 30° in liquid haploid selection medium. The *MAT***a** progeny were diluted in sterile water and plated out on medium that selects for the query-gene mutation [clonNAT], the deletion mutant array mutation [G418], or both the query-gene and deletion mutant array mutations [clonNAT/G418], and then incubated at 30° for ~2 days. Colony growth under the three conditions was compared and the double mutants were scored as synthetic sick (SS), synthetic lethal (SL), or no interaction (No). Tetrad analysis was used to test synthetic interactions in 42 cases. In all, 248 interactions were positive.

Accuracy of the procedure: Since screens were done in triplicate followed by a confirmation procedure, we expect our data set to be largely devoid of false positives. However, some interactions may have been missed (false negatives). A search in the literature and databases indicated that 10, 1, 1, 11, 5, and 8 synthetic-lethal interactions were reported for *FKS1*, *FKS2*, *FKS3*, *GAS1*, *SMI1*, and *CHS3*, respectively. Of these 36 interactions, 6 engaged essential genes and thus were not seen with our procedure. Of the 30 remaining "observable" interactions, 22 were also found in our screen. On the basis of this, we estimate the rate of false negatives to be ~30%, which is consistent with an estimate made on a larger SGA data set (Tong *et al.* 2004). Some true interactions would be missed if they involve one of the ~500 genes whose deletion led to a systematic defect in our assay and were excluded from analysis (Tong *et al.* 2001). For example, 25 of the 45 nonessential genes whose deletion leads to caspofungin hypersensitivity fall into this group.

Caspofungin sensitivity/resistance screening procedures: *Robotic procedures:* Mutants were pinned onto YEPD plates with or without caspofungin. Final caspofungin concentrations were 500 ng/ml (from a 1-mg/ml stock in 1% DMSO) for sensitivity testing and 5 μg/ml (from a 10-mg/ml stock in 1% DMSO) for resistance screening. Growth was scored after overnight incubation at 25°. Strains showing significant growth defects on a 0.5-μg/ml caspofungin plate (mutants in 157 nonessential genes and 103 essential genes) or growing on a 5-μg/ml caspofungin plate (mutants in 116 nonessential genes) were individually confirmed by the spotting assay described below.

Confirmation and scoring procedure: Due to high cell density (768 colonies/plate) and the pinning geometry of the plate during the robotic screening, the caspofungin concentrations used during the screening were higher than those used in the confirmation test. Cells were grown in liquid YEPD to log-phase, diluted to OD_{600} 0.5, serial diluted 10-fold four times, and 2.5 μl was spotted onto YEPD ± caspofungin. Since haploids are less sensitive to the drug than diploids, confirmation of the hypersensitivity phenotype was performed at 100 and 200 ng/ml caspofungin for the former and at 150 ng/ml caspofungin for the latter. Phenotypes were scored after overnight incubation at 25°, by checking growth of mutants in the presence or absence of the drug and comparison to growth of the wild-type strain. Sensitive haploid mutants that failed to grow at 100 ng/ml, or at 200 ng/ml, or grew very slowly at 200 ng/ml were scored as - - -, - -, or -, respectively. Sensitive diploid strains were scored - - - if they did not grow in the presence of drug and - - when they grew poorly. Confirmation of the resistance phenotype was performed at 400 ng/ml.

Probability of overlap between networks: The probability P that N_{hit} genes are found in two data sets composed of N_1 and N_2 interacting genes was estimated using the formula

$$P = \frac{P(N_1,\ N_{hit})}{P(N,\ N_{hit})}\ \frac{P(N_2,\ N_{hit})}{P(N,\ N_{hit})}$$

(Parsons *et al.* 2004), where N is the total number of interactions tested (*i.e.*, the number of strains in the array), and $P(N, M) = N!/(M!(N - M)!)$.

RESULTS

Synthetic interactions with mutants in β-1,3-glucan assembly

To identify genes buffering defects in β-1,3-glucan synthesis, we searched for genes required for viability or optimal growth in *fks1*Δ, *fks2*Δ, *fks3*Δ, *gas1*Δ, or *smi1*Δ backgrounds. Haploid deletion mutants in 4598 genes were arrayed and crossed with strains individually deleted for *FKS1*, *FKS2*, *FKS3*, *GAS1*, or *SMI1*. The resulting diploids were selected, sporulated, and haploid double mutants scored for growth. Double mutants showing a growth defect were scored as candidate interactants. Random spore analysis was used to confirm a synthetic interaction. In all, 76, 71, 48, and 1 genes were found to interact with *FKS1*, *SMI1*, *GAS1*, and *FKS2*, respectively. No synthetic interactions were found with the *fks3* null strain. We found 77 synthetic lethal interactions and 118 double mutant combinations leading to growth defects. The 135 genes involved in these interactions are grouped in eight categories according to their cellular function (Table 2) and are displayed as a network of 195 interactions depicted as edges linking two nodes (Figure 1).

Synthesis and regulation of the cell wall: In addition to *FKS1*, *FKS2*, *GAS1*, and *SMI1*, 27 genes group here. Some are involved in the synthesis of cell wall components such as chitin (*BNI4*, *CHS3*, *CHS4*, *CHS5*, *CHS6*, and *CHS7*), β-1,6-glucan (*KRE1*), or protein glycosylation (*CWH41*, *MNN10*, *MNN11*, *ROT2*, and *VAN1*). This indicates that when β-1,3-glucan synthesis is impaired, correct synthesis of other cell wall constituents is required for normal cell growth or viability.

Components of the *PKC1-SLT2* cell integrity pathway, such as sensors (*MID2* and *SLG1/WCS1*), regulators (*BEM2* and *ROM2*), kinases (*BCK1* and *SLT2*), and transcription factors (*RLM1*, *SSD1*, and *SWI4*), occur in the interaction network. This mitogen-activated protein (MAP) kinase cascade orchestrates morphological change by regulating cell wall assembly in response to stress and low osmolarity (Heinisch *et al.* 1999). Moreover, the ability to respond to low osmolarity with the glycerol channel Fps1p is essential in the *fks1* and *smi1* mutants. Thus, appropriate osmosensing and a functional cell integrity pathway buffer mutants defective in β-1,3-glucan synthesis.

Finally, five poorly characterized genes (*DFG16/ECM41*, *ECM7*, *ECM21*, *IMG1*, and *RIM20*) are candidates for involvement in cell wall assembly, with their mutants having altered sensitivity to environmental stresses or cell surface perturbing agents.

Polarity and secretory pathway function: Since cell polarity, vesicular transport, endocytosis, and mem-

