Genetic and Molecular Characterization of *GAL83*: Its Interaction and Similarities With Other Genes Involved in Glucose Repression in *Saccharomyces cerevisiae*

James R. Erickson and Mark Johnston

Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

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ABSTRACT

Expression of the *GAL* genes of *Saccharomyces cerevisiae* is subject to glucose repression, a global regulatory mechanism that requires several gene products. We have isolated *GAL83*, one of these genes required for glucose repression. The sequence of the predicted Gal83 protein is homologous to two other yeast proteins, Sip1p and Sip2p, which are known to interact with the *SNF1* gene product, a protein kinase required for expression of the *GAL* genes. High-copy clones of *SIP1* and *SIP2* cross-complement the *GAL83-2000* mutation (as well as *GAL82-1*, a mutation in another gene involved in glucose repression), suggesting that these four genes may perform similar functions in glucose repression. Consistent with this hypothesis, a *gal83* null mutation does not affect glucose repression, and only dominant or partially dominant mutations exist in *GAL83* (and *GAL82*). Two other observations were made that suggests that *GAL83* functions interdependently with *GAL82* and *REG1* (another gene involved in glucose repression) to effect glucose repression: 1) *REG1* on a low-copy plasmid cross-complements *GAL82-1* and *GAL83-2000* mutations, and 2) all pairwise combinations of *reg1*, *GAL82-1* and *GAL83-2000* fail to complement one another. Such unlinked noncomplementation suggests that Gal83p, Gal82p and Reg1p may interact with one another. Possible roles for *GAL83*, *GAL82* and *REG1* are discussed in relation to *SNF1*, *SIP1* and *SIP2*.

THE expression of many genes involved in utilization of alternate carbon sources, such as galactose, sucrose and maltose, is repressed in yeast growing on glucose. This global regulatory mechanism, referred to as glucose repression, causes cells to use glucose preferentially when presented with a variety of carbon sources.

Studies of the *GAL* genes have revealed that glucose repression occurs by three mechanisms. First, glucose reduces the level and function of the inducer of the *GAL* genes by repressing expression the *GAL2* [encoding the galactose permease (TSCHOPP *et al.* 1986)] and *GAL3* [encoding a protein required for inducer function (TORCHIA and HOPPER 1986)]. The lower functional inducer levels leads to increased activity of Gal80p, the inhibitor of Gal4p function, thereby lowering *GAL* gene expression. Because all strains used in this study are deleted for *GAL80*, we do not need to consider this mode of regulation in the experiments described here. Second, expression of *GAL4*, which encodes the positive activator of *GAL* gene expression, is transcriptionally repressed about fivefold in cells growing on glucose (GRIGGS and JOHNSTON 1991; NEHLIN, CARLBERG and RONNE, 1991; LAMPHIER and PTASHNE 1992). This modest reduction in Gal4p levels results in approximately a 50-fold reduction in expression of *GAL1* (GRIGGS and JOHNSTON 1991) because several Gal4ps activate *GAL* gene transcription cooperatively (GINIGER and PTASHNE 1988). Third, glucose repression acts directly on the promoter of the *GAL1* gene at sites located between the Gal4p binding sites (UAS_{GAL}) and the TATA box (FLICK and JOHNSTON 1991a, 1992). A key component of the latter two mechanisms of repression is Mig1p, a DNA binding protein that binds to the *GAL1* and *GAL4* promoters (NEHLIN and RONNE 1990; NEHLIN, CARLBERG and RONNE 1991). Neither the nature of the signal for glucose repression nor the mechanism by which it is transduced to Mig1p is known.

Several genes are required for glucose repression of the *GAL* genes. In addition to *MIG1*, these genes include *HXK2*, *GRR1*, *REG1*, *GAL82*, *GAL83*, *TUP1* and *SSN6* (NEHLIN and RONNE 1990; ZIMMERMAN and SCHEEL 1977; BAILEY and WOODWARD 1984; MATSUMOTO, Yoshimatsu and OSHIMA 1983; WICKNER 1974; NEIGEBORN and CARLSON 1987, reviewed in TRUMBLY 1992; GANCEDO 1992; JOHNSTON and CARLSON 1993). *SSN6* and *TUP1* encode proteins that probably associate with Mig1p to form the actual repressor complex (KELEHER *et al.* 1992). *HXK2* encodes a hexokinase, but its role in glucose repression is unclear (ENTIAN and FROLICH 1984; MA and BOTSTEIN 1986; WALSH *et al.* 1991). *REG1* and *GRR1* have been cloned (NIEDERACHER and ENTIAN 1987, 1991; FLICK and JOHNSTON 1991b), but no biochemical functions have been assigned to them. Analysis of

these genes will be essential for understanding the mechanism of glucose repression.

GAL82 and *GAL83* are unusual because mutations in these genes appear to affect glucose repression of the *GAL* genes, but not other glucose repressed genes (MATSUMOTO, Toh-E and OSHIMA 1981; MATSUMOTO, YOSHIMATSU and OSHIMA 1983) and all existing alleles of these genes are dominant or partially dominant. Because isolation of *GAL83* by conventional procedures proved difficult, we developed a method to recover DNA inserts from genomic lambda clones by homologous recombination with yeast centromere plasmids (ERICKSON and JOHNSTON 1993). By testing recombinant lambda phage clones from the region of chromosome V to which *GAL83* maps (RILES *et al.* 1993) for their ability to complement the partially dominant *GAL83-2000* mutation, a single lambda clone that contained the *GAL83* gene was identified.

We report here our analysis of *GAL83*. The predicted sequence of Gal83p revealed that it is similar to Sip1p and Sip2p, two proteins that are known to interact physically with the *SNF1* gene product (YANG, ALBERT-HUBBARD and CARLSON 1992; X. YANG, R. JIANG and M. CARLSON, manuscript submitted for publication). Our results suggest that the function of Gal83p may be redundant with Sip1p and Sip2p, and that Gal83p functionally interacts with Reg1p and Gal83p.

