Multicopy *FZF1* (*SUL1*) Suppresses the Sulfite Sensitivity but Not the Glucose Derepression or Aberrant Cell Morphology of a *grr1* Mutant of *Saccharomyces cerevisiae*

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ABSTRACT

An *ssu2* mutation in *Sacccharomyces cerevisiae,* previously shown to cause sulfite sensitivity, was found to be allelic to *GRR1,* a gene previously implicated in glucose repression. The suppressor *rgt1,* which suppresses the growth defects of *grr1* strains on glucose, did not fully suppress the sensitivity on glucose or nonglucose carbon sources, indicating that it is not strictly linked to a defect in glucose metabolism. Because the Cln1 protein was previously shown to be elevated in *grr1* mutants, the effect of *CLN1* overexpression on sulfite sensitivity was investigated. Overexpression in *GRR1* cells resulted in sulfite sensitivity, suggesting a connection between *CLN1* and sulfite metabolism. Multicopy *FZF1,* a putative transcription factor, was found to suppress the sulfite sensitive phenotype of *grr1* strains, but not the glucose derepression or aberrant cell morphology. Multicopy *FZF1* was also found to suppress the sensitivity of a number of other unrelated sulfite-sensitive mutants, but not that of *ssu1* or *met20,* implying that *FZF1* may act through Ssu1p and Met20p. Disruption of *FZF1* resulted in sulfite sensitivity when the construct was introduced in single copy at the *FZF1* locus in a *GRR1* strain, providing evidence that *FZF1* is involved in sulfite metabolism.

SULFITE is a potentially toxic metabolite in *Saccharomyces cerevisiae* and other organisms in which it occurs naturally as an intermediate in the reductive sulfate assimilation pathway or as a product of the catabolism of sulfur-containing amino acids. In yeast, sulfite is formed by reduction of 3′-phosphoadenosine phosphosulfate (PAPS) through the action of PAPS reductase. It is then reduced to hydrogen sulfide in a six-electron transfer catalyzed by sulfite reductase. Hydrogen sulfide condenses with *O*-acetylhomoserine to form homocysteine leading directly to methionine, or to cysteine via cystathionine (CHEREST and SURDIN-KERJAN 1992; THOMAS *et al.* 1992). Catabolic formation in mammals occurs through the oxidation of cysteine leading to β-sulfinyl pyruvate that spontaneously hydrolyzes to form sulfite and pyruvate. The sulfite formed is oxidized to sulfate in mitochondria by sulfite oxidase (HUXTABLE 1986).

Because sulfite is toxic to many organisms, those that produce it are presumed to have evolved mechanisms to prevent deleterious reactions from occurring *in vivo.* While yeast has a basal tolerance for sulfite, we previously isolated mutants with heightened sensitivity or resistance in expectation that sulfite-protective mechanisms may be impaired in the former and enhanced in the latter (XU *et al.* 1994). The differences in levels of sulfite tolerated among the mutants were found to be small. The resistant mutant *RSU1* was found to tolerate a threefold greater concentration of sulfite than wild type, which in turn was tolerant of a twofold higher level than the most sensitive mutants. One of the sensitive mutants, *ssu2,* was found to have an elongated cell morphology, enhanced sensitivity to the reducing agents dithiothreitol, nitrite, and thiosulfate, to excrete less acetaldehyde, and to contain a reduced amount of glutathione. While the lattter two compounds are reactive with sulfite and represent potential routes of detoxification, a mutant severely deficient in glutathione was found not to be sensitive (XU *et al.* 1994). CASALONE *et al.* (1992) reported that a sulfite-resistant mutant excreted significantly more acetaldehyde than wild type, suggesting that the reaction between acetaldehyde and sulfite may be important *in vivo.* STRATFORD *et al.* (1987) found that exogenous sulfite induced excretion of acetaldehyde by wild-type strains of *S. cerevisiae* and *Saccharomycodes ludwigii.*

Here we show that *SSU2* is the same as *GRR1* and identify a multicopy suppressor of the sulfite sensitivity, but not the glucose derepression or aberrant cell morphology previously observed in *grr1* strains. *GRR1* encodes a 135-kDa weakly expressed protein that is presumed to play a regulatory role, but whose biochemical function(s) is unknown (FLICK and JOHNSTON 1991). *GRR1* has been implicated in a variety of functions: glucose repression (BAILEY and WOODWARD 1984; FLICK and JOHNSTON 1991), glucose transport (ÖZCAN *et al.* 1994; VALLIER *et al.* 1994), *SUC2* gene expression (VALLIER and CARLSON 1991), divalent cation transport

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TABLE 1

Yeast strains

Strain	Genotype	Source
2757-4d-T4[a]	*MAT***a** *LEU2::pRY181 ura3-52 ade2*	This study
3090-9d	*MATα ura3-52 leu2-3, 112*	This study
3118-19c	*MATα ssu2-6 ura3-52 leu2-3, 112 arg4*	This study
3118-27d	*MAT***a** *ssu2-6 ura3-52 leu2-3, 112 arg4 lys9*	This study
3118-27d-T1	*MAT***a** *LEU2::pRY181 ssu2-6 ura3-52 arg4 lys9*	This study
3089-1d	*MAT***a** *ssu3-7 ura3-52 leu2-3, 112*	This study
3100-5b	*MAT***a** *ssu4-11 ura3-52 leu2-3, 112 pet*	This study
3149-1a	*MAT***a** *LEU2::pRY181 his3 trp1-1 ura3-52 ade2*	This study
3150-14	*MAT***a** *LEU2::pRY181 ssu2-6 ura3-52*	This study
3154-2b	*MAT***a** *grr1::URA3 ade2 leu2-3, 112 lys2-801 ura3-52*	
3090-9d-T6	*MATα ura3-52 leu2-3, 112 grr1Δ::URA3*	This study
3090-9d-T6-L1	*MATα ura3-52 leu2-3, 112 grr1Δ6-1*	This study
3090-9d-T4	*MATα ssu1Δ::URA3 leu2-3, 112 ura3-52*	This study
3090-9d-T10	*MATα fzf1Δ99-182::URA3 leu2-3, 112 ura3-52*	This study
3090-9d-T6-L1-T10	*MATα grr1Δ6-1 fzf1Δ99-182::URA3 leu2-3, 112 ura3-52*	This study
3152	*MAT***a**/*MATα LEU2::pRY181/leu2-3, 112 ura3-52/ura3-52 ssu2-6/grr1Δ::URA3* HIS3/*his3Δ-200 ADE2/ade2-101 LYS2/lys2-801 MET/met arg4/ARG4*	This study
YM2061	*MAT***a** *LEU2::pRY181ura3-52 his3Δ-200 ade2-101 lys2-801 met*	M. JOHNSTON
YM3499	*MAT***a** *LEU2::pRY181 ura3-52 grr1Δ::URA3 his3Δ-200 ade2-101 lys2-801 met*	M. JOHNSTON
YM3502[b]	*MATα LEU2::pRY181 ura3-52 grr1Δ::URA3 his3Δ-200 ade2-101 lys2-801 met*	M. JOHNSTON
YM2957	*MATα grr1Δ-1829 ura3-52 hisΔ-200 ade2-101 lys2-801 gal80ΔLEU2::pRY181*	M. JOHNSTON
YM3378	*MAT***a** *grr1Δ-1829 rgt1-101 ura3-52 his3Δ-200 ade2-101 lys2-801 gal80ΔLEU2::pRY181*	M. JOHNSTON
YM3478	*MAT***a** *rgt1-101 ura3-52 his3Δ-200 ade2-101 lys2-801 gal80ΔLEU2::pRY181*	M. JOHNSTON
YPH499	*MAT***a** *ura3-52 his3Δ-200 ade2-101 lys2-801 leu2Δ1 trp1Δ63*	C. MANN
CC359-OL2	*MATα leu2 ura3 his3*	Y. SURDIN-KERJAN
CC363-20B	*MAT***a** *leu2 ura3 his3*	Y. SURDIN-KERJAN
CC370-8C	*MATα leu2 ura3 met20*	Y. SURDIN-KERJAN

[a] pRY181 contains a *Gal1-lacZ* fusion (YOCUM *et al.* 1984).