TABLE 2

Genes showing synthetic interaction with *FKS1*, *FKS2*, *GAS1*, or *SMI1*

ORF	Gene	Description of gene product	Interaction
		Cell wall synthesis and regulation (31 genes)	
YJL095W	*BCK1*	MAPKK-kinase of the cell integrity pathway	*GAS1*, *SMI1*
YER155C	*BEM2*	GTPase-activating protein for Rho1p	*FKS1*, *GAS1*, *SMI1*
YNL233W	*BNI4*	Anchors Chs3p-Chs4p to the bud neck septin ring	*FKS1*, *SMI1*
YBR023C	*CHS3*	Chitin synthase III	*FKS1*, *GAS1*, *SMI1*
YBL061C	*CHS4*	Activator of Chs3p	*FKS1*, *SMI1*
YLR330W	*CHS5*	Involved in transport of Chs3p from the late Golgi to the chitosome	*FKS1*, *GAS1*, *SMI1*
YJL099W	*CHS6*	Involved in transport of Chs3p from the late Golgi to the chitosome	*FKS1*, *SMI1*
YHR142W	*CHS7*	Facilitates exit of Chs3p from the ER	*FKS1*, *SMI1*
YGL027C	*CWH41*	Glucosidase I, mutant has a reduced level of β-1,6-glucan	*GAS1*
YOR030W	*DFG16*	Involved in invasive growth, mutant hypersensitive to calcofluor white	*FKS1*, *SMI1*
YBL101C	*ECM21*	Mutant hypersensitive to calcofluor white	*SMI1*
YLR443W	*ECM7*	Mutant hypersensitive to calcofluor white	*GAS1*
YLR342W	*FKS1*	β-1,3-Glucan synthase subunit	*FKS2*
YGR032W	*FKS2*	β-1,3-Glucan synthase subunit	*FKS1*
YLL043W	*FPS1*	Glycerol channel protein	*FKS1*, *SMI1*
YMR307W	*GAS1*	GPI-anchored surface glycoprotein with β-1,3-glucanosyltransferase activity	*SMI1*
YCR046C	*IMG1*	Putative mitochondrial ribosomal protein, null mutant K1 toxin resistant	*GAS1*
YNL322C	*KRE1*	Cell wall protein involved in β-1,6-glucan synthesis	*GAS1*, *SMI1*
YLR332W	*MID2*	Sensor for the *PKC1-SLT2* cell wall integrity pathway	*FKS1*, *SMI1*
YDR245W	*MNN10*	Subunit of the Mannan polymerase II complex	*SMI1*
YJL183W	*MNN11*	Subunit of the Mannan polymerase II complex	*FKS1*, *SMI1*
YOR275C	*RIM20*	Transcription factor involved in stress resistance	*FKS1*, *SMI1*
YPL089C	*RLM1*	Transcription factor mediating cell integrity pathway response	*FKS1*, *GAS1*, *SMI1*
YLR371W	*ROM2*	GDP/GTP exchange factor for Rho1p	*FKS1*, *GAS1*, *SMI1*
YBR229C	*ROT2*	Glucosidase II, mutant has a reduced level of β-1,6-glucan	*GAS1*
YOR008C	*SLG1*	Sensor for the *PKC1-SLT2* cell integrity pathway	*GAS1*
YHR030C	*SLT2*	MAP-kinase of the cell integrity pathway	*FKS1*, *GAS1*, *SMI1*
YGR229C	*SMI1*	Regulator of β-1,3-glucan synthesis	*GAS1*
YDR293C	*SSD1*	mRNA-binding protein, may regulate expression of cell integrity pathway targets	*GAS1*
YER111C	*SWI4*	Transcription factor mediating the cell integrity pathway response	*FKS1*, *GAS1*, *SMI1*
YML115C	*VAN1*	Component of the Mannan polymerase I complex	*SMI1*
		Polarity and secretory pathway function (29 genes)	
YBR200W	*BEM1*	SH3-domain protein maintaining Cdc42p-Cdc24p at the bud tip	*SMI1*
YPL161C	*BEM4*	Bud emergence protein that activates Cdc42p	*FKS1*, *GAS1*
YNL271C	*BNI1*	Member of the polarisome with Bud6p, Pea2p, and Spa2p. This complex binds activated Cdc42p and its effector Ste20p and acts as an apical scaffold regulating actin filament assembly at the bud tip	*FKS1*, *SMI1*
YNR051C	*BRE5*	Activator of Ubp3p that regulates COPII coat assembly	*FKS1*, *SMI1*
YJL188C	*BUD19*	Mutant defective in bud site selection and bipolar budding	*GAS1*, *SMI1*
YBR131W	*CCZ1*	Protein involved in vesicular transport and vacuolar assembly	*SMI1*
YNL298W	*CLA4*	Cdc42p effector regulating septin assembly at the bud neck	*SMI1*
YPR030W	*CSR2*	Overexpression rescues *chs5 spa2* synthetic lethality	*FKS1*
YBL047C	*EDE1*	Cortical actin patch protein with a role in endocytosis	*FKS1*
YKL048C	*ELM1*	Serine/threonine protein kinase regulating septin network organization	*FKS1*, *SMI1*
YGL200C	*EMP24*	COPII-coated vesicle protein required for sorting of GPI-anchored proteins	*GAS1*
YBR041W	*FAT1*	Very long-chain acyl-CoA synthetase	*SMI1*
YCR034W	*FEN1*	Involved in fatty acids elongation	*FKS1*
YDL223C	*HBT1*	Target of Hub1p ubiquitination, mutant shows morphological defects	*FKS1*
YGR166W	*KRE11*	Subunit of transport protein particle II complex	*GAS1*, *SMI1*
YJR073C	*OPI3*	Involved in phosphatidylcholine biosynthesis	*GAS1*
YDR137W	*RGP1*	Acts in a complex with Ric1p as a GTP-GDP exchange factor for Ypt6p	*FKS1*, *GAS1*
YLR039C	*RIC1*	Acts in a complex with Rgp1p as a GTP-GDP exchange factor for Ypt6p	*GAS1*, *SMI1*
YDR388W	*RVS167*	Affects cortical actin patch distribution, mutant shows defective endocytosis	*FKS1*, *SMI1*
YDR351W	*SBE2*	Golgi protein involved in targeting Chs3p and Chr2p to the bud neck	*SMI1*
YOR035C	*SHE4*	Involved in cortical actin patch assembly and endocytosis	*FKS1*

(*continued*)

TABLE 2

(Continued)