MATERIALS AND METHODS

Strains and growth medium: The yeast strains used are listed in Table 1. Most strains carrying glucose repression mutants are isogenic. The *GAL83-2000* mutant obtained from Oshima (MATSUMOTO, Yoshimatsu and OSHIMA 1983) was backcrossed to our wild-type strain twice to yield YM3033. Yeast were grown on standard YEP or SD synthetic medium (ROSE, WINSTON and HIETER 1990). 2-deoxy-D-galactose (2dGal; PLATT 1984) was added to 0.5% to score the expression of the *GAL1* gene on 2% glucose plates. Yeast was made competent for transformation with lithium acetate (ITO *et al.* 1983). To determine the phenotype of *snf1* mutants, strains were grown anaerobically in a gas pack (BBL) on YP + 2% sucrose or 2% raffinose. *Eschericiha coli* strain DH5α was used as a host for all plasmids.

Plasmids: Yeast genomic DNA encompassing the *GAL83* gene was originally recovered from lambda clone λPM5083 (RILES *et al.* 1993) by recombination with pBM2240 as previously described (ERICKSON and JOHNSTON 1993). All plasmids were constructed using standard techniques (MANIATIS, FRITSCH and SAMBROOK 1982). An 11-kb *Xba*I fragment containing the *GAL83* gene was subcloned into pRS316 (SIKORSKI and HIETER 1989) to generate pBM2385 (see Figure 1). pBM2424 was constructed by subcloning the 6-kb *Eco*RI fragment from pBM2385 into the *Eco*RI site of YCp50. pBM2431 was constructed by subcloning a 2.5-kb *Eco*RI-*Hin*dIII fragment that contained the *GAL83* gene (from pBM2424, see Figure 1) into the *Eco*RI-*Hin*dIII site of a pRS316-derived vector in which the *Cla*I-*Xho*I sites were deleted. All other subclones were made with pRS316 using the indicated restriction sites (see Figure 1). pBM2439 was constructed by subcloning the 2.5-kb *Eco*RI-*Hin*dIII fragment from pBM2431 (see Figure 1) into the 2 μm-based vector, pRS426 (CHRISTIANSON *et al.* 1992). pBM2452 was constructed by digesting pBM2431 with *Xho*I and religating to delete the 536 base pairs between the two *Xho*I sites.

Plasmids containing cloned copies of genes involved in glucose repression are as follows: pBM1624 contains *GRR1* (FLICK and JOHNSTON 1991b), pBM1962 contains *REG1* (J. ERICKSON and M. JOHNSTON, UNPUBLISHED DATA), pJH3 (pBM2453) and pXY28 (pBM2454) contain *SIP1* and *SIP2* respectively (generously provided by X. YANG and M. CARLSON). To determine linkage between *GAL82-1* and *SIP2*, the *ADE5* locus was disrupted with the *URA3* gene using the *ADE5* disruption plasmid pBM2441.

Sequencing *GAL83*: DNA sequence analysis was performed with the dideoxynucleotide chain termination method (SANGER, NICKLEN and COULSON 1977) using double strand DNA and sequenase (United States Biochemical). DNA sequence data was analyzed using the Geneworks (Intelligenetics) software package.

Construction of gene disruptions: A *GAL83::URA3* disruption was constructed by inserting the 1.8-kb *Cla*I-*Sal*I fragment containing *URA3* from YEp24 between the *Cla*I and *Xho*I sites of pBM2431. This construction deletes amino acids 99–278 from the *GAL83* coding region. The resultant plasmid (pBM2460) was digested with *Eco*RI and *Kpn*I, and the 3.8-kb *gal83::URA3* containing fragment was gel purified and used to transform YM2169 to Ura$^+$. Since Ura$^+$ transformants were obtained in this haploid at high frequency, we assumed that the disruption mutant was viable. PCR analysis on genomic DNA using primers that flank the disrupted region of *GAL83* confirmed that proper insertion had occurred (data not shown). Transformants were scored for sensitivity to 2-deoxygalactose and assayed for β-galactosidase activity from the integrated *GAL1-lacZ* fusion contained in pRY181 (YOCUM *et al.* 1984). Disruptions of *SIP1* and *SIP2* were made and provided by X. YANG and M. CARLSON (YANG, ALBERT-HUBBARD and CARLSON 1992; X. YANG, R. JIANG and M. CARLSON, unpublished data). Disruptions were confirmed by Southern blots.

Enzyme assays: β-galactosidase expressed from a *GAL1-lacZ* fusion (contained in pRY181) intergrated into the chromosome at *LEU2* was assayed as described previously (YOCUM *et al.* 1984; FLICK and JOHNSTON 1991a). All β-galactosidase assays are reported as Miller units and are the average of at least two assays. Assays for each table were performed on the same day. The highest variance for the assays in each table (typically 14%) is reported in the footnote. Yeast strains were grown on YEP + 2% glucose (repressing) or 2% raffinose (or 2% galactose) (nonrepressing). Yeast strains transformed with a plasmid were grown on SD-Uracil media containing either 2% glucose or 2% raffinose. Invertase was assayed as described previously (GOLDSTEIN and LAMPEN 1975; NEIGEBORN and CARLSON 1984).