[b] Strains YM3502-T1 and YM3502-T2 are YM3502 transformed with YEplac195 and YEplac195::FZF1, respectively.

(CONKLIN *et al.* 1993), turnover of G_1 cyclins (BARRAL *et al.* 1995), and suppression of *bem2* mutations involved in cellular morphogenesis (KIM *et al.* 1994). We add sensitivity to sulfite to a growing list of phenotypes associated with mutations in *GRR1*, provide evidence for a link between sulfite sensitivity and *CLN1* overexpression, and implicate *FZF1* in sulfite metabolism.

MATERIALS AND METHODS

Yeast strains, media, growth conditions, and genetic techniques: Yeast strains are listed in Table 1. Standard yeast genetic techniques were used (ROSE *et al.* 1990). Yeast transformations were performed using the method of GIETZ *et al.* 1992. Yeast media and plates used to test sulfite sensitivity are described elsewhere (XU *et al.* 1994). SM is glucose-based synthetic complete medium (SD plus required amino acids and bases at the prescribed concentrations, except for uracil, which was added to a final concentration of 10 μg/ml). Dropout media are SM lacking the indicated amino acid or base (ROSE *et al.* 1990). Sulfite sensitivity on different carbon sources was determined on YEP (1% yeast extract, 2% peptone) containing 2% glucose (YEPD), 2% galactose (YEPgal), 2% maltose (YEPmal), 2% ethanol (YEPE), 3% glycerol (YEPgly), and 0.5% acetate (YEPac). All media containing sulfite were buffered at pH 3.5 with 75 mM L(+)tartaric acid (XU *et al.* 1994), with the exception of YEPac, which was buffered at pH 4.8, to avoid a precipitate that formed at the lower pH. 5-fluoro-orotic acid (5-FOA) plates, for selection of *ura3* segregants, were prepared as described (BOEKE *et al.* 1984). An *in vivo* assay for hydrogen sulfide formation was performed on "BiGGY" agar (Nickerson medium, Difco Laboratories, Detroit, Michigan) according to the manufacturer's instructions.

β-galactosidase activity was assayed as described by KIPPERT (1995).

Sulfite chemistry: As used in this paper, sulfite is an inclusive term referring to all species and salts of sulfurous acid, including sulfur dioxide, its anhydride. Free sulfite includes all unbound species of sulfurous acid: H_2SO_3, HSO_3^-, and SO_3^{-2}. All species are reactive with carbonyl groups present in ingredients commonly found in microbiological media or produced during fermentation, such as sugars, acetaldehyde, and pyruvate. The resultant sulfonates (bound forms of sul-

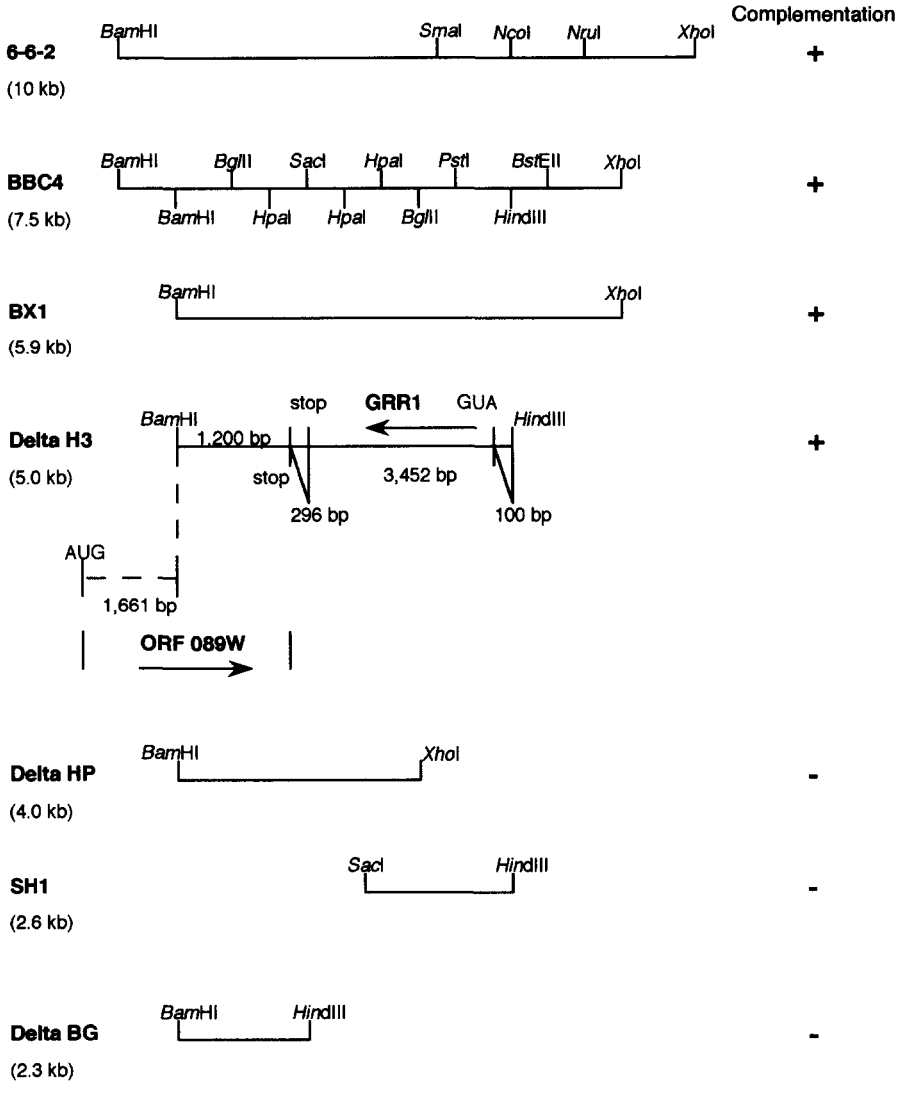

FIGURE 1.—Restriction map of the primary *SSU2*-containing fragment and derived subclones (not drawn to scale). The primary clone was designated 6-6-2. Subclone BBC4 was derived by removal of a 2.5-kb *SmaI*/*NruI* fragment. Subclone BX1 was derived from BBC4 by deletion of a 1.6-kb *Bam*HI fragment. The remaining subclones were derived from BX1: Delta H3 as a 5.0-bp *Bam*HI/*Hin*dIII fragment; Delta HP as a 4.0-kb fragment with a 1.9-kb *Hpa*I deletion spanning all three sites; SH1 as a 2.6-kb *Sac*I/*Hin*dIII fragment; and Delta BG as a fragment with a 3.6-kb *Bgl*II deletion. The positions of start and stop codons, direction of transcription (arrows), and ORF designations in Delta H3 are from the DNA sequence of chromosome *X* (GALIBERT *et al.* 1995).

fite) are not inhibitory to yeast growth, but their formation reduces the effective free sulfite concentration. The only species of sulfite that is inhibitory to yeast is undissociated sulfurous acid, H_2SO_3, as it freely traverses the cell membrane, whereas the other free forms apparently cannot (STRATFORD *et al.* 1987). Because the concentration of the various sulfite species of sulfite is pH-dependent [pKa_1 and pKa_2 are 1.77 and 7.2, respectively (KING *et al.* 1981)] media containing sulfite were buffered.