ORF	Gene	Description of gene product	Interaction
YBL007C	*SLA1*	Cortical actin patch assembly control protein, mutation affects endocytosis	*FKS1*, *SMI1*
YLL021W	*SPA2*	Member of the polarisome with Bni1p, Bud6p, and Pea2. This complex binds activated Cdc42p and its effector Ste20p and acts as an apical scaffold regulating actin filament assembly at the bud tip	*FKS1*, *SMI1*
YER151C	*UBP3*	Ubiquitin-specific protease, Ubp3p-Bre5p regulates COPII coat assembly	*FKS1*, *SMI1*
YDR136C	*VPS61*	Class B vacuolar sorting protein	*GAS1*
YLR261C	*VPS63*	Vacuolar protein sorting	*GAS1*
YKR020W	*VPS67*	Involved in maintenance of actin cytoskeleton and apical bud growth	*FKS1*, *SMI1*
YLR262C	*YPT6*	GTP-binding protein that regulates vesicle fusion during retrograde transport	*GAS1*, *SMI1*
YLR338W		Questionable ORF, overlaps with *VRP1*, a cortical actin patch component	*FKS1*
		Transcriptional regulation and stress response (22 genes)	
YDL243C	*AAD4*	Putative aryl-alcohol dehydrogenase	*GAS1*
YNL027W	*CRZ1*	Calcineurin responsive zinc-finger transcription factor	*FKS1*
YKR024C	*DBP7*	RNA helicase required for 60S ribosomal subunit assembly	*FKS1*
YBR121C	*GRS1*	Glycyl-tRNA synthetase, also involved in 3′-end formation of mRNA	*FKS1*
YOR358W	*HAP5*	Transcription factor required for activity of the CCAAT-binding complex	*GAS1*
YJR055W	*HIT1*	Required for growth at high temperature	*FKS1*
YLR384C	*IKI3*	RNA polymerase II transcriptional elongation component	*SMI1*
YIL154C	*IMP2′*	Transcriptional activator with a role in DNA repair upon oxidative stress	*FKS1*
YKL032C	*IXR1*	Transcription factor mediating oxygen repression	*FKS1*
YJL124C	*LSM1*	Sm-like RNA-binding protein involved in control of mRNA decay	*FKS1*
YDR378C	*LSM6*	U6 snRNA-associated protein of the Sm-like group	*FKS1*
YMR038C	*LYS7*	Copper chaperone essential for the oxidative protective function of Sod1p	*SMI1*
YML017W	*PSP2*	Suppressor of DNA polymerase α mutation	*FKS1*
YLR204W	*QRI5*	Transcription profile suggests involvement in stress responses	*FKS1*
YDR156W	*RPA14*	RNA polymerase I subunit A14	*SMI1*
YLR357W	*RSC2*	Component of the RSC chromatin remodeling complex regulating RNA polymerase II and III transcription	*GAS1*
YKR072C	*SIS2*	Involved in ion homeostasis	*GAS1*
YDR477W	*SNF1*	Protein kinase with roles in glucose derepression and filamentation	*FKS1*
YOL006C	*TOP1*	DNA topoisomerase I	*FKS1*
YNL064C	*YDJ1*	Hsp40 chaperone required for protein entry into the ER	*FKS1*
YJL046W		Lipoate-protein ligase A-related, null mutant hypersensitive to oxidative stress	*FKS1*
YMR073C		Possible NAD(P)H oxidoreductase involved in stress response	*FKS1*
		Ion homeostasis and signal transduction (5 genes)	
YGR217W	*CCH1*	Calcium channel protein	*FKS1*, *SMI1*
YKL190W	*CNB1*	Regulatory subunit of the phosphatase calcineurin	*FKS1*, *GAS1*, *SMI1*
YJL117W	*PHO86*	ER protein facilitating incorporation of Pho84p into secretory vesicles	*SMI1*
YDL006W	*PTC1*	Protein phosphatase, acts on MAP kinases such as Hog1p	*FKS1*, *GAS1*, *SMI1*
YEL031W	*SPF1*	ATP-dependent calcium pump required for normal ER calcium homeostasis	*SMI1*
		Ubiquitin-regulated protein degradation (4 genes)	
YGR133W	*PEX4*	E2 ubiquitin-conjugating enzyme induced in the unfolded protein response	*FKS1*
YGR135W	*PRE9*	20S (catalytic) proteasome subunit Y13 (α3)	*FKS1*, *SMI1*
YHR200W	*RPN10*	19S (regulatory) proteasome subunit, homolog of the mammalian S5a protein	*FKS1*, *SMI1*
YBR082C	*UBC4*	E2 ubiquitin-conjugating enzyme	*FKS1*, *SMI1*
		Cell cycle (3 genes)	
YGL240W	*DOC1*	Component of the anaphase-promoting complex	*FKS1*, *SMI1*
YPL031C	*PHO85*	Cyclin-dependent protein kinase that interacts with cyclin Pho80p	*FKS1*, *GAS1*, *SMI1*
YNL171C		Overlaps with *APC1*, an essential subunit of the anaphase-promoting complex	*FKS1*, *SMI1*

(*continued*)

TABLE 2

(Continued)

ORF	Gene	Description of gene product	Interaction
		Other (17 genes)	
YHR129C	*ARP1*	Centractin	*SMI1*
YMR116C	*ASC1*	40S ribosomal protein that affects cell size	*SMI1*
YPR135W	*CTF4*	DNA-directed DNA polymerase α-binding protein	*SMI1*
YHR191C	*CTF8*	Putative kinetochore protein	*SMI1*
YDR440W	*DOT1*	Involved in telomere silencing and in the meiotic arrest checkpoint	*GAS1*
YDR508C	*GNP1*	High-affinity glutamine permease	*FKS1*, *GAS1*
YDR162C	*NBP2*	Nap1p-binding protein	*GAS1*, *SMI1*
YHR004C	*NEM1*	Protein required for nuclear morphology	*GAS1*
YJL208C	*NUC1*	Nuclease, mitochondrial	*FKS1*
YER178W	*PDA1*	Pyruvate dehydrogenase complex E1-α-subunit	*SMI1*
YMR205C	*PFK2*	β-subunit of the 6-phosphofructokinase	*SMI1*
YNL069C	*RPL16B*	Ribosomal protein L16, nearly identical to Rpl16Ap	*FKS1*
YOR312C	*RPL20B*	Ribosomal protein L20, nearly identical to Rpl20Ap	*FKS1*
YFR032C-A	*RPL29*	Ribosomal protein L29	*SMI1*
YGR214W	*RPS0A*	40S ribosomal protein	*SMI1*
YJL136C	*RPS21B*	Ribosomal protein S21B	*FKS1*
YBR166C	*TYR1*	Prephenate dehydrogenase in the tyrosine biosynthesis pathway	*SMI1*
		Unknown (24 genes)	
YMR318C	*ADH6*	NADPH-dependent cinnamyl-alcohol dehydrogenase	*GAS1*
YJR118C	*ILM1*	Null mutant shows increased loss of mitochondrial DNA	*FKS1*, *SMI1*
YLR320W	*MMS22*	Unknown	*FKS1*, *SMI1*
YNL294C	*RIM21*	Protein of unknown function	*FKS1*, *GAS1*
YAL053W		Expression is upregulated in an *fks1* mutant	*GAS1*
YBL062W		Questionable ORF, overlaps with *CHS4*	*FKS1*
YGL046W		Hypothetical protein, merged with *RIM8/YGL045W*	*FKS1*, *SMI1*
YGL081W		Phosphopeptide-binding protein with a forkhead-associated (FHA) domain	*FKS1*
YGL110C		Member of the CUE domain family, which binds ubiquitin-conjugating enzymes	*FKS1*, *GAS1*
YGL196W		Hypothetical protein	*FKS1*
YGR237C		Weak similarity to *YOR019W*	*SMI1*
YIL121W		Member of the multidrug-resistance 12-spanner family	*GAS1*
YLR021W		Hypothetical protein	*FKS1*
YLR358C		Unknown	*SMI1*
YML117W		Unknown	*GAS1*, *SMI1*
YMR313C		Unknown	*GAS1*
YMR316C-A		Protein of unknown function	*GAS1*
YMR317W		Unknown	*GAS1*
YMR326C		Protein with high similarity to Ynr077p	*GAS1*
YOL003C		Similar to Erf2p, Ydr459p, and others in a cysteine-rich domain	*FKS1*, *SMI1*
YPL041C		Hypothetical protein	*FKS1*, *GAS1*
YPL077C		Weak similarity to *YBR197C*	*SMI1*
YPL144W		Unknown	*FKS1*
YPL261C		Putative GPI-anchored protein that may be involved in cell wall maintenance	*FKS1*

ORF, open reading frame; ER, endoplasmic reticulum.

brane biogenesis are needed for coordinating cell wall assembly during yeast growth (Pruyne and Bretscher 2000a,b), the 29 genes involved in these cellular functions are grouped in a single category. Genes involved in cell polarity and showing interaction in the network encode regulators of the Cdc42p GTPase (*BEM1*, *BEM4*), scaffold proteins regulating the directionality of actin polymerization from the bud tip (*BNI1*, *SPA2*), regulators of septin assembly at the bud neck (*CLA4*, *ELM1*), and factors with a role in cell morphology and budding (*BUD19*, *HBT1*). Stages of secretion found among interacting genes involved in vesicular transport are ER to Golgi (*BRE5*, *EMP24*, and *UBP3*), intra-Golgi (*KRE11*), Golgi to bud neck (*CSR2* and *SBE2*), and vacuole assem-