Sequencing the *GAL83-2000* allele: A 2.2-kb PCR product was generated from genomic DNA from YM3033 (*GAL83-2000*). To eliminate the possibility of PCR-induced errors, products from four separate PCR reactions were pooled and subcloned using the TA cloning system (Invitrogen). Ten independent subclones were pooled and sequenced using the same oligonucleotide primers that were used to sequence *GAL83-2000*. To confirm the identity of the missense mutations, the *GAL83-2000* allele was also recovered by gapped plasmid rescue as follows: pBM2431 was digested with *Xho*I and *Cla*I and used to transform YM3033 to Ura$^+$. Ura$^+$ transformants were scored for the ability to complement the *GAL83-2000* mutations. Noncom-

TABLE 1

List of yeast strains[a]

Strain	Genotype
YM2169	*MAT***a** *met*$^-$
YM2170	*MATα tyr1-501*
YM2180	*MAT***a** *GAL82-1*
YM2201	*MAT***a** *met*$^-$ *reg1-547*
YM2213	*MATα snf1Δ3*
YM3032	*MAT***a** *URA3 GAL83-2000*
YM3033	*MATα GAL83-2000*
YM3196	*MATα reg1Δ::LEU2*
YM3205	*MAT***a** *met*$^-$ *reg1Δ::LEU2*
YM2957	*MAT***a** *grr1Δ1829*
YM4237	*MAT***a**/*MATα URA3/ura3-52 tyr1/tyr1 met/met GAL83/GAL83-2000 REG1/reg1Δ::LEU2*
YM4237	*MAT***a**/*MATα MET/met GAL83/GAL83-2000 REG1/reg1Δ*
YM4239	*MAT***a**/*MATα GAL83/GAL83-2000 REG1/reg1-547*
YM4240	*MAT***a**/*MATα TYR1/tyr1 MET/met REG1/reg1Δ::LEU2*
YM4241	*MAT***a**/*MATα REG1/reg1-547*
YM4245	*MAT***a**/*MATα GAL82/GAL82-1*
YM4246	*MAT***a**/*MATα GAL82/GAL82-1 GAL83/GAL83-2000*
YM4247	*MAT***a**/*MATα GAL82/GAL82-1 reg1-547/REG1*
YM4248	*MAT***a**/*MATα GAL82/GAL82-1 reg1Δ/REG1*
YM4266	*MAT***a** *gal83::URA3*
YM4268	*MAT*A *sip1::URA3*
YM4270	*MAT***a** *URA3::GAL1/lacZ leu2*$^-$ *sip2::LEU2*
YM4310	*MAT***a** *URA3::GAL1/lacZ gal83::URA3 sip2::LEU2*
YM4312	*MAT***a** *gal83::URA3 sip1::URA3*
YM4313	*MAT***a** *URA3::GAL1/lacZ sip1::URA3 sip2::LEU2*
YM4314	*MAT***a** *URA3::GAL1/lacZ gal83::URA3 sip1::HIS3 sip2::LEU2*

[a] All strains carry the *ura3-52 his3Δ200 ade2-101 lys2-801 gal80Δ-542* and *LEU2::pRY181* alleles unless otherwise noted.

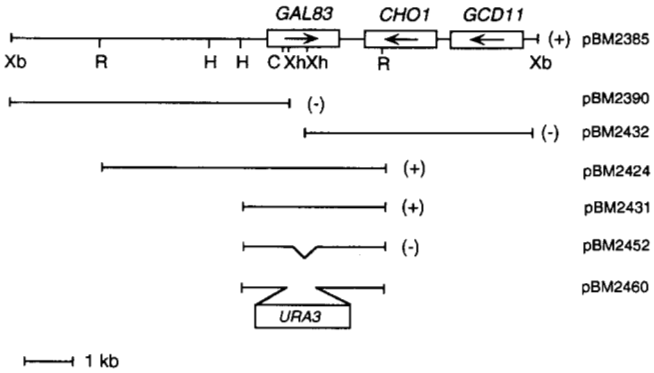

FIGURE 1.—Restriction map of the *GAL83* containing fragment from λ clone λPM5083. The boxes represent the open reading frames of *GAL83*, *CHO1* and *GCD11*. The symbol in parentheses indicates the ability of the particular restriction fragment to complement the *GAL83-2000* mutation, scored by sensitivity to 2-deoxygalactose and by assaying *GAL1/lacZ* expression. Restriction sites: R = *Eco*RI; H = *Hin*dIII; C = *Cla*I; Xh = *Xho*I; Xb = *Xba*I.

plementing plasmids were recovered into *Escherichia coli* and sequenced as before. To determine which missense mutation was responsible for the *GAL83-2000* phenotype, the *GAL83-2000* gene was recovered from FM133 (*GAL82-1*), (a congenic *GAL83* strain from Y. Oshima), by PCR and sequenced.

RESULTS

Molecular analysis of *GAL83*: We isolated the wild-type *GAL83* gene by a genetic technique that converted the lambda clone that contains *GAL83* into a yeast centromere plasmid (ERICKSON and JOHNSTON 1993; RILES *et al.* 1993). The lambda clone that carried *GAL83* contained approximately 15 kb of yeast sequence. To delimit the location of the *GAL83* coding sequences, restriction fragments were subcloned and tested for the ability to complement the partially recessive character of *GAL83-2000* by plating transformants carrying various subclones onto plates containing glucose + 2-deoxygalactose. 2-deoxygalactose (2dGal) is a galactose analog that is converted to a form that is toxic to yeast by the action of galactokinase, the *GAL1* gene product. A wild-type strain is resistant to 2dGal in the presence of glucose because of glucose repression of *GAL1* expression; a *GAL83-2000* mutant is sensitive to 2dGal in the presence of glucose because it expresses the *GAL1* gene. Results of plate assays were confirmed by assaying β-galactosidase expressed from a *GAL1/lacZ* fusion present in our strains. As summarized in Figure 1 and Table 2, the smallest complementing fragment was the 2.5-kb *Eco*RI-*Hin*dIII fragment in pBM2431. The cloned *GAL83* gene never restored completely wild-type levels of *GAL1-lacZ* expression, consistent with the partially dominant character of the *GAL83-2000* allele used in these experiments (MATSUMOTO, YOSHIMATSU and OSHIMA 1983; compare lines 1–3 in Table 2). A

TABLE 2

Complementation of *GAL83-2000*

Yeast strain[b]	Plasmid	*GAL1-lacZ* activity[a]		Growth on 2-deoxy-galactose
		Glucose	Galactose	
1. *GAL83*	None	9.5	630	+
2. *GAL83-2000*	None	200	600	−
3. *GAL83-2000/GAL83*	None	135	715	+
4. *GAL83-2000*	pRS316 (vector)	313	872	−
5. *GAL83-2000*	pBM2384	113	568	+
6. *GAL83-2000*	pBM2431	104	527	+
7. *GAL83-2000*	pBM2439[c]	66.0	563	+
8. *GAL83-2000*	pBM2452	312	909	−

[a] Maximum error for values in this table is ±14%.
[b] Wild-type strain = YM2169; *GAL83-20000* = YM3033; heterozygous diploid is the a/α diploid of YM2169 × YM3033.
[c] High-copy plasmid.

high-copy plasmid containing the 2.5-kb *Eco*RI-*Hin*dIII fragment (pBM2439) was able to restore glucose repression closer to wild-type levels (Table 2, line 7).