Hydrogen sulfide, sulfite, and protein determinations: Intracellular hydrogen sulfide and sulfite were assayed as fluorescent adducts of monobromobimane (Calbiochem, La Jolla, CA) following separation by HPLC (FAHEY and NEWTON 1987) as modified by VETTER *et al.* (1989). Briefly, cells were grown to ~1 × 10^7 cells/ml in 100 ml of SM-met, pelleted by centrifugation, washed twice in 20 mM HEPES pH 8.0, and resuspended in 100 μl of the same buffer to which was added 10 μl of 50 mM monobromobimane in acetonitrile. A volume of acid-washed glass beads equal to the cell pellet was added, the mixture was vortexed six times at high speed in 30-sec bursts, and a fresh 110 μl of the HEPES-monobromobimane mixture was added. The liquid fraction was then centrifuged at 12,000 × *g* for 5 min at 4°. A portion of the supernatant (10 μl) was removed for protein determination and the remainder was held 10 min in the dark at room temperature to allow the monobromobimane to react completely with thiols. Per 110 μl of supernatant, 100 μl of acetonitrile were added, the mixture was incubated at 60° for 10 min, and 300 μl of 25 mM methane sulfonic acid were then added. The samples were centrifuged at 12,000 × *g* at room temperature for 5 min to pellet precipitated protein, the supernatants were filtered through 0.45 μm PTFE membranes (VWR), and held at −80° until HPLC analysis on a Beckman C18 reversed phase column, #235329, (via Rainin Instrument Co., Inc., Ridgefield, NJ) using an integrator and gradient maker from OMS Tech (Miami, FL), and an Alltech 325 HPLC pump (Alltech Associates, Inc., Deerfield, IL).

Protein was measured by the Bradford method using ovalbumin as a standard (BRADFORD 1976).

Subcloning, plasmids, DNA sequencing, and PCR: Standard procedures for the manipulation of plasmid DNA and bacterial transformation were used (SAMBROOK *et al.* 1989). Subcloning involved YCplac and YEplac, centromeric and episomal vectors, respectively (GIETZ and SUGINO 1988), and pBluescript KS II (Stratagene, La Jolla, CA). pDR1 (BARRAL *et al.* 1995) was obtained from C. MANN. pRY181 (YOCUM *et al.* 1984), used to introduce a *GAL1-lacZ* fusion to monitor glucose derepression, and pBM2101, which contains a null allele of *GRR1* (FLICK and JOHNSTON 1991), were obtained from M. JOHNSTON. pDIS vectors were obtained from C. MARCIREAU (personal communication, 1994).

DNA was sequenced using the dideoxy dye terminator

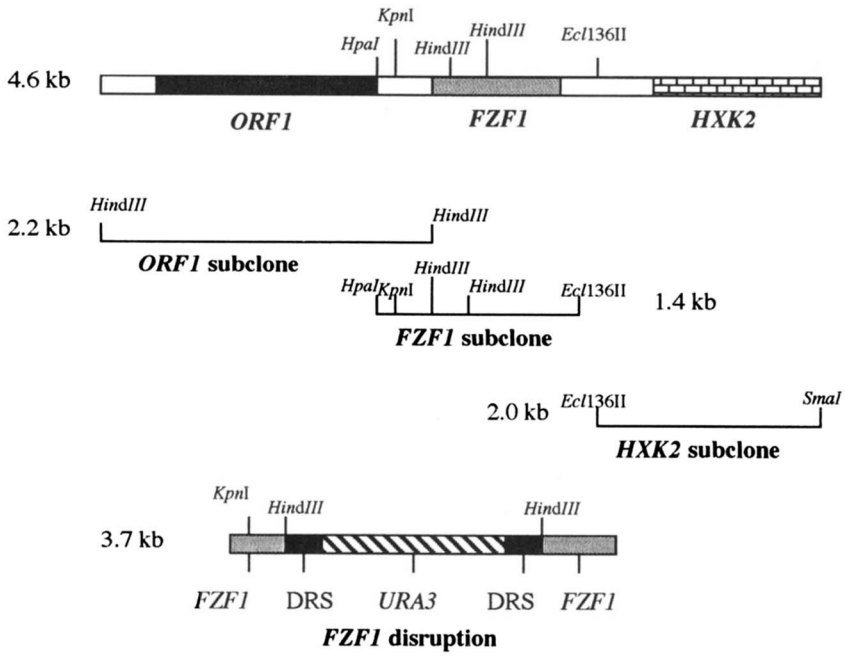

FIGURE 2.—Restriction map and subcloning of *FZF1*. Unshaded regions in the initial 4.6-kb clone refer to noncoding sequences of *ORF1*, *FZF1*, and *HXK2*. DRS in the 3.7-kb subclone refers to a direct repeated sequence from pDIS3. Shaded regions designated *FZF1* in this clone refer to both coding and adjacent noncoding *FZF1* sequences.

method on an ABI Model 373A sequencer (Applied Biosystems, Inc., Foster City, CA) at the Central Services Laboratory of the Oregon State University Center for Gene Research and Biotechnology.

Escherichia coli strain DH5α was used in all subcloning experiments (HANAHAN 1983).

PCR was performed using Taq polymerase (Promega, Madison, WA) in an Easycycler (Ericomp, Inc., New Haven, CT) or a Robocycler 40 (Stratagene, La Jolla, CA) thermal cycler.

Cloning of *SSU2*: *SSU2* was cloned by complementation of the sulfite sensitivity of mutant 3118-27d (*ssu2-6*) by transformation with a yeast genomic library in the centromeric vector p366 (F. SPENCER and P. HIETER, unpublished data). Transformants were selected on SM-leu and screened on YEPD containing 2 mM sulfite. One positive clone was isolated from 11,323 transformants. The complementing clone, 6-6-2, contained an insert of ~10 kb (Figure 1). Subclone BBC4 was constructed by deleting an internal 2.5-kb *Nru*I/*Sma*I fragment. Subclone BX1 was derived from BBC4 by deletion of a 1.6-kb *Bam*HI fragment. The remaining subclones were derived from BX1: Delta H3 as a 5.0-kb *Bam*HI/*Hin*dII fragment; Delta HP as a 4.0-kb fragment with a 1.9-kb *Hpa*I deletion spanning all three sites; SH1 as a 2.6-kb *Sac*I/*Hin*dIII fragment; and Delta BG as a fragment with a 3.6-kb *Bgl*II deletion. SH1 was subcloned into YCplac33 cut with *Sac*I and *Hin*dIII, and sequenced at both ends, using YCplac33 primers 5′TTAGGCACCCCAGGCTTTACACTTT3′ and 5′GCTGGCGAAAGGGGGATGTG3′.