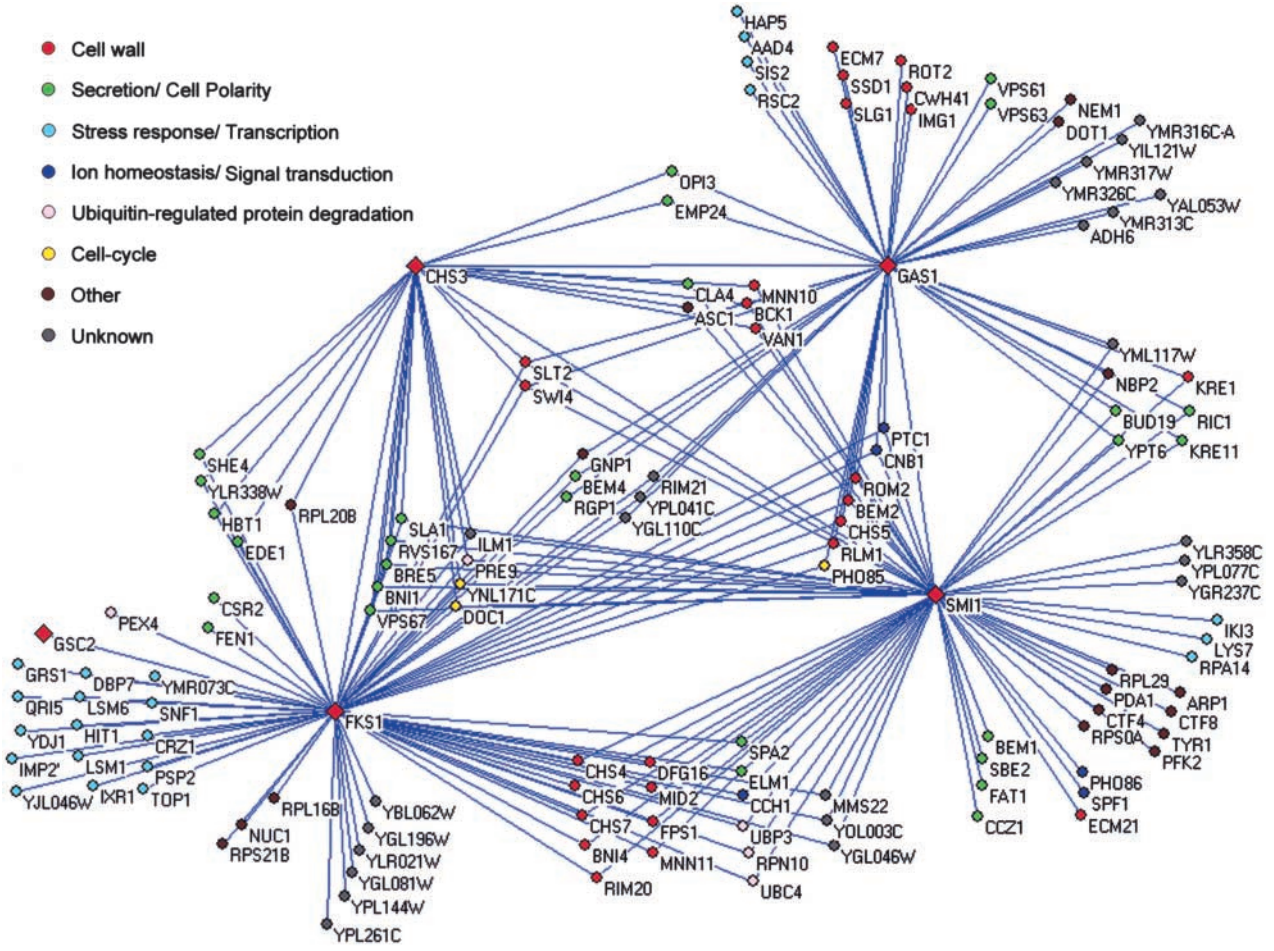

FIGURE 1.—Network of synthetic interactions with *CHS3*, *FKS1*, *GSC2/FKS2*, *GAS1*, and *SMI1*. Genes engaged in interactions are represented as nodes. Nodes are colored according to functional categories, assigned on the basis of information from the literature.

bly (*CCZ1*, *VPS61*, *VPS63*, and *VPS67*). Genes required for correct endocytosis are found in the glucan network, encoding regulators of cortical actin patch assembly (*EDE1*, *RVS167*, *SHE4*, *SLA1*, and *YLR338W*) or regulators of vesicle trafficking from endosomes to the Golgi (*RIC1*, *RGP1*, and *YPT6*). A further three genes are involved in membrane biogenesis (*FAT1*, *FEN1/ELO2/GNS1*, and *OPI3*).

Transport of cell wall assembly components to sites of cell wall expansion requires cell polarity control, forward transport through the secretory pathway, and endocytosis-mediated recycling. In mutants defective in β-1,3-glucan synthesis, such polarized transport is essential to bring compensatory components to the cell wall.

Transcription regulation and stress response: Genes encoding transcription factors (*CRZ1*, *HAP1*, and *IXR1*), transcription factor regulators (*RSC2* and *SNF1*), a subunit of RNA polymerase I (*RPA14*), and RNA processing factors (*DBP7*, *GRS1*, *LSM1*, *LSM6*, *PSP2*, and *TOP1*) were found. An additional set of nine genes putatively involved in stress responses also falls into this group (*AAD4*, *HIT1*, *IMP2'/YIL154C*, *LYS2*, *QRI5*, *SIS2*, *YDJ1*, *YJR046W*, and *YMR073C*). The regulated expression of genes compensating for defects in β-1,3-glucan synthesis may depend upon these gene products.

Ion homeostasis and signal transduction: Excluded here are genes involved in the cell integrity pathway discussed above. The five remaining genes are involved in the downregulation of the high-osmolarity glycerol response (HOG) pathway (*PTC1*), calcium signaling (*CCH1*, *CNB1*, and *SPF1/COD1*), and phosphate transport (*PHO86*). Ion homeostasis, by acting through signaling cascades, may contribute to the onset of processes essential for viability of β-1,3-glucan mutants.

Ubiquitin-regulated protein degradation: Constituents of the 26S proteasome (*PRE9* and *RPN10*) and ubiquitin-conjugating enzymes (*PEX4* and *UBC4*) were found. Ubiquitination regulates a number of processes required in β-1,3-glucan mutants, such as endocytosis or cell cycle progression.

Cell cycle: Regulation of cell cycle progression by cyclin action (*PHO85*) or destruction (*DOC1* and *YNL171C*) is

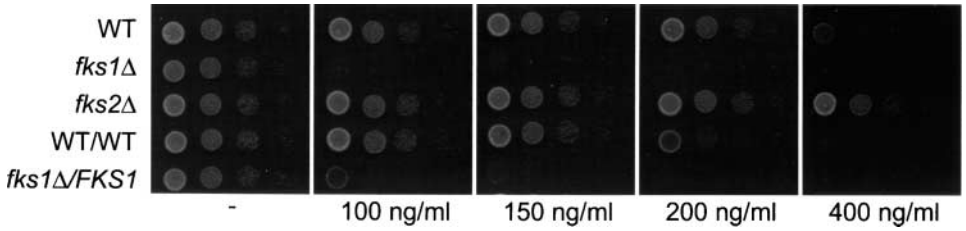

FIGURE 2.—Assay for altered sensitivity to caspofungin. Dilutions of exponentially growing wild-type haploid or diploid (WT or WT/WT, respectively) and mutant strains were spotted onto YEPD plates containing the indicated caspofungin concentration.

crucial in coordinating cell wall synthesis and cell growth and buffers β-1,3-glucan mutant defects.

Other genes and poorly characterized genes: Of the other genes interacting with *FKS1*, *GAS1*, or *SMI1* (Table 2), 17 have a known function not discussed above, and 24 are poorly characterized or of unknown function.