The DNA sequence of the 2.5-kb *Eco*RI-*Hin*dIII insert in pBM2431 was determined on both strands. This fragment contained a single, long open reading frame of 1251 bp that can encode a protein of 417 amino acids. We confirmed that this reading frame is indeed *GAL83* by deleting the sequences between the two *Xho*I sites contained within the putative open reading frame (see Figure 1). This plasmid, pBM2452, failed to complement the *GAL83-2000* mutation (Table 2, line 8). The complete nucleotide sequence of *GAL83* and the predicted amino acid sequence of its encoded protein are shown in Figure 2. Partial sequence analysis of the large 11-kb *Xba*I insert in pBM2385 revealed that is also contains two genes, *CHO1* (NIKAWA *et al.* 1987) and *GCD11* (HARASHIMA and HINNEBUSCH 1986), which are known to be tightly linked to *GAL83-2000*. This confirms that the cloned piece of DNA that complemented *GAL83-2000* is from the correct genetic locus. *GAL83* is identical to *SPM1*, a gene identified as a high-copy suppressor of a mutation in the zinc-finger of the largest subunit of RNA polymerase II (J. Friessen, unpublished data).

Predicted protein sequence of *GAL83* reveals homology with two functionally related yeast proteins: The predicted sequence of Gal83p did not reveal any notable motifs. The sequence of Gal83p has homology with two other yeast proteins, Sip1p and Sip2p (YANG, Albert-Hubbard and CARLSON 1992; X. YANG, R. JIANG and M. CARLSON, manuscript submitted for publication). Both *SIP1* and *SIP2* were identified as genes whose products physically interact with Snf1p (YANG, Albert-Hubbard and CARLSON 1992), a protein kinase that is required for derepression of several glucose repressed genes, including the *GAL* genes (CARLSON, OSMOND and BOTSTEIN 1981; CELENZA and CARLSON 1984). Alignment of Gal83p with Sip1p and Sip2p revealed that these three proteins have significant homology with each other over much of the Gal83 protein sequence (see Figure 3). The Gal83 and Sip2 protein sequences are 45% identical, with the majority of the homology in the C-terminal 60% of the gene; the homology between Gal83p and Sip1p is limited to two regions, one approximately in the center of the Gal83p sequence and the other near the C-terminus of Gal83p (see Figure 3). A high-copy clone of *SIP2* was able to cross complement *GAL83-2000*, raising the possibility that these two genes have similar functions. Despite its more limited homology to Gal83p, a cloned copy of *SIP1* was also able to cross complement *GAL83-2000* on a high-copy plasmid (see below).

A *gal83* disruption allele allows normal glucose repression: To determine the null phenotype of *GAL83*, the gene was disrupted with *URA3*, deleting amino acids 97–181. Surprisingly, the null mutant exhibited normal glucose repression of *GAL1* expression (see Table 3, line 3). Null mutations in *SIP1* and *SIP2* (disruption plasmids generously supplied by X. YANG, R. JIANG and M. CARLSON) also had no effect on *GAL1* expression (Table 3, lines 4 and 5).

Since null mutations in *GAL83*, *SIP1* and *SIP2* do not affect regulation of the *GAL1* gene, it was of interest to determine if combinations of these three mutations would cause a glucose repression phenotype. Double mutants were constructed by genetic crosses of single null mutants, and the *gal83*Δ, *sip1*Δ, *sip2*Δ triple mutant was constructed by a subsequent cross of two double mutants. As shown in Table 3, no combination of *gal83*, *sip1* and *sip2* had a significant effect on the regulation of the *GAL1/lacZ* reporter gene. This suggests that these three genes are redundant, and that other members of this gene family remain to be identified.