Null alleles of *SSU2* were obtained by transforming 2757-4d-T4 and 3090-9d with *Bam*HI-digested pBM206 (FLICK and JOHNSTON 1991), which contained a *grr1::URA3* disruption to direct integration to the *GRR1* locus. The null allele was confirmed by PCR (SATHE *et al.* 1991) with *GRR1* primers 5′CGA[cchGATATTCAAGGCAGTTC3′ and 5′TACAGCACGCAAAGTCCAC3′, and 5′CTAAACTCACAAATTAGAGCTTC3′, a *URA3* primer.

Cloning of *FZF1*: A yeast genomic library in the episomal vector pGAD (CHIEN *et al.* 1991) was screened to isolate multicopy suppressors of the sulfite sensitivity of 3154-2b (*grr1::URA3*). Three positive clones were isolated from 3025 transformants plated on SM −leu, containing 1.5 mM sulfite. Two contained vector sequences only. The third contained an insert of ~5 kb that was shown not to be *GRR1* by PCR using the *GRR1* primers given above. The ends of the insert were sequenced using pGAD primers.

Subclones were constructed as follows (Figure 2): *ORF1* was subcloned as a 2.2-kb *Hin*dIII fragment in the *Hin*dIII site of YEplac195; *FZF1* was subcloned as a 1.4-kb *Hpa*I/*Ecl*136II fragment in the *Ecl*136II site of YEplac195; and *HXK2* was subcloned as a 2.0-kb *Ecl*136II/*Sma*I fragment in the *Ecl*136II site of YEplac195. The subclones were confirmed by restriction analysis and PCR, using *FZF1* primers: 5′GGACAATAAATACGCTAAAG3′ and 5′CACATGAGTAGAGGACGGAA3′.

Disruption of *FZF1*: *FZF1* was disrupted using the one-step gene replacement method (ROTHSTEIN 1983). The 1.4-kb *Hpa*I/*Ecl*136II *FZF1* fragment was subcloned into *Bam*HI/*Sal*I-digested Bluescript KS II that had been pretreated with Klenow polymerase. A *Hin*dIII fragment, encoding amino acids 99–182, was replaced by the disruption cassette of pDIS3, containing *URA3* flanked by two direct repeated sequences, DRS, (C. MARCIREAU, personal communication 1994) (Figure 2). The final construct was digested with *Kpn*I that cuts once in noncoding sequences adjacent to the 5′ end of *FZF1* and once in the vector within 30 bp of the 3′ end of *FZF1*. The digest was then used to transform 3090-9d (*GRR1 ura3*) and 3090-9d-T6-L1 (*grr1D6-1 ura3*) to yield 3090-9d-T10 (*fzf1D99-182::URA3*) and 3090-9d-T6-L1-T10 (*fzf1D:99-182:URA3 grr1D6-1*), respectively. Integration of the disruption construct at the *FZF1* locus was confirmed by PCR (SATHE *et al.* 1991) using the two *FZF1* primers and the *URA3* primer given above.

RESULTS

***SSU2* is identical to *GRR1*:** A sulfite-sensitive mutant, *ssu2-6*, was previously shown to have an elongated cell morphology, to excrete less acetaldehyde than wild type, and to have 50% of the wild-type level of glutathione (XU *et al.* 1994). The one positive clone obtained by transformation with DNA from a centromeric wild-type genomic library grew on selective media, YEPD containing 2 mM sulfite, and exhibited wild-type cell

Strain	Relevant genotype	Sulfite (mM) 0	0.5	0.75	1.0	1.5	2	2.5
3118-19c	*ssu2-6*	+	+	+	+	+/-	-	-
YM2957	*grr1Δ–1829*	+	+	+/-	-	-	-	-
YM3378	*grr1Δ-1829 rgt1-101*	+	+	+	+/-	-	-	-
YM3478	*rgt1-101*	+	+	+	+	+	-	-
3152	*ssu2-6/grr1Δ::URA3*	+	+	+	+	-	-	-
3090-9d-T6-L1	*grr1Δ6-1*	+	+	+	-	-	-	-
YM2061	*GRR1*	+	+	+	+	+	+/-	-
3090-9d	*GRR1*	+	+	+	+	+	+	-
3090-9d-T10	*fzf1Δ 99-182 GRR1*	+	+	+	+	-/+	-	-
3090-9d-T6-L1-T10	*grr1Δ6-1 fzf1Δ 99-182*	+	+	-/+	-	-	-	-

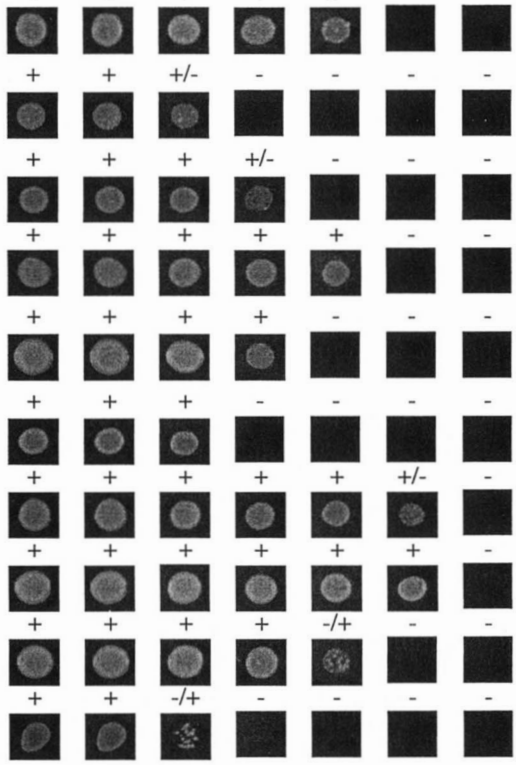

FIGURE 3.—Growth of *grr1* (*ssu2*) mutants on YEPD containing sulfite. Cells were grown overnight in liquid YEPD, washed once, and resuspended in distilled water to yield 2×10^9 cells/ml. Aliquots of 5 μl (10^7) cells were plated and scored after 2 days at 30°. Growth of strain YM 3502, not tabulated above, was identical to that of YM 2957. *rgt1-101* was originally isolated as a suppressor of *grr1* (ERICKSON and JOHNSTON 1994). +, normal growth; −, no growth; +/-, poorer than normal growth; -/+, very poor growth.

morphology. The transforming plasmid was rescued from yeast, amplified in *E. coli*, and subcloned (Figure 1). Subclone Delta H3, the smallest complementing fragment, was presumed to contain the intact gene. Three smaller subclones derived from Delta H3 (Delta BG, Delta HP, and SH1) failed to complement the sulfite sensitivity of *ssu2-6*, indicating that sequences necessary for function had been deleted. The ends of one of these, SH1, were partially sequenced. Analysis of 218 and 231 bp of noncontiguous terminal sequences, showed identity to sequences 412–629 and 2931–3161, respectively, of *GRR1* (FLICK and JOHNSTON 1991). Once *GRR1* sequences were localized, we were able to determine that subclone Delta H3 contained the intact *GRR1* open reading frame and a promoterless 1.2-kb carboxyl terminal fragment of ORF 089W (GALIBERT *et al.* 1995).

To confirm that *SSU2* was identical to *GRR1*, 3118-19c (*ssu2-6*) was crossed with YM3502 (*grr1Δ::URA3*) to yield the diploid 3152, which was found to be sensitive to sulfite indicating noncomplementation (Figure 3). To establish allelism, 24 tetrads were dissected of which 12 produced four viable spores (overall spore viability was 85%). All progeny in these 12 tetrads were tested and shown to be sensitive to sulfite, confirming that *ssu2-6* is an allele of *GRR1*. Mutant *ssu2-6* was also found to exhibit glucose derepression, a key phenotype of *grr1* strains, as measured in a *GAL1-lacZ* fusion construct (strain 3150-14 in Table 2).