Screen for genetic interactions with *CHS3*

As chitin synthesis can compensate for mutational defects in β-1,3-glucan synthesis, *CHS3* and other genes required for Chs3p function show genetic interactions with *FKS1*, *SMI1*, and *GAS1*. To further investigate this compensation process, we reasoned that genes involved in balancing chitin and β-1,3-glucan synthesis should be required for the normal growth of mutants with defects in chitin synthase or β-1,3-glucan synthase. Thus, we searched among the genes required in the absence of *CHS3* for those that are also required in the absence of *FKS1*, *SMI1*, or *GAS1*. An SGA analysis was performed with the *chs3* null strain, and 53 gene deletions affected growth, with 26 of these also found in the glucan network (Figure 1). The remaining genes are listed in TONG *et al.* (2004). These 26 overlapping genes fall mainly into two categories: secretory pathway polarization (12 genes) and synthesis and cell wall regulation (8 genes). Thus, the proper localization of cell wall synthesis components buffers both glucan and Chs3p-dependent chitin synthesis.

Screens for altered sensitivity to caspofungin

To broaden our view of β-1,3-glucan biology, we searched for genes whose deletion led to altered sensitivity to caspofungin, a glucan synthase inhibitor. As caspofungin is thought to inhibit both Fks1p and Fks2p, such an analysis should give insights distinct from our interaction approach that examines the buffering effects of genes on mutants individually deleted for the *FKS1* or *FKS2* target genes. A screen for growth in the presence of caspofungin was made with 4598 haploid strains deleted for nonessential genes and 1058 strains heterozygous for essential genes. As the wild-type diploid had a higher sensitivity to caspofungin than the wild-type haploid (Figure 2), screens were performed at concentrations specific for these two cell types. The search for hypersensitive mutants was performed at a subinhibitory caspofungin concentration, while for screening resistant mutants a drug concentration that inhibited growth of the wild type was used (Figure 2). Strains were first grown on YEPD and then replicated onto YEPD with or without caspofungin. The hits were then confirmed by a spotting assay (see MATERIALS AND METHODS and Figure 2). We found 45 haploid deletion mutants to be hypersensitive to caspofungin. Of these, 23 were also tested for haplo-insufficiency, with 16 (69%) showing a haplo-insufficient sensitivity phenotype as heterozygous diploids (Table 3 and supplementary Table 1 at http://www.genetics.org/supplemental/). In addition, among the ~1100 heterozygous null mutants in essential genes, 7 were caspofungin hypersensitive (Table 3). Finally, a screen for haploid deletion mutants able to grow at high caspofungin concentration gave mutants in 39 genes with caspofungin resistance (Table 4 and supplementary Table 2 at http://www.genetics.org/supplemental/).

Genes involved in multidrug sensitivity: Recently, a set of yeast mutants that are hypersensitive to a range of inhibitory compounds has been identified (PARSONS *et al.* 2004). A number of these mutants also show hypersensitivity to caspofungin (supplementary Table 1). These genes are involved in a wide range of cellular functions: assembly of the vacuolar H^+-ATPase (*PPA1/VMA16*, *TFP3/VMA11*, *VMA2*, *VMA4*, *VMA5*, *VMA7*, *VMA10*, *VMA13*, *VMA22*, and *VPH2*), late endosomal trafficking (*SNF7* and *STP22*), ergosterol synthesis (*ERG6*), transcription (*CCR4* and *SPT20*), nuclear migration (*SPC72*), glycogen turnover (*GPH1*), and signal transduction (*SLT2*).

Although no global compendium of multidrug-resistant yeast mutants is currently available, a literature search revealed that mutations in 12 additional genes confer resistance to a number of drugs as well as caspofungin (supplementary Table 2). These genes are involved in lipid biosynthesis (*CSG2*, *FEN1*, *MCT1*, *SUR1*, and *SUR4*), ER-to-Golgi trafficking (*ERV14*), and signal transduction (*CKA2* and *CWH43*) or are of unknown function. As mutants in the genes in supplementary Tables 1 and 2 show altered sensitivity to a diverse set of bioactive compounds, their altered sensitivity to caspofungin is likely nonspecific.

Genes specifically involved in caspofungin toxicity: These are grouped in five categories (Tables 3 and 4).

Synthesis and regulation of the cell wall: Deletion of *FKS1* leads to hypersensitivity, while deletion of *FKS2* leads to caspofungin resistance relative to a wild type. Mutants

TABLE 3

Genes whose deletion confers hypersensitivity to caspofungin

ORF	Gene	Comment	Caspofungin sensitivity[a]	
			Haploid	Heterozygous diploid
		Cell wall components synthesis and cell wall assembly		
YBR023C	*CHS3*	Chitin synthase III	- -	- -
YBL061C	*CHS4*	Activator of Chs3p	- -	
YLR330W	*CHS5*	Involved in Chs3p transport from the late Golgi to the chitosome	- -	
YJL099W	*CHS6*	Involved in Chs3p transport from the late Golgi to the chitosome	- -	
YHR142W	*CHS7*	Facilitates exit of Chs3p from the ER	- -	
YLR342W	*FKS1*	β-1,3-Glucan synthase subunit	- - -	- - -
YDR245W	*MNN10*	Subunit of the Mannan polymerase II complex	-	
YDL047W	*SIT4*	Protein phosphatase that negatively regulates Slt2p	-	
YGR229C	*SMI1*	Regulator of β-1,3-glucan synthesis	-	- - -
		Cytoskeleton assembly and vesicular transport		
YIL142W	*CCT2*	Subunit of the TCP-1 ring complex required for the folding of actin and tubulin	NA	- -
YJR064W	*CCT5*	Subunit of the TCP-1 ring complex required for the folding of actin and tubulin	NA	- - -
YJL204C	*RCY1*	Involved in endocytic membrane traffic and recycling out of an early endosome	- -	
YBL007C	*SLA1*	Cortical actin patch assembly control protein, mutation affects endocytosis	-	0
YPR139C	*VPS66*	Class B vacuolar sorting protein	-	
		Transcription and protein synthesis		
YER017C	*AFG3*	Mitochondrial protease, degrades nonassembled inner membrane proteins	-	- -
YHR013C	*ARD1*	Protein N-acetyltransferase	-	
YDR173C	*ARG82*	Inositol phosphate kinase	- -	
YDR364C	*CDC40*	Protein required for the second catalytic step of mRNA splicing	- -	
YDR432W	*NPL3*	Involved in nuclear export of poly(A)+ mRNA	- -	- - -
YIL021W	*RPB3*	RNA polymerase II subunit	NA	- -
YBL014C	*RRN6*	RNA polymerase I core transcription factor	NA	- - -
YGR215W	*RSM27*	Component of mitochondrial ribosomal subunit	- -	
YOR290C	*SNF2*	Transcription factor acting in the SWI/SNF chromatin remodeling complex	-	0
YJL127C	*SPT10*	General transcription repressor	-	
YLR182W	*SWI6*	Component of SBF and MBF transcription factors, G1/S transition	-	0
YOL072W	*THP1*	Transcription factor involved in transcription elongation	- - -	- - -
YGR285C	*ZUO1*	Component of ribosome-associated complex	- -	- - -
YHR085W		May be involved in rRNA processing	NA	- -
		Other and unknown functions		
YEL027W	*CUP5*	Component of V0 sector of the vacuolar H^+-ATPase.	- -	- - -
YDR052C	*DBF4*	Regulatory subunit of Cdc7p kinase, required for G1/S transition	NA	- - -
YEL046C	*GLY1*	Threonine aldolase, required for glycine biosynthesis	-	- -
YJR118C	*ILM1*	Null mutant shows increased loss of mitochondrial DNA	- -	- - -
YNL126W	*SPC98*	Spindle pole body component that interacts with γ-tubulin	NA	- -
YGR105W	*VMA21*	Required for export of V-ATPase V0 sector out of the ER	- -	

[a] Scores for caspofungin sensitivity are as follows: - - -, no growth of haploid on 100 ng/ml, no growth of heterozygous diploid on 150 ng/ml; - -, slow growth of haploid on 100 ng/ml and no growth on 200 ng/ml, slow growth of heterozygous diploid on 150 ng/ml; -, slow growth of haploid on 200 ng/ml; 0, wild-type growth; NA, nonapplicable (essential gene).

deleted for genes required for chitin synthase III-dependent chitin deposition (*CHS3–7*) and protein mannosylation (*MNN10*) are also hypersensitive to the drug.