Recovery of the *GAL83-2000* mutation: Since the

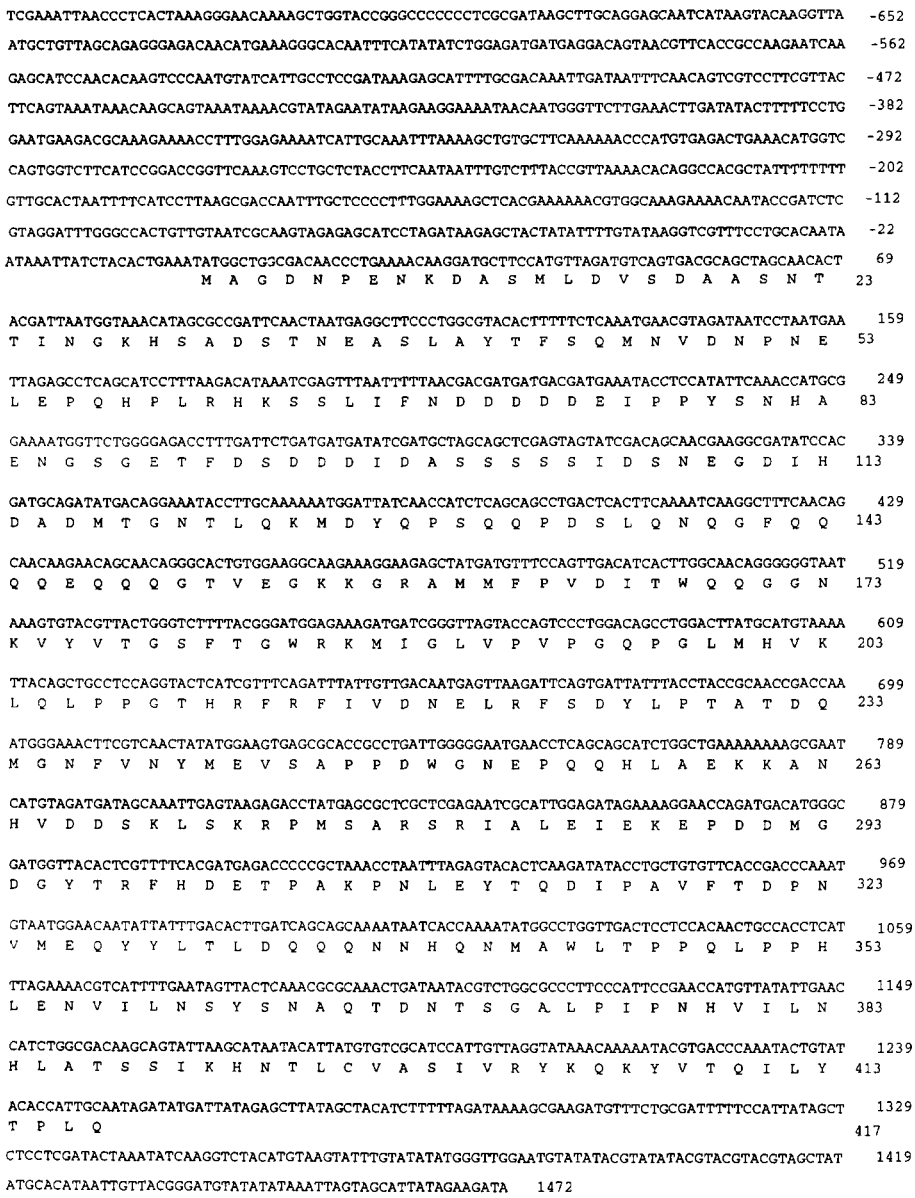

FIGURE 2.—DNA sequence of *GAL83* and predicted amino acid sequence of the protein product.

gal83 null mutation produced no glucose repression phenotype, it was of interest to identify the nature of the *GAL83-2000* allele. To identify the mutation, a 2.2-kb PCR product that contains the *GAL83-2000* coding region was subcloned and sequenced (see MATERIALS AND METHODS for details). Two differences between our wild-type allele (S288C derived) and the *GAL83-2000* allele (obtained from Y. OSHIMA) were found. First, a C to T transition was identified that results in a glycine to arginine substitution at position 235. This residue lies in a region that is conserved between Gal83p, Sip1p and Sip2p (see Figure 3). The second mutation is a T to C transition that results in a leucine to serine change at position 270. This amino acid lies in a region that is not conserved with Sip1p or Sip2p. To determine which of these mutations contribute to the *GAL83-2000* phenotype, we determined the sequence of this region of *GAL83* in a wild-type strain congenic for *GAL83* (a *GAL82-1* mutant from Y. OSHIMA). This strain carries the L270→S mutation, but possesses the G235 residue present in our wild-type strain. Therefore, it seems likely that the G235→R mutation is responsible for the *GAL83-2000* phenotype; the L270→S change is probably a phenotypically silent sequence polymorphism between our strains and OSHIMA's strains, although it is possible that both missense mutations are necessary to confer the observed phenotype.

***snf1* is epistatic to *GAL82-1* and *GAL83-2000*:** Mutations in *SNF1* cause a phenotype opposite to glucose repression resistant mutations: *snf1* mutants are unable to derepress *GAL1* in the absence of glucose (CARLSON, OSMOND and BOTSTEIN 1981). Since Gal83p is so similar to two proteins known to interact with Snf1p, it was of interest to determine the epistatic relationship of *snf1* and *GAL83-2000*. A diploid het-