***grr1* mutants are sensitive to sulfite and are partially defective in hydrogen sulfide formation:** A number of *grr1* mutants, isolated or constructed on the basis of their glucose derepression phenotype (FLICK and JOHNSTON 1991), were tested for sensitivity to sulfite. All were found to be sensitive, with the null mutants YM3502 and YM2957 exhibiting the greatest sensitivity (Figure 3). A *grr1* null mutant was constructed in the same genetic background in which the *ssu2-6* allele had originally been isolated by transforming strain 3090-9d with *Bam*HI-digested pBM206. The null mutant obtained, 3090-9d-T6-L1, was found to be sulfite-sensitive (Figure 3).

Because an inability to reduce sulfite to hydrogen sulfide via the reductive sulfate assimilation pathway would explain potential accumulation of the com-

TABLE 2

Expression of β-galactosidase in *GAL1-lacZ* fusion strains grown under inducing or repressing conditions

Strain	Relevant genotype	2% gal	2% gal + 2% glu	Level of repression (gal/gal + glu)
3150-14	*ssu2-6 LEU2::GAL1-lacZ*	637 ± 42	145 ± 10	4.4
2757-4d-4T	*SSU2 LEU2::GAL1-lacZ*	592 ± 55	2.8 ± 0.5	211
YM3502-T1	*grr1ΔLEU2::GAL1-lacZ/YEplac195*	682 ± 39	277 ± 12.8	2.5
YM3502-T2	*grr1ΔLEU2::GAL1-lacZ/YEplac195::FZF1*	768 ± 44	361 ± 4.2	2.1

Values are means ± SD for three experiments. β-galactosidase activity is expressed as OD_{420} normalized to cell number (KIPPERT 1995).

pound and sensitivity to exogenous sulfite, a qualitative estimate of intracellular hydrogen sulfide was made on BiGGY agar. This medium allows a qualitative, visual estimate of hydrogen sulfide formation, an obligate intermediate in methionine biosynthesis, by its intracellular precipitation as brown bismuth sulfide. Relative to wild type, the *grr1Δ* mutants 3090-9d-T6 and 3154-2b exhibited a weak ability to form hydrogen sulfide *in vivo* on BiGGY agar (data not shown). The fact that *grr1* mutants are not methionine auxotrophs indicates that enzymatic formation of hydrogen sulfide by sulfite reductase is operative, but apparently not at wild-type levels. Measurement of intracellular hydrogen sulfide by reaction with monobromobimane confirmed that significantly less ($P = 0.05$, Student's *t*-test) was formed in a *grr1Δ* mutant than in a *GRR1* strain: 0.61 ± 0.22 *vs.* 2.56 ± 0.67 pmoles/μg protein, respectively ($n = 3$). Free sulfite could not be detected in either strain.

The sulfite-sensitivity caused by *grr1* and partially suppressed by *rgt1* is independent of carbon source: *grr1* mutants grow poorly on glucose as a result of reduced glucose uptake caused by a significant decrease in expression of genes encoding hexose transporters (ÖZCAN *et al.* 1994; ÖZCAN and JOHNSTON 1995). The Grr1 protein is thought to be a negative regulator of the product of the *RGT1* gene, which itself negatively regulates glucose transporters (ERICKSON and JOHNSTON 1994). Mutations in *RGT1* suppress the slow growth of *grr1* mutants on glucose (ERICKSON and JOHNSTON 1994). To determine if the sulfite sensitivity of *grr1* mutants was related to the defect in transport and resultant slow growth on glucose, *grr1* mutants and a *grr1 rgt1* double mutant (YM3378) were tested for sensitivity during growth on glucose and nonglucose carbon sources. On glucose, the double mutant was more sulfite-tolerant than the *grr1* mutant, poor growth on 1.0 mM sulfite relative to no growth by the *grr1* strain, although wild-type tolerance was not observed, indicating partial suppression of the sensitivity (Figure 3). On nonglucose carbon sources, where the absolute sulfite levels tolerated varied slightly, *grr1* strains were consistently sensitive relative to wildtype (Table 3). While the *rgt1* strain by itself exhibited mild sensitivity to sulfite when grown on glucose (Figure 3), and greater sensitivity on acetate and maltose, it partially suppressed the sensitivity of the *grr1* mutant on all the nonglucose carbon sources tested: galactose, maltose, glycerol, ethanol, and acetate (Table 3). Taken together, these data indicate that the sulfite sensitivity of *grr1* is not strictly linked to the growth defect on glucose and that *rgt1* is mediating suppression through a route likely unrelated to its role as a negative regulator of glucose transporters.

An interesting observation was made relative to *RGT1* that is clearly unrelated to sulfite. In the absence of added sulfite, the *rgt1* mutant was found to grow on acetate, to grow very poorly on ethanol, and not to grow at all on glycerol. However, the *rgt1 grr1* double mutant grew on glycerol and grew well on ethanol, indicating that *grr1* was able to suppress the *rgt1*-specific growth defect (Table 3).

The acetate plates (YEPac) were buffered at pH 4.8 rather than at 3.5 to avoid a precipitate that formed at the lower pH. At pH 4.8, wild type was expected to tolerate an ~20-fold greater concentration than in the medium buffered at pH 3.5 for the following reasons. (1) The concentration of the toxic form of sulfite, H_2SO_3, is pH-dependent and is 20 times lower at pH 4.8 than at 3.5 ($pK_{a_1} = 1.77$). (2) Acetate is not known to react with and detoxify sulfite. The observation that wild type failed to grow in the presence of significantly higher total sulfite in acetate suggests significantly greater sensitivity under these conditions. This conclusion assumes that H_2SO_3 is the only species that freely traverses the cell membrane (STRATFORD *et al.* 1987). Acetaldehyde is not an obligate intermediate of acetate catabolism, whereas it is likely formed during metabolism of the other carbon sources tested (FRAENKEL 1982). These results suggest that the detoxification reaction that occurs nonenzymatically between sulfite and acetaldehyde, and that does not occur during growth on acetate, is an important mechanism in mediating the basal sulfite tolerance of Saccharomyces.