Deletion of *SLG1* or *TUS1*, two genes acting upstream of the cell integrity pathway, confers caspofungin resistance. In contrast, deletion of genes acting in the downstream part of the pathway such as *SMI1* or *SIT4* leads to caspofungin hypersensitivity.

Cytoskeleton and vesicular transport: Two essential genes (*CCT2* and *CCT5*) have heterozygous diploids that are hypersensitive. In addition, the deletion of *SLA1*, *RCY1*, components of the endocytic pathway, or the vacuolar protein-sorting gene *VPS66* leads to a moderate increase in caspofungin sensitivity. Among genes whose deletion decreases susceptibility to caspofungin are *DNF2* and *LEM3*, both involved in membrane trafficking, and *SEC66* and *VID24*, both involved in protein trafficking.

Signal transduction and stress: Deletion of any of the

TABLE 4

Genes whose haploid deletion mutants show enhanced resistance to caspofungin

ORF	Gene	Comment
		Cell wall assembly
YGR032W	*FKS2*	β-1,3-Glucan synthase subunit
YOR008C	*SLG1*	Sensor for the *PKC1-SLT2* cell integrity pathway
YLR425W	*TUS1*	GDP-GTP exchange factor for Rho1p
		Cytoskeleton and vesicular transport
YDR093W	*DNF2*	Putative flippase, aminophospholipid transporter
YNL323W	*LEM3*	Required for plasma membrane translocation of phosphatidylcholine and phosphatidylethanolamine
YBR171W	*SEC66*	Component of ER protein-translocation subcomplex
YBR105C	*VID24*	Required for ubiquitin-regulated transport of Fbp1p into the vacuole by the cytoplasm to vacuole pathway
		Stress and signal transduction
YOR197W	*MCA1*	Cysteine protease of the metacaspase family
YDL079C	*MRK1*	Member of the glycogen synthase kinase-3 family, involved in stress signaling
YJR153W	*PGU1*	Endopolygalacturonase induced upon filamentation
YHR050W	*SMF2*	Manganese transporter, mutant resistant to osmotic stress
YNR031C	*SSK2*	MAPKK kinase involved in the high-osmolarity signal transduction pathway
		Transcription and protein synthesis
YNL027C	*CRZ1*	Calcineurin responsive zinc-finger transcription factor
YNL301C	*RPL18B*	Ribosomal protein
		Other and unknown functions
YDR528W	*HLR1*	Low similarity to Lre1p, a gene involved in the regulation of β-1,3-glucan biosynthesis
YHR004C	*NEM1*	Protein required for nuclear morphology
YJR033C	*RAV1*	Regulator of the vacuolar H^+-ATPase
YAR035W	*YAT1*	Outer carnitine acetyltransferase
YCR087C-A		Unknown function
YDR326C		Protein with a GRAM domain, found on glucosyltransferases
YDR479C		Peroxisomal integral membrane protein
YIL110W		Repressed under stress conditions
YJR024C		Enterobacterial L-ribose-5-phosphate 4-epimerases-like protein, possibly involved in carbohydrate metabolism
YLR338W		Questionable ORF, overlaps with *VRP1*, a cortical actin patch component
YNL080C		May play a role in respiratory growth
YOR024W		Unknown function
YOR118W		Unknown function

five genes in this group increases caspofungin resistance. These genes encode components of signaling cascades (*MRK1* and *SSK2*) or factors activated by or mediating sensitivity to various stresses (*MCA1*, *PGU1*, and *SMF2*).

Transcription and protein synthesis: A set of 11 nonessential and 3 essential genes in this group shows increased caspofungin sensitivity when deleted. They are involved in all stages from transcription to translation: histone acetylation (*SPT10*), chromatin remodeling (*ARG82* and *SNF2*), transcription regulation (*SWI6*), RNA-polymerase I and II transcription (*RPB3*, *RRN6*, and *THP1*), RNA processing and transport (*CDC40*, *NPL3*, and *YHR085W*), and regulation of translation (*ARD1*, *RSM27*, and *ZUO1*). The absence of the Crz1p transcription factor or the ribosomal protein L18B leads to increased resistance to caspofungin.

Genes of other and unknown function: Deletion in 6 and 13 other genes was found to confer hypersensitivity and resistance, respectively (Tables 3 and 4).

DISCUSSION

A network of genetic interactions with *FKS1*, *FKS2*, *GAS1*, and *SMI1*

By compiling the synthetic genetic interactions of a set of mutants with defects in β-1,3-glucan synthesis, we have generated a network of 135 genes involved in 195 interactions.

A set of genes compensates for defects in glucan synthesis: Many (51/135, 37%) of the genes in the glucan network are connected to more than one query gene. This core set of genes is engaged in 112/195

(57%) interactions and 10 genes interact with *FKS1*, *GAS1*, and *SMI1*. *FKS1* shares 43/76 interactions with *GAS1* or *SMI1*, *GAS1* shares 24/48 interactions with *FKS1* or *SMI1*, while *SMI1* shares 45/71 interactions with *FKS1* or *GAS1*. The majority of multiply connected genes (62%) occupy two categories: synthesis and regulation of the cell wall (18 genes) and polarization and secretory pathway function (14 genes). This reflects the underlying coordination of polarized growth and cell wall assembly in the mitotic cell cycle. The regulation and orchestration of these processes depend on the integrity of the actin cytoskeleton, the cell polarity machinery, and a functional *PKC1-SLT2* pathway. Other categories represented are ion homeostasis and signal transduction, cell cycle, and ubiquitin-related protein degradation. Importantly our glucan network core identifies genes of unknown function that appear central to the buffering of glucan defects and that are likely new components of the pathways discussed above. These include *ILM1*, *MMS22*, *RIM21*, *YGL046W*, *YGL110C*, *YML117W*, *YOL003C*, and *YPL041C*.

Cell integrity pathway: Eight of the 10 genes interacting with *FKS1*, *SMI1*, and *GAS1* are involved in regulation of cell wall assembly through the cell integrity pathway. These genes encode components (*BEM2*, *ROM2*, and *SLT2*) or downstream targets (*RLM1* and *SWI4*) of the *PKC1-SLT2* pathway. In addition, *CNB1* and *PHO85* act in concert with this pathway under stress conditions (Zhao *et al.* 1998; Huang *et al.* 2002). Furthermore, overactivation of the HOG signaling pathway by deletion of the protein phosphatase *PTC1*, as well as deletion of the glycerol channel *FPS1*, is deleterious to *fks1* and *smi1* mutants. These findings support the view that the HOG and *PKC1-SLT2* pathways play opposing roles in regulating cell wall synthesis (Reynolds *et al.* 1998).

Chitin compensation: CHS3 and *CHS5* both interact synthetically with *FKS1*, *SMI1*, and *GAS1*, as Chs3p-dependent chitin synthesis compensates for stress generated by defects in glucan synthesis (Garcia-Rodriguez *et al.* 2000b; Valdivieso *et al.* 2000; Carotti *et al.* 2002). This chitin stress response is regulated, at least in part, by components of the *PKC1-SLT2* pathway (Mazzoni *et al.* 1993; Igual *et al.* 1996; Valdivia and Schekman 2003). In addition, a group of genes (*BNI1*, *ELM1*, *RVS167*, *SLA1*, *SPA2*, and *VPS67*) required for normal polarized growth and morphogenesis interact with both *FKS1* and *SMI1*, suggesting that compensatory chitin synthesis at the bud neck is essential in both *fks1* and *smi1* mutants. These gene products may also participate in the targeting of other cell wall synthesis components.