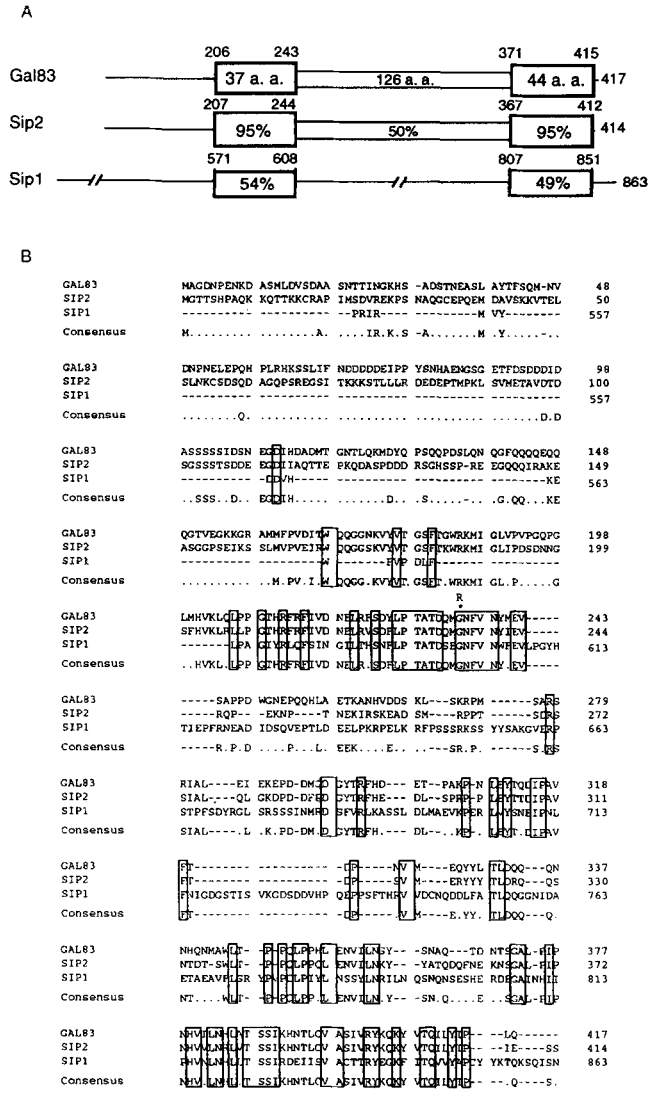

FIGURE 3.—(A) Schematic of homologous regions of Gal83, Sip1 and Sip2. Gal83, Sip1 and Sip2 contain two regions of homology that are designated by shaded boxes. The length of the region is shown in the Gal83 box and the homology to Gal83 is shown in the Sip1 and Sip2 boxes. A third region of homology between Gal83 and Sip2 is represented by the open box. Amino acid residue numbers above boxes indicate limits of homologous regions. (B) Comparison of the sequences of Gal83p with Sip1p and Sip2p. The comparison was made with the complete sequences of Gal83p and Sip2p but only the C-terminal 313 amino acids of Sip1p were shown. Only identical amino acids are blocked. Sequence for Sip1 was previously published (YANG, Albert-Hubbard and CARLSON 1992) and the Sip2 sequence was provided by R. JIANG and M. CARLSON. The numbers on the right are the numbers of the last amino acid residue for each protein. The mutation that is present in the *GAL83-2000* allele is indicated at position 235.

erozygous for *snf1Δ3* and *GAL83-2000* was sporulated and tetrads were dissected. The *GAL83-2000* phenotype was scored by its sensitivity to glucose + 2-deoxygalactose; the *snf1* phenotype was indicated by the inability to grow on sucrose or raffinose. Analysis of tetrads showed that resistance to 2dGal predominantly segregated 3^r: 1^s, suggesting that *snf1* is epistatic to *GAL83-2000*. This was confirmed by assaying

TABLE 3

Effects of null mutations in *GAL83*, *SIP1* and *SIP2* on *GAL1* regulation

Strain genotype	Strain	*GAL1/lacZ* activity[a] Glucose	*GAL1/lacZ* activity[a] Galactose	Growth on 2-deoxygalactose
1. Wild-type	(YM2169)	45	670	+
2. *GAL83-2000*	(YM3033)	191	605	–
3. *gal83Δ::URA3*	(YM4266)	34	400	+
4. *sip1Δ::URA3*	(YM4268)	38	595	+
5. *sip2Δ::LEU2*	(YM4270)	13	642	+
6. *gal83Δ*; *sip1Δ*	(YM4312)	42	608	+
7. *gal83Δ*; *sip2Δ*	(YM4310)	17	565	+
8. *sip1Δ*; *sip2Δ*	(YM4313)	54	537	+
9. *gal83Δ*; *sip1Δ*; *sip2Δ*	(YM4314)	10	443	+

[a] Maximum error for values in this table is ±14%.

GAL1-lacZ expression. Data from two sample tetrads (a tetratype and a nonparental ditype) is shown in Table 4. Similar results were obtained for epistasis of *GAL82-1* and *snf1* (see Table 4). These results suggest that Gal83p and Gal82p may act "upstream" of Snf1p in the glucose signaling pathway.

***GAL82* is not allelic to *SIP1* or *SIP2*:** Mutants of *GAL82* and *GAL83* share several genetic properties. First, mutations isolated in both genes confer resistance to glucose repression that is *GAL* gene specific. Second, both *GAL82-1* and *GAL83-2000* are cross-complemented by a cloned copy of *REG1* (see below; Table 5). Since Gal83p and Sip1p and Sip2p appear related, we considered the possibility that *GAL82* may be allelic to *SIP1* or *SIP2*. Indeed, both *SIP1* and *SIP2* on high-copy plasmids cross-complement the *GAL82-1* mutation (see Table 5). To test for genetic linkage between *GAL82-1* and *SIP2*, we examined the linkage between *GAL82-1* and *ade5* (*ADE5* is approximately 16 cM from the predicted position of *SIP2*). An *ade5::URA3* disruption strain was crossed to a *GAL82-1* mutant and tetrads were dissected. Results from this cross demonstrated that *GAL82-1* and *ade5* are unlinked (7PD; 6NPD; 20T). Thus, *GAL82-1* and *SIP2* are different genes. To determine linkage between *GAL82-1* and *SIP1*, a *sip1::URA3* strain (YM4268) was crossed to the *GAL82-1* mutant. Tetrad analysis showed that *sip1* (Ura^+) and *GAL82-1* (sensitivity to 2dGal) segregated independently and are therefore unlinked (1PD; 2NPD; 5T)

Cross-complementation by *GAL83* and *REG1*: In our attempts to clone *GAL83* by complementation from genomic plasmid libraries, another gene involved in glucose repression, *REG1*, was isolated several times. As shown in Table 5 and summarized in Table 6, *REG1* on a *CEN* plasmid cross-complements the *GAL83-2000* allele (Table 5, line 3). *REG1* on a *CEN* plasmid also cross complements *GAL82-1* to the same extent as the *GAL83-2000* allele (Table 5, line

TABLE 4

Epistatic relationship of *snf1* to *GAL82-1* and *GAL83-2000*

Cross and inferred genotype	Growth on Glucose	Raffinose	2dGal	*GAL1/lacZ* expresson on glucose[a]
snf1 × *GAL83-2000*				
Tetratype				
a *snf1, GAL83* (or *GAL83-2000*)	+	−	+	28
b *SNF1, GAL83-2000*	+	+	−	176
c *SNF1, GAL83*	+	+	+	18
d *snf1, GAL83-2000* (or *GAL83*)	+	−	+	42
Nonparental ditype				
a *SNF1, GAL83*	+	+	+	37
b *SNF1, GAL83*	+	+	+	17
c *snf1, GAL83-2000*	+	−	+	21
d *snf1, GAL83-2000*	+	−	+	40
snf1 × *GAL82-1*				
Tetratype				
a *snf1, GAL82* (or *GAL82-1*)	+	+	+	16
b *snf1, GAL82-1* (or *GAL82*)	+	−	+	34
c *SNF1, GAL82-1*	+	+	−	186
d *SNF1, GAL82*	+	+	+	54
YM2213 *snf1Δ3*	+	−	+	25
YM3033 *GAL83-2000*	+	+	−	212
YM2180 *GAL82-1*	+	+	−	201
YM2169 w.t.	+	+	+	22

[a] Maximum error for values in this table is ±15%.