Overexpression of *CLN1* causes a *GRR1* strain to become sulfite-sensitive: BARRAL *et al.* showed that in *grr1* strains, Cln1 and Cln2 proteins are stabilized and speculated that this might promote the characteristic change in cell morphology, from round to elongate (BARRAL *et al.* 1995). They also showed that overexpression of *CLN1* in a *GRR1* background induced a similar change in cell shape. To determine if overexpression of *CLN1*

TABLE 3

Growth of *grr1* mutants on different carbon sources containing sulfite

Medium[b]	Strains[a]				
	GRR1	*grr1Δ-1829*	*grr1Δ-1829 rgt1-101*	*rgt1-101*	*ssu2-6*
YEPgal					
0 mM sulfite	+	+	+	+	+
0.125 mM sulfite	+	+	+	+	+
0.25 mM sulfite	+	+	+	+	+
0.5 mM sulfite	+	−	+	+	−
0.75 mM sulfite	+	−	−	−	−
1.0 mM sulfite	−	−	−	−	−
YEPmal					
0 mM sulfite	+	+	+	+	+
0.125 mM sulfite	+	+	+	+	+
0.25 mM sulfite	+	+	+	+/−	+
0.5 mM sulfite	+	−	+	−	−/+
0.75 mM sulfite	+	−	−	−	−
1.0 mM sulfite	−	−	−	−	−
YEPE					
0 mM sulfite	+	+	+	−/+	+
0.125 mM sulfite	+	+	+	−	+
0.25 mM sulfite	+	+	+	−	+
0.5 mM sulfite	+	−	+	−	+/−
0.75 mM sulfite	+	−	−	−	−
1.0 mM sulfite	−	−	−	−	−
YEPgly					
0 mM sulfite	+	+	+	−	+
0.125 mM sulfite	+	+	+	−	+
0.25 mM sulfite	+	−	+	−	−
0.5 mM sulfite	+	−	−	−	−
0.75 mM sulfite	−	−	−	−	−
YEPac					
0 mM sulfite	+	+	+	+	+
0.125 mM sulfite	+	+	+	+	+
0.25 mM sulfite	+	+	+	+	+
0.5 mM sulfite	+	+	+	+	+
0.75 mM sulfite	+	−	+	−	+/−
1.0 mM sulfite	+	−	+	−	−/+
1.5 mM sulfite	+	−	−	−	−
2.0 mM sulfite	−	−	−	−	−

+, normal growth; −, no growth; +/−, poorer growth than normal; −/+, very poor growth. Refer to Figure 3 for a visual indication of these scores.

[a] Growth of strain *grr1Δ*, not tabulated above, was identification to that of *grr1Δ-1829*.

[b] YEPac plates were buffered at pH 4.8, all others at 3.5.

in a *GRR1* strain would cause sulfite sensitivity, 2757-4d-T4 was transformed with a vector containing the *CLN1* gene under the control of the *GAL10* promoter (pDR1) and grown on galactose. Transformants overexpressing *CLN1* became twice as sensitive to sulfite as wild type, suggesting a connection between the sensitive phenotype and *CLN1* expression (Table 4). Expression of *GAL10*-driven *CLN1* in a *grr1* strain resulted in such so slow growth that assessment of sulfite sensitivity was difficult.

Multicopy *FZF1/SUL1* suppresses the sulfite sensitivity, but not the glucose derepression or aberrant cell morphology of a *grr1* mutant: To identify proteins that might be functionally related to Grr1, multicopy genes were sought that would suppress the sulfite sensitivity of a *grr1* mutant. A null *grr1* mutant was transformed with DNA from a multicopy genomic library and a single clone containing an insert of ~4.6 kb was identified. The ends of the suppressing DNA were sequenced and indicated identity with sequences 1292–1592 of *HXK2* and sequences 109–409 upstream of *ORF1*. The intact DNA contained a part of the *HXK2* gene (lacking the carboxy terminal end), the entire *FZF1* ORF, identified on the basis of restriction analysis and PCR using *FZF1*-specific primers (BREITWIESER *et al.* 1993), and an open reading frame whose identity is unknown, *ORF1* (Figure 2).

The three fragments were subcloned separately in YEplac195 and tested for suppressing activity. *FZF1*, en-

TABLE 4

Growth of *GRR1* cells carrying multicopy *CLN1*

Relevant genotype	Sulfite (mM)				
	0	0.5	1	1.5	2.0
GRR1	+	+	+	−	−
GRR1, GAL10-driven *CLN1*, YEplac195 (vector alone)	+	−	−	−	−
GRR1, GAL10-driven *CLN1*, YEplac 195::FZF1	+	+	+	−	−

+, growth; −, no growth. Refer to Figure 3 for a visual indication of these scores. The parental strain (*GRR1*) is 2757-4d-T4. *GAL10*-driven *CLN1* was introduced by transformation with plasmid pDR1, and *FZF1* was introduced on plasmid YEplac195::FZF1. All strains were grown on galactose-based SM plates buffered at pH 3.5, under selective conditions (no uracil or leucine) if necessary to maintain plasmids. The apparent increased sensitivity to sulfite on galactose relative to glucose (compare with *GRR1* grown on YEPD, Figure 3) is an artifact due to a lack of a chemical detoxification reaction between the added sulfite and galactose. In glucose-based media, a portion of the added sulfite is always quenched in a chemical reaction with glucose.

coding a putative transcription factor containing five zinc fingers, was the only DNA to suppress the sulfite sensitivity of the *grr1* mutant. Others have shown that substitution of glutamic acid for histidine in position 180 of the Fzf1 protein conferred a sulfite-resistant phenotype (Casalone *et al.* 1994). *FZF1* failed to suppress the aberrant cell morphology and weak ability to form hydrogen sulfide (data not shown). Furthermore, *FZF1* did not suppress the glucose derepression phenotype of *grr1*. A null mutant of *grr1* carrying an integrated *GAL1-lacZ* fusion construct (YM3502) transformed with multicopy *FZF1* (TM3502-T2) or vector alone (YM3502-T1) exhibited the same level of derepression as a *grr1* strain (*ssu2-6*) (Table 2).

Sulfite sensitivity caused by mutations not allelic to *grr1* and by *CLN1* overexpression is suppressed by multicopy *FZF1*: To determine if multicopy *FZF1*-mediated suppression of sulfite sensitivity was specific to *grr1*, a number of other sulfite-sensitive mutants were examined. The growth of the strains in the presence and absence of multicopy *FZF1* as a function of sulfite concentration is shown in Table 5. *ssu1*, *ssu3*, and *ssu4* were previously isolated on the basis of their sulfite sensitivity (Xu *et al.* 1994). *SSU1* has since been cloned and sequenced and does not share significant similarity with any other gene in public data banks, GenBank accession number U20254 (D. Avram and A. T. Bakalinsky, unpublished data). The sulfite sensitivity of *ssu1* and *met20* was not suppressed by multicopy *FZF1*. However, the sensitivity of *ssu4* and *met18* was suppressed and that of *ssu3* was partially suppressed. The petite character of *ssu4*, which cosegregates with its sulfite sensitivity, was not suppressed. *met20* mutants are defective, and *met18* mutants are partially defective in sulfite reductase, and both accumulate sulfite intracellularly (Thomas *et al.* 1992). Interestingly, multicopy *FZF1* in a *GRR1* strain caused a modest increase in its sulfite tolerance (3090-9d in Table 5). As noted above, overexpression of *CLN1* in a *GRR1* strain was found to cause sulfite sensitivity (Table 4). Transformation of such a strain (2757-4d-T4/pDR1) with multicopy *FZF1* suppressed the sensitive phenotype.