Gene-specific interactions: *FKS1* interacts specifically with *FKS2* and the double mutant is synthetically lethal. Since deletion of *FKS1* triggers expression of *FKS2*, a fraction of *FKS1*-specific interactions involve genes required for *FKS2* expression or for Fks2p function. For example, Crz1p and Snf1p are both positively involved in *FKS2* induction (Stathopoulos and Cyert 1997; Zhao *et al.* 1998). In this category are genes required for or induced during stress responses (*IMP2′/YIL154C*, *IXR1*, *HIT1*, *QRI5*, *YDJ1*, *YJR046W*, and *YMR073C*). Many of these genes are important for the oxidative stress response, a process known to involve calcium signaling through calcineurin (Serrano *et al.* 2002), and thus, could influence *FKS2* expression. *FKS1* also interacts with genes involved in transcription, RNA processing, and translation, again suggesting that their buffering of Fks1p loss is through an altered Fks2p level. Finally, genes involved in endocytosis (*EDE1* and *SHE4*) and cell polarity (*CSR2* and *HBT1*) that interact with *FKS1* may be required for cellular targeting of Fks2p.

The *SMI1* deletion is buffered by genes acting in different areas of cell wall assembly, such as crosslinking glucan fibrils (*GAS1*), efficient β-1,6-glucan synthesis (*KRE1*), or crosslinks between β-1,3-glucan and Van1p- and Mnn10p-dependent protein mannosylation. In addition, *SMI1* shows interactions with genes required for chromosome segregation (*ARP1*, *CTF4*, and *CTF8*) and polarity establishment (*CLA4* and *BEM1*), two processes requiring bud neck integrity. This suggests that a *SMI1* deletion results in defective bud neck assembly or function.

Survival of a *gas1* null mutant appears to require the correct synthesis and assembly of cell wall β-1,6-glucan. We found a set of *gas1*-interacting mutants in genes affecting this process or resistance to K1 killer toxin, which requires β-1,6-glucan as a receptor (*BUD19*, *CWH41*, *IMG1*, *KRE1*, *KRE11*, *NBP2*, *PHO85*, *RGP1*, *RIC1*, *ROT2*, *RSC2*, *SMI1*, *VPS61*, *VPS63*, and *YPT6*; see Page *et al.* 2003). A number of potential cell wall regulating genes also interact with *GAS1* and are candidates for involvement in β-1,6-glucan biology. These include *ECM7* (Lussier *et al.* 1997; Giaever *et al.* 2002), *YAL053W* (Lagorce *et al.* 2003), and *SSD1*, a regulator of cell wall composition (Kaeberlein and Guarente 2002).

Genetic interactions with *FKS2* and *FKS3*: In contrast with the many interactions found for *FKS1*, *FKS2* interacted only with *FKS1*, and no interactions were found with *FKS3*. Differential expression of these genes likely underlies their interaction patterns. *FKS1* is expressed during vegetative growth on glucose, a growth condition where the *FKS2* transcript is largely undetectable. *FKS2* is, however, induced under specific conditions such as starvation, stress, and in stationary phase (Mazur *et al.* 1995). Little is known about *FKS3* function; its expression is regulated by Ste12p upon pheromone exposure (Zeitlinger *et al.* 2003), and the *fks3* null mutant shows a slight sporulation defect (Deutschbauer *et al.* 2002).

Functional links between glucan and chitin synthesis: Defective β-1,3-glucan assembly is compensated for by an increased synthesis of chitin. In our synthetic analysis of Chs3p-dependent chitin synthesis, we found that, as with glucan mutants, this is largely buffered by genes involved in the regulation of cell wall assembly and

secretory pathway polarization. Indeed almost half of the genes interacting with *CHS3* are found in the glucan network, highlighting their common function in buffering the cell wall from adversity. A significant overlap of *CHS3* interactants with *FKS1* (16 genes, $P = 5 \times 10^{-62}$) and *SMI1* (17 genes, $P = 1 \times 10^{-66}$) interactants was found, with 11 genes interacting with *FKS1*, *SMI1*, and *CHS3* (*BNI1*, *BRE5*, *DOC1*, *ILM1*, *PRE9*, *RVS167*, *SLA1*, *SLT2*, *SWI4*, *VPS67*, and *YNL171C*), 5 genes interacting with *CHS3* and *FKS1* (*EDE1*, *HBT1*, RPL20B, *SHE4*, and *YLR338W*), and 6 genes interacting with *CHS3* and *SMI1* (*ASC1*, *BCK1*, *CLA4*, *GAS1*, *MNN10*, and *VAN1*). This further indicates that proper localization of cell wall building components, through polarization of the secretory apparatus, is essential in achieving a balance of chitin and glucan levels.

Genes involved in caspofungin sensitivity

A synthetic-lethal analysis reveals pairwise interactions among genes. Application of this approach to the FKS gene family is complicated by the need to compare more complex combinations of mutants. In this situation, a drug inhibiting a protein family offers a powerful alternative "chemogenomics" strategy. As caspofungin differentially inhibits both Fks1p and Fks2p, targets that are singly dispensable but together are essential, the basis for phenotypes of deletion mutants with altered sensitivity to this drug is likely to be complex. In general, deletion of genes required to maintain Fks1p and Fks2p activity would lead to lower glucan synthase activity levels and hypersensitivity to caspofungin. In addition, the absence of genes whose products buffer cells from loss of glucan synthesis would be more vulnerable to such loss, and thus caspofungin hypersensitive. In this case, the mutant-caspofungin interaction can be viewed as being "synthetic" (Parsons *et al.* 2004). As the complete loss of the Fks1p and Fks2p targets is lethal, resistance of this kind cannot occur, but is possible with mutant alleles (Douglas *et al.* 1994; Mazur *et al.* 1995) or if targets are overproduced (Rine *et al.* 1983). Altered sensitivity to caspofungin can also arise through detoxification by vacuole enzymes or mutant defects that affect membrane permeability and hence accessibility of the drug to its targets.

Caspofungin toxicity and regulation of glucan synthesis: The two FKS targets show different levels of sensitivity to this drug class, with Fks2p being more sensitive to echinocandin and aerothricin than Fks1p (Mazur *et al.* 1995; Kondoh *et al.* 2002). Our work accords with these findings, with *fks1* and *fks2* mutants being caspofungin hypersensitive and resistant, respectively. Consistent with this, the *crz1* mutant, known to be defective in *FKS2* induction (Stathopoulos and Cyert 1997), is more resistant to caspofungin than the wild type. Mutants in regulatory components affecting glucan synthase activity show a complex set of responses. The *slg1/wsc1* mutant defective in Rho1p-dependent activation of Fks1p/Fks2p (Mazur and Baginsky 1996; Sekiya-Kawasaki *et al.* 2002) is caspofungin resistant, as is a *tus1* mutant that is also implicated in Rho1p signaling. In contrast, the ability to activate the Rho1p-dependent *PKC1-SLT2* pathway buffers cells against caspofungin, as *slt2* and *smi1* mutants are hypersensitive. However, deletion of *SSK2*, leading to defective activation of the HOG pathway, as well as that of *HLR1*, a multicopy suppressor of osmosensitivity of a *ste11ssk2ssk22* triple mutant (Alonso-Monge *et al.* 2001), confers resistance to caspofungin. These findings, together with results on calcofluor white sensitivity (Garcia-Rodriguez *et al.* 2000a) highlight the crosstalk between the cell integrity and the HOG pathways and the importance of coordinating these opposing signaling pathways for cell wall assembly.