9). A cloned copy of *REG1* does not cross-complement other glucose repression mutations including *grr1*, *hxk2* and *mig1* (data not shown). This suggests that *REG1* may be functionally related to the *GAL82* and *GAL83* gene products.

To test if *GAL83* is able to cross-complement other mutations, high-copy *GAL83* (pBM2439) was used to transform *reg1* and *GAL82-1* mutants. The results shown in Table 5 indicate that *GAL83* was not able to cross-complement either mutant. Since both *SIP1* and *SIP2* cross-complement *GAL83-2000* or *GAL82-1*, but *GAL83* does not cross-complement *GAL82-1*, the function of *GAL83* is not strictly equivalent to *SIP1* or *SIP2* (see Table 6).

Unlinked, noncomplementation between *reg1*, *GAL82-1* and *GAL83-2000*: To further examine the relationship between *REG1*, *GAL82* and *GAL83*, a series of isogenic diploids heterozygous for each of the mutations was made and assayed for glucose repression. Since the diploids heterozygous for both *GAL83-2000* and *reg1* (Table 7, lines 10 and 11) exhibit much less glucose repression than diploids heterozygous for either mutation alone (lines 6 and 8), the two mutations are genetically defined as noncomplementing, unlinked alleles. Note that a *reg1Δ* allele gave the same noncomplementing phenotype as a *reg1-547* point mutation. To test the gene specificity of noncomplementation, heterozygous diploids were made between *GAL82-1* and *reg1-547*, *reg1Δ* and *GAL83-2000*. Like *GAL83-2000*, *GAL82-1* also failed to complement *reg1* (line 13 and 14). Furthermore, *GAL82-1* and *GAL83-2000* also failed to complement each other (line 12). Although the effect was not as substantial as with *reg1*, the trans-heterozygote conferred enough resistance to glucose repression to confer sensitivity to 2-deoxygalactose. This noncomplementing phenotype is suggestive of an interaction between the *GAL82*, *GAL83* and *REG1* gene products.

DISCUSSION

GAL83 as well as *GAL82* are exceptional among the several genes required for glucose repression in that they seem specific for the *GAL* genes (MATSUMOTO, TOH-E and OSHIMA 1981; our unpublished data). This specificity may reflect a *GAL* gene specific branch of the glucose repression pathway, or may simply be a property of the particular *GAL82-1* and *GAL83-2000* mutations obtained by the *GAL*-specific genetic screen used by MATSUMOTO, TOH-E and OSHIMA (1981). Mutations in *GAL82* and *GAL83* indeed seem rare, as exhaustive mutant hunts in our laboratory using *GAL1* as the reporter failed to uncover any new alleles of these two genes. Since the *gal83* null mutation causes no glucose repression phenotype and three different genes can cross complement the *GAL83-2000* mutation, we believe that the *GAL* specificity of *GAL82-1* and *GAL83-2000* is unique to these alleles.

Gal83p may be involved in the function of the *SNF1* gene product, because its sequence is similar to two proteins (Sip1p and Sip2p) known to interact physi-

TABLE 5

Cross complementation of glucose repression genes

	Yeast strain	Cloned gene	*GAL1-lacZ* activity[a] after growth on: Glucose	Galactose	% Fully induced
1.	*GAL83-2000* (YM3033)	None[b]	313	589	33
2.		***GAL83***[c]	**66.0**	**574**	**11**
3.		***REG1***	**57.3**	**642**	**8.9**
4.		***SIP1***[c]	**57.5**	**548**	**10.5**
5.		***SIP2***[c]	**56.4**	**532**	**10.6**
6.		*GRR1*	224	608	37
7.	*GAL82-1* (YM2180)	None[b]	197	589	33
8.		*GAL83*	233	772	30
9.		***REG1***	**25.6**	**725**	**3.5**
10.		***SIP1***	**55.8**	**675**	**8.3**
11.		***SIP2***	**44.0**	**700**	**6.2**
12.		*GRRI*	268	844	32
13.	*reg1*Δ (YM3205)	None[b]	511	622	82
14.		*GAL83*	437	493	89
15.		***REG1***	**10.3**	**475**	**4.3**
16.		*SIP1*	346	472	73
17.		*SIP2*	226	379	60
18.		*GRR1*	465	563	83
19.	Wild-type (YM2169)	None[b]	9.5	630	1.5
20.		*GAL83*	14.8	596	2.5
21.		*REG1*	13.1	955	1.4
22.		*SIP1*	33.5	571	5.8
23.		*SIP2*	34.6	681	5.3
20.		*GRR1*	17.0	600	2.8

[a] Maximum error for values in this table is ±14%.
[b] Vector (pRS316) only.
[c] *GAL83*, *SIP1* and *SIP2* clones for these experiments are all high-copy clones.

cally with Snf1p. Interestingly, high-copy clones of *SIP1* and *SIP2* are able to cross-complement *GAL83-2000*, suggesting that these gene products may have a similar function with respect to Snf1p activity (see Table 5). High-copy clones of *SIP1* and *SIP2* also cross-complement *GAL82-1*. This suggests that these four proteins may associate with the Snf1p kinase and affect its function. However, since a cloned copy of *GAL83* does not cross-complement *GAL82-1*, the function of Gal82p may be distinct from that of Gal83p (see Table 6 for summary).