Disruption of *FZF1* causes sulfite sensitivity in a *GRR1* strain: A deletion allele of *FZF1* was constructed by replacing sequences corresponding to amino acids 99–182, which includes the fourth zinc finger and most of

TABLE 5

Suppression of sulfite sensitivity by *FZF1* in high copy number

Strain	Relevant mutation	Sulfite concentration (mM)				
		0	1	1.5	2	2.5
3090-9d	Wild type	+(+)	+(+)	+(+)	+(+)	+/−(−)
YM3502	*grr1*	+(+)	+(−)	+(−)	+/−(−)	−(−)
3090-9d-T4	*ssu1*	+(+)	−(−)	−(−)	−(−)	−(−)
3089-1d	*ssu3*	+(+)	+(−)	+(−)	−(−)	−(−)
3100-5b	*ssu4*	+(+)	+(−/+)	+(−)	+/−(−)	−(−)
CC370-8C	*met20*	+(+)	+(+)	−(−)	−(−)	−(−)
CC363-20B	*met18*	+(+)	+(−)	+(−)	+(−)	−(−)

+, normal growth; −, no growth, +/−, poorer growth than normal; −/+, very poor growth. Refer to Figure 3 for a visual indication of these scores. The values in parentheses indicate the level of tolerance of strains not carrying multicopy *FZF1* and are preceeded by values for the same strains transformed with the multicopy *FZF1* construct. Cells were grown on SM-ura buffered at pH 3.5. Strains not carrying multicopy *FZF1* carried the vector alone, YEplac195.

the bipartite motif, with the disruption cassette from pDIS3, and integrating the construct at the *FZF1* locus into a *GRR1* strain (Figure 2). The disruptant strain, 3090-9d-T10, exhibited sensitivity to sulfite, whereas in a *grr1* background (strain 3090-9d-T6-L1-T10), the disruption caused significantly greater sensitivity (Figure 3).

DISCUSSION

A mutation causing sulfite sensitivity, *ssu2-6*, was identified as an allele of *GRR1*. *grr1* mutants were also found to be partially deficient in the formation of hydrogen sulfide, but not to the extent that methionine auxotrophy was evident. Sulfite sensitivity was observed during growth on glucose, galactose, maltose, acetate, ethanol, and glycerol, indicating that it is not strictly associated with a defect in glucose metabolism. The suppressor *rgt1*, which restores the transport and derepression defects of *grr1* mutants on glucose, was found to partially suppress the sulfite-sensitive phenotype on glucose and other carbon sources. Overexpression of *CLN1* in *GRR1* cells resulted in sulfite sensitivity, and an elongated cell morphology characteristic of *grr1* strains. Multicopy *FZF1*, encoding a putative zinc finger protein, was found to suppress the sulfite sensitive phenotype of *grr1* strains, but not the glucose derepression, aberrant cell morphology, or partial deficiency in hydrogen sulfide formation. Multicopy *FZF1* was also found to suppress the sensitivity of a number of unrelated sulfite-sensitive mutants: a *CLN1*-overexpressing strain, *ssu3*, *ssu4*, and *met18*, but not that of *ssu1* or *met20*. Disruption of *FZF1* resulted in sulfite sensitivity when the construct was introduced in single copy at the *FZF1* locus in a *GRR1* strain.

Different functions have been attributed to Grr1, a weakly expressed, putative regulatory protein of 135 kDa: involvement in glucose repression (BAILEY and WOODWARD 1984; FLICK and JOHNSTON 1991), positive regulation of glucose transport (ÖZCAN *et al.* 1994; VALLIER *et al.* 1994; ÖZCAN and JOHNSTON 1995), *SUC2* gene expression (VALLIER and CARLSON 1991), regulation of divalent cation transport (CONKLIN *et al.* 1993), involvement in turnover of G_1 cyclins (BARRAL *et al.* 1995), and suppression of *bem2* mutations (KIM *et al.* 1994). *grr1* mutants grow slowly on glucose apparently due to impaired high affinity glucose uptake (VALLIER *et al.* 1994). The sulfite sensitivity of *grr1* may, in part, be related to this defect. XU *et al.* (1994) previously found that a *grr1* (*ssu2-6*) mutant excreted a reduced amount of acetaldehyde relative to wild type during growth on glucose. Because acetaldehyde can react with and detoxify sulfite, it is reasonable that the mutant has a reduced capacity to tolerate added sulfite. CASALONE *et al.* (1992) reported that a sulfite-resistant mutant excreted more acetaldehyde than wild type. The sulfite sensitivity of *grr1* mutants is likely due in part to distinct consequences of defective glucose uptake. One is slow growth on glucose leading to the reduction in acetaldehyde production. The other is a possible impairment in a glucose-dependent process, such as transport, that may slow or abolish a hypothetical sulfite-efflux pump, or other sulfite-detoxifying pathway. However, the observation that *grr1* mutants are sensitive to sulfite on carbon sources other than glucose, requiring other transporters (*i.e.*, maltose) (LAGUNAS 1993), or on which growth defects are restored (galactose) (FLICK and JOHNSTON 1991), or on nonfermentable carbon sources (glycerol and ethanol), indicates that sensitivity is not only caused by growth defects on glucose. The poor growth of *grr1* mutants on glucose is suppressed by a mutation in *RGT1* (ERICKSON and JOHNSTON 1994). The Rgt1 protein is thought to be a negative regulator of glucose transporters and has been proposed to be negatively regulated by Grr1 (ERICKSON and JOHNSTON 1994). A slight suppression of sulfite sensitivity was observed in the double mutant *grr1 rgt1*, not only on glucose but on all other carbon sources tested, providing further indirect evidence that the defect in glucose transport cannot account entirely for the sensitive phenotype. These data together with the observation that *grr1* was able to suppress the poor growth and lack of growth of an *rgt1* mutant on ethanol and glycerol, respectively, suggest that *RGT1* may interact with *GRR1* in functions unrelated to glucose metabolism.

grr1 mutants were found to produce a reduced amount of hydrogen sulfide, but were not methionine auxotrophs, indicating a functional sulfite reductase. One explanation is a defective sulfite reductase that produces an adequate but limiting amount of hydrogen sulfide sufficient to avoid methionine auxotrophy, but insufficient to form a prominent precipitate with the bismuth indicator on BiGGY agar. It is also possible that flux through the reductive sulfate assimilation pathway is slowed in *grr1* strains, so that intermediates such as hydrogen sulfide do not accumulate significantly.

BARRAL *et al.* (1995) showed that in *grr1* mutants, Cln1 and Cln2 proteins are stabilized and suggested that this might cause the change in cell morphology. When *CLN1* was overexpressed in a *GRR1* strain, cells became sensitive to sulfite and also acquired the elongated morphology characteristic of *grr1* mutants. These workers postulated that in a *grr1* mutant, the high level of Cln1 and Cln2 proteins would advance the time at which START is executed. The execution of START results in the expression of functions required for DNA replication. Among these, the reduction of ribonucleotides to deoxyribonucleotides requires NADPH as does sulfite reduction to sulfide. Premature execution of START may result in a deficit of NADPH, inadequate for reduction of exogenous sulfite, leading to the observed sensitivity.

We found that multicopy *FZF1*, encoding a putative transcription factor (BREITWIESER *et al.* 1993), suppressed the sulfite sensitivity of *grr1* mutants, but not

the aberrant cell morphology, glucose derepression, or partial deficiency in hydrogen sulfide formation. CASALONE *et al.* (1994) found that replacement of histidine with glutamic acid in position 180 of Fzf1 resulted in a dominant sulfite-resistant phenotype, characterized by a reduced accumulation of sulfite (CASALONE *et al.* 1992), consistent with diminished uptake or enhanced efflux. The dominant nature of the resistant phenotype suggests possible hyperactivation. BREITWIESER *et al.* (1993), who cloned *FZF1* in a search for cell-cycle-regulated genes having a five zinc finger motif, proposed that histidine in position 179 is the second histidine involved in tetrahedral coordination of the zinc atom. PAVLETICH and PABO (1993) suggested that the fourth zinc finger of GLI is important for interactions with DNA. Our finding that disruption of *FZF1* resulted in sensitivity to sulfite suggests that the Fzf1 protein plays a role in sulfite metabolism. The observation that multicopy *FZF1* suppressed the sulfite sensitivity of several sensitive mutants but not that of *ssu1* or *met20* is consistent with a role as a positive regulator of Ssu1p and Met20p. The function of Ssu1p is presently unknown but is the subject of investigation in this laboratory. However, Met20p is required for sulfite reductase activity, and increased sulfite reduction is a reasonable route through which exogenous sulfite can be detoxified.