Genes involved in processes compensating for the inhibition of a target are required for survival in presence of a drug. For example, chitin synthesis is upregulated by cell wall stress, and as with the genetic interac-

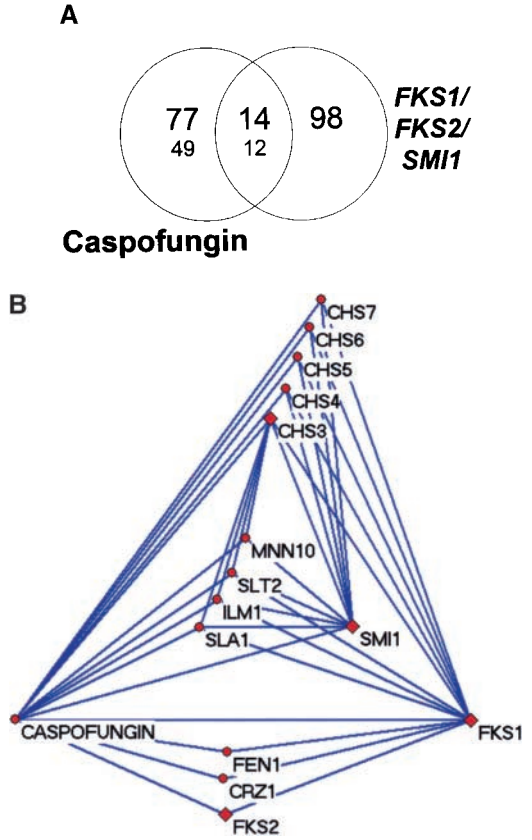

Figure 3.—Overlap of the *FKS1/FKS2/SMI1* genetic interaction network and the caspofungin chemical-genetic network. (A) Venn diagram summarizing the number of genes leading to altered caspofungin sensitivity when deleted and/or showing synthetic interaction with *FKS1*, *FKS2*, or *SMI1*. The numbers of genes not classified as multidrug sensitive are indicated in small font. (B) Network of chemical genetic interactions with caspofungin and the genetic interactions with *FKS1*, *FKS2*, and *SMI1*.

TABLE 5

Genes whose transcription is increased and whose deletion mutant shows altered growth in a *fks1*, *smi1*, or *gas1* null background or upon caspofungin exposure

	fks1[a]		*smi1*[a]		*gas1*[a]		Caspofungin[b]	
Gene/ORF	Induced	Interaction	Induced	Interaction	Induced	Interaction	Induced	Sensitivity
CHS3	+	SSL	+	SSL	+	SSL		Hs
FKS2	+	SSL	+	SSL	+	SSL	+	Res
GPH1							+	Hs
KRE11	+			SSL	+	SSL	+	
SLT2	+	SSL	+	SSL	+	SSL	+	Hs
YAL053W	+	SSL	+	SSL	+	SSL		Res

[a] Genes whose mRNA level is increased in the *fks1*, *smi1*, or *gas1* null mutants are scored "+" (Lagorce *et al.* 2003). Genetic interaction is indicated. SSL, synthetic sick or lethal.

[b] Genes whose mRNA level is increased by caspofungin treatment are scored "+" (Agarwal *et al.* 2003). Caspofungin sensitivity is indicated. Hs, hypersensitive; Res, resistant.

tion data, deletion of *CHS3* or the ancillary genes (*CHS4–7*) leads to caspofungin hypersensitivity. Deletion of components of the endocytic pathway (*RCY1* or *SLA1*) also leads to a moderate increase in caspofungin sensitivity. Thus, transport of cell surface components likely buffers perturbed cell wall synthesis in caspofungin-treated cells, with defects in the proper recycling of these components resulting in increased drug sensitivity. For instance, cortical actin patches are important for dynamic Fks1p localization, with cell wall remodeling and *SLA1* deletion resulting in mislocalization of Fks1p (Li *et al.* 2002; Utsugi *et al.* 2002). In this context, the dynamics of Fks1p and/or Chs3p localization in an *rcy1* null mutant merit examination.

Comparison of the synthetic and chemical-genetic networks

Both the synthetic interaction and the caspofungin phenotype data sets should identify genes involved in buffering cells against defective β-1,3-glucan synthesis. However, each set has limitations, such as gene family issues with the synthetic interactions and the multidrug sensitivities and possible "off target" side effects of caspofungin. Integration of the 189 genes in the two networks (Figure 3A) shows a central overlapping core of 14 genes. Of these 14 genes with altered caspofungin sensitivity, 11 show synthetic interactions with *FKS1* and 9 with *SMI1* (Figure 3B). The overlap of these two sets is highly significant ($P = 1 \times 10^{-39}$ and 9×10^{-33} for *FKS1* and *SMI1*, respectively) and is consistent with caspofungin acting at the level of Fks1p and Fks2p in inhibiting β-1,3-glucan synthesis.

Deletion mutants in 98 genes of the glucan network have wild-type sensitivity to caspofungin (Figure 3A), indicating that caspofungin treatment does not phenocopy cell wall mutations. This may be because at the subMIC concentration used here, caspofungin does not fully inhibit its target. A prediction of this is that the viable synthetic double mutants should show enhanced caspofungin sensitivity when compared to the single mutants. Finally, a set of 77 genes whose deletion alters caspofungin sensitivity is absent from the glucan network (Figure 3A). Mutation in a fraction of these could affect growth of an *fks1 fks2* double mutant but not that of the singly deleted mutants. In addition, some of these genes may actually show synthetic interactions, and be false negatives (see materials and methods), while others may buffer against off target side effects of caspofungin.

Genetic interactions, fitness under stress condition, and transcription

We have compared our data on synthetic interactions and altered caspofungin sensitivity with the relevant transcriptional profiling data (Table 5). Three genes found in our functional core (Figure 3B), *CHS3*, *FKS2*, and *SLT2*, show altered transcriptional profiles. These three core genes capture much of glucan buffering, namely the need for compensatory chitin, an alternative glucan synthase component, Fks2p, and a cell integrity signal transduction pathway. Other genes found in the functional network that are transcriptionally regulated are *KRE11*, *YAL053W*, and *GPH1*.

It is striking that most genes that genetically buffer *fks1*, *smi1*, and *gas1* mutants or lead to altered caspofungin sensitivity show no changes in transcriptional levels. Thus, it appears that most genes functionally involved in responding to glucan defects do so in ways that are transcription independent. Presumably, the existing cellular location and activity of these gene products coupled with their normal levels of synthesis are sufficient to achieve an effective buffering.

Our work also emphasizes that many transcriptionally regulated genes have no apparent effect on fitness during perturbation of β-1,3-glucan synthesis, a situation seen previously for a range of conditions (Giaever *et*

al. 2002). This indicates that the yeast repertoire of transcriptional responses may be limited and stereotyped. Here, no β-1,3-glucan-specific response is invoked, but rather a more general response occurs, in which only a fraction of genes are functionally effective, but where the stereotypic response set has been evolutionarily selected as a "tool box" to cope with a more broadly based set of insults.

Issues of drug resistance

Drug resistance is a major clinical issue. Our work on resistance is confined to null mutants in nonessential genes and so is not comprehensive; for example, point mutants in *FKS1* or *FKS2* leading to resistance would not be seen. However, despite these limitations, we found 39 *S. cerevisiae* genes leading to decreased caspofungin sensitivity when deleted (see Table 3 and supplementary Table 1). In particular, deletion of several genes encoding putative membrane-associated proteins with unknown function led to enhanced resistance to caspofungin; they could encode either additional targets for caspofungin (*YDR326C* and *YDR479C*) or proteins mediating effects of the drug (*YBR144C*, *YGR283C*, *YIL110W*, and *YPL056C*). Mutations in the fungal pathogen orthologs of these 39 genes could lead to increased resistance.

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