Genetic analysis has shown that *snf1* is epistatic to *GAL83-2000* and *GAL82-1* (Table 4). This suggests that *GAL83* and *GAL82* act upstream of *SNF1* in the glucose repression pathway. However, we state this conclusion with caution because the *gal83* null mutant exhibits no phenotype with respect to glucose repression of *GAL* gene expression. The only *GAL82-1* and *GAL83-2000* mutations we possess are partially dominant. Since *SIP1* and *SIP2* cross-complement *GAL82-1* and *GAL83-2000* and encode proteins similar to Gal83p, it seems reasonable also to place *SIP1* and *SIP2* at a position upstream of the *SNF1*-encoded kinase. Our data are consistent with a model in which Gal82p, Gal83p, Sip1p and Sip2p may act in a redundant fashion to regulate Snf1p activity, as opposed to acting as downstream targets of Snf1p. However, *SIP1* was shown to suppress a defect in the *snf4* gene (a positive activator of Snf1p kinase) apparently without stimulating Snf1p kinase activity. This result is consistent with the possibility that Sip1p acts downstream of Snf1p (YANG, ALBERT-HUBBARD and CARLSON 1992).

TABLE 6

Summary of complementation of glucose repression mutants with cloned copies of glucose repression genes

Mutation	Complementation by cloned gene: *GAL83*	*SIP1*	*SIP2*	*REG1*	*GRR1*
1. *GAL83-2000*	+	+	+	+	−
2. *GAL82-1*	−	+	+	+	−
3. *reg1-547*	−	−	−	+	−
4. *grr1-1121*	−	−	−	−	+

The *GAL83* and *GAL82* gene products seem to interact or function closely with the *REG1* gene product. This hypothesis is based on two observations: First, a cloned copy of *REG1*, on a low copy plasmid, significantly cross-complements the effects of *GAL82-1* and *GAL83-2000* mutations (Table 5). Cross-complementation of particular mutations by high copy clones has been a successful approach to identifying genes in the same regulatory pathway (ANDREWS and HERSKOWITZ 1989; NEWMAN, SHIM and FERRO-NOVICK 1990; BENDER and PRINGLE 1989, 1991). The rationale behind such experiments is that many regulatory pathways require a balance of positive and negative factors that combine to produce a specific signal. The reduction or loss of one factor may be compensated for by an increased amount of another factor. In the case of *GAL82-1* and *GAL83-2000*, a modest increase in the *REG1* gene dosage can restore nearly wild-type levels of glucose repression. However, a cloned copy of *GAL83* does not reciprocally complement *reg1*. This result, along with the fact that a *reg1* null mutation almost completely relieves glucose repression (see Table 5), suggests that *REG1* has a more significant role than *GAL82* or *GAL83* in effecting glucose repression. Interestingly, *REG1* also appears to be involved in RNA processing (TUNG *et al.* 1992). The observation that *REG1* has pleiotropic effects beyond glucose repression suggests that its function in not limited to glucose repression.

The second observation that suggests *GAL82*, *GAL83* and *REG1* interact is that mutations in these genes do not complement one another. This unlinked, noncomplementing phenotype is often interpreted as suggestive of a protein-protein interaction. Noncomplementation is thought to occur for either of two reasons: 1) either specific alleles combine with each other to produce a complex that has a dominant

TABLE 7

Unlinked, noncomplementation between *GAL82-1*, *GAL83-20000* and *reg1*

Yeast strain	Relevant genotype	*GAL1-lacZ* activity[a] after growth on: Glucose	Galactose	%Fully induced
1. YM2169	Wild-type	35.4	635	5.5
2. YM3033	*GAL83-2000*	449	992	45
3. YM2180	*GAL82-1*	339	839	40
4. YM2201	*reg1-547*	615	1158	53
5. YM3205	*reg1Δ*	683	696	98
6. YM4238	*GAL83-2000*/wild-type	135	715	19
7. YM4245	*GAL82-1*/wild-type	116	1143	10
8. YM4241	*reg1-547*/wild-type	53.8	707	7.6
9. YM4240	*reg1Δ*/wild-type	39.0	840	4.6
10. YM4239	*GAL83-2000/reg1-547*	350	450	78
11. YM4237	*GAL83-2000/reg1Δ*	359	652	55
12. YM4246	*GAL82-1/GAL83-2000*	241	950	25
13. YM4247	*GAL82-1/reg1-547*	365	1270	29
14. YM4248	*GAL82-1/reg1Δ*	233	600	39

[a] Maximum error for values in this table is ±11%.

inhibitory effect (poisoned subunits), or 2) the relative amounts of active complex fall below a threshold level due to reduced levels of functional proteins in the heterozygote (STEARNS and BOTSTEIN 1990; HAYS *et al.* 1988). Because the noncomplementation is allele independent with respect to *REG1*, we favor the latter explanation (insufficient complex formation) for this case of noncomplementation. Genetic analysis of other regulatory systems in yeast have revealed similar patterns of interplay between mutations. For example, SWANSON and WINSTON (1992) demonstrated that mutations in *SPT4*, *SPT5* and *SPT6* fail to complement one another and that the *SPT5* and *SPT6* gene products physically interact in co-immunoprecipitation experiments. Our data, together with the observation that mutations in a single gene will suppress the glucose repression defect of all three of these mutants (J. ERICKSON and M. JOHNSTON, unpublished data), suggests that the protein products of *GAL82*, *GAL83* and *REG1* act in concert or at the same level in the regulatory hierarchy to effect glucose repression. It is also possible that these results are due to altered *GAL83* (and *GAL82*) expression in *reg1* mutants.

The genetic and molecular analysis suggests that the mechanism of glucose repression is indeed complex. Other regulatory phenomena in yeast are similarly complex. For example, mutations in *CDC24*, a putative calcium binding protein involved in bud site assembly is complemented by three heterologous genes, *BUD1/RSR1*, *MSB1* and *MSB2* (BENDER and PRINGLE 1989, 1991; for review, see DRUBIN 1991), some of which display unlinked, noncomplementation. While sequence analysis of *GAL83* failed to suggest its biochemical function, our results suggest that *GAL83* is directly associated with a signaling pathway involving the *SNF1*-encoded protein kinase and the *REG1* gene product. Understanding how these several proteins functionally interact will be essential for determining their roles in the complex process of glucose repression.

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