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LITERATURE CITED

BAILEY, R. B., and A. WOODWARD, 1984 Isolation and characterization of a pleiotropic glucose repression resistant mutant of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **193:** 507–512.

BARRAL, Y., S. JENTSCH and C. MANN, 1995 G1 cyclin turnover and nutrient uptake are controlled by a common pathway in yeast. Genes Dev. **9:** 402–410.

BOEKE, J. D., F. LACROUTE and G. R. FINK, 1984 A positive selection for mutants lacking orotidine-5′-phosphate-decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:** 345–346.

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

BREITWIESER, W., C. PRICE and T. SCHUSTER, 1993 Identification of a gene encoding a novel zinc finger protein in *Saccharomyces cerevisiae*. Yeast **9:** 551–556.

CASALONE, E., C. M. COLELLA, S. DALY, E. GALLORI, L. MORIANI *et al.*, 1992 Mechanism of resistance to sulfite in *Saccharomyces cerevisiae*. Curr. Genet. **22:** 435–440.

CASALONE, E., C. M. COLELLA, S. DALY, S. FONTANA, I. TORRICELLI and M. POLSINELLI, 1994 Cloning and characterization of a sulfite-resistance gene of *Saccharomyces cerevisiae*. Yeast **10:** 1101–1110.

CHEREST, H., and Y. SURDIN-KERJAN, 1992 Genetic analysis of a new mutation conferring cysteine auxotrophy in *Saccharomyces cerevisiae*: updating of the sulfur-metabolism pathway. Genetics **130:** 51–58.

CHIEN, C.-T., P. L. BARTEL, R. STERNGLANZ and S. FIELDS, 1991 The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:** 9578–9582.

CONKLIN, D. S., C. KUNG and M. R. CULBERTSON, 1993 The *COT2* gene is required for glucose-dependent divalent cation transport in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:** 2041–2049.

ERICKSON, J. R., and M. JOHNSTON, 1994 Suppressors reveal two classes of glucose repression genes in the yeast *Saccharomyces cerevisiae*. Genetics **136:** 1271–1278.

FAHEY, R., and G.L. NEWTON, 1987 Determination of low-molecular weight thiols using monobromobimane fluorescent labeling and high-perfomance liquid chromatography. Methods Enzymol. **143:** 85–96.

FLICK, J. S., and M. JOHNSTON, 1991 *GRR1* of *Saccharomyces cerevisiae* is required for glucose repression and encodes a protein with leucine-rich repeats. Mol. Cell. Biol. **11:** 5101–5112.

FRAENKEL, D. G., 1982 Carbohydrate metabolism, pp. 1–37 in *The Molecular Biology of the Yeast Saccharomyces, Metabolism and Gene Expression*, edited by J. N. STRATHERN, E. W. JONES, and J. R. BROACH. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

GALIBERT, F., D. ALEXANDRAKI, A. BAUR, E. BOLES, N. CHALWATZIS *et al.*, 1995 The complete sequence of chromosome X of the yeast *Saccharomyces cerevisiae*. Martinsried Institute for protein sequences (MIPS).

GIETZ, D., and A. SUGINO, 1988 New yeast-*Escherichia coli* shuttle vectors constucted with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene **74:** 527–534.

GIETZ, D., A. ST. JEAN, R. A. WOODS and R. H. SCHIELSTL, 1992 Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:** 1425–1426.

HANAHAN, D., 1983 Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:** 557–580.

HUXTABLE, R. J., 1986 *Biochemistry of Sulfur*. Plenum Press, New York.

KIM, Y. J., L. FRANCISCO, G. C. CHEN and C. S. CHAN, 1994 Control of cellular morphogenesis by the Ip12/Bem2 GTPase-activating protein: possible role of protein phosphorylation. J. Cell. Biol. **127:** 1381–1394.

KING, A. D., J. D. PONTING, D. W. SANSHUCK, R. JACKSON and K. MIHARA, 1981 Factors affecting death of yeast by sulfur dioxide. J. Food Prot. **44:** 92–97.

KIPPERT, F., 1995 A rapid permeabilization procedure for accurate quantitative determination of β-galactosidase activity in yeast cells. FEMS Microbiol. Lett. **128:** 201–206.

LAGUNAS, R., 1993 Sugar transport in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. **104:** 229–242.

ÖZCAN, S., and M. JOHNSTON, 1995 Three different regulatory mechanisms enable yeast hexose transporter (*HXT*) genes to be induced by different levels of glucose. Mol. Cell. Biol. **15:** 1564–1572.

ÖZCAN, S., F. SCHULTE, K. FREIDEL, A. WEBER and M. CIRIACY, 1994 Glucose uptake and metabolism in *grr1/cat80* mutants of *Saccharomyces cerevisiae*. Eur. J. Biochem. **224:** 605–611.

PAVLETICH, N. P., and C. O. PABO, 1993 Crystal structure of a five-finger GLI-DNA complex: new perspectives on zinc-fingers. Science **261:** 1701–1707.

ROSE, M. D., F. WINSTON and P. HIETER, 1990 *Methods in Yeast Genetics, a Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

ROTHSTEIN, R., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

SATHE G. M., S. O'BRIEN, M. M. MCLAUGHLIN, F. WATSON and G. P. LIVI, 1991 Use of polymerase chain reaction for rapid detection of gene insertions in whole yeast cells. Nucleic Acids Res. **19:** 4775.

STRATFORD, M., P. MORGAN and A. H. ROSE, 1987 Sulphur dioxide resistance in *Saccharomyces cerevisiae* and *Saccharomycodes ludwigii*. J. Gen. Micro. **133:** 2173–2179.

THOMAS, D., R. BARBEY, D. HENRY and Y. SURDIN-KERJAN, 1992 Physiological analysis of mutants of *Saccharomyces cerevisiae* impaired in sulfate assimilation. J. Gen. Micro. **138:** 2021–2028.

VALLIER, L. G., and M. CARLSON, 1991 New *SNF* genes, *GAL11* and *GRR1* affect *SUC2* expression in *Saccharomyces cerevisiae.* Genetics **129:** 675–684.

VALLIER L. G., D. COONS, L. F. BISSON and M. CARLSON, 1994 Altered regulatory responses to glucose are associated with a glucose transport defect in *grr1* mutants of *Saccharomyces cerevisiae.* Genetics **136:** 1279–1285.

VETTER, R. D., P. A. MATRAI, B. JAVOR and J. O'BRIEN, 1989 Reduced sulfur compounds in the marine environment: analysis by high-performance liquid chromatography, pp. 243–261 in *Biogenic Sulfur in the Environment,* edited by E. S. SALTZMAN and W. J. COOPER. American Chemical Society symposium series 393, American Chemical Society, Washington, D.C.

XU, X., J. D. WIGHTMAN, B. L. GELLER, D. AVRAM and A. T. BAKALINSKY, 1994 Isolation and characterization of sulfite mutants of *Saccharomyces cerevisiae.* Curr. Genet. **25:** 488–496.

YOCUM, R. R., S. HANLEY, R. WEST, JR., and M. PTASHNE, 1984 Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **4:** 1985–1998.

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