Mutations in *GCR1*, a Transcriptional Activator of *Saccharomyces cerevisiae* Glycolytic Genes, Function as Suppressors of *gcr2* Mutations

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Manuscript received May 3, 1994

Accepted for publication October 11, 1994

ABSTRACT

The *Saccharomyces cerevisiae* *GCR1* and *GCR2* genes affect expression of most of the glycolytic genes. Evidence for Gcr1p/Gcr2p interaction has been presented earlier and is now supported by the isolation of mutations in Gcr1p suppressing *gcr2*, as assessed by growth and enzyme assay. Four specific mutation sites were identified. Together with use of the two-hybrid system of FIELDS and SONG, they show that Gcr1p in its N-terminal half has a potential transcriptional activating function as well as elements for interaction with Gcr2p, which perhaps acts normally to expose an otherwise cryptic activation domain on Gcr1p. Complementation of various *gcr1* mutant alleles and results with the two-hybrid system also indicate that Gcr1p itself normally functions as an oligomer.

THE glycolytic pathway is a major metabolic route in *Saccharomyces cerevisiae*. Most of the genes are highly expressed, and the glycolytic enzymes constitute a major fraction of the soluble proteins (HESS *et al.* 1969; FRAENKEL 1982). Thus, the genes encoding glycolytic enzymes are among the most highly expressed genes in yeast.

Analyses of the regulatory regions of many glycolytic genes have provided evidence for a common *cis*-acting regulatory element(s) and for binding of the *trans*-acting factor Rap1p (also called Grf1p or Tuf1p) (HUET and SENTENAC 1987; SHORE and NASMYTH 1987; BUCHMAN *et al.* 1988a). In some cases mutational analysis has shown Rap1p binding to be essential for expression (BUCHMAN *et al.* 1988b; CHAMBERS *et al.* 1989, 1990; McNEIL *et al.* 1990; NISHIZAWA *et al.* 1989; BUTLER *et al.* 1990; SCOTT *et al.* 1990). In several genes CTTCC motifs have also been implicated in expression (BUCHMAN *et al.* 1988b; CHAMBERS *et al.* 1988; BITTER *et al.* 1991; SCOTT and BAKER 1993) and are often located adjacent to a Rap1p-binding site (HUET *et al.* 1992). Some of the genes also have Abf1p- (BRINDLE *et al.* 1990; CHAMBERS *et al.* 1990) and Reb1p- (CHASMAN *et al.* 1990; SCOTT and BAKER 1993; WILLETT *et al.* 1993) binding sites. However, neither Rap1- (HUET and SENTENAC 1987; SHORE and NASMYTH 1987; LUSTIG *et al.* 1990), Abf1p- (BUCHMAN *et al.* 1988a) nor Reb1p- (MORROW *et al.* 1989) binding sites are restricted to glycolytic genes. The respective roles of the factors bound at adjacent sites in the UAS elements of glycolytic genes and how they act together to promote their high-level expression remain to be elucidated.

Thus, any special mechanism for glycolytic gene expression likely involves other elements, perhaps those coded by *GCR1* (CLIFTON *et al.* 1978), *GCR2* (UEMURA and FRAENKEL 1990) and *GCR3* (UEMURA and JIGAMI 1992b). *gcr1* mutants (CLIFTON *et al.* 1978; CLIFTON and FRAENKEL 1981; KAWASAKI and FRAENKEL 1982; BAKER 1986) are severely reduced in expression of most glycolytic enzymes at the transcriptional level, and BAKER (1991) showed that the Gcr1p binds to the sequence containing CTTCC motif. Recently, SCOTT and BAKER (1993) proposed a model that the binding of Gcr1p is the most critical step for the activation of glycolytic genes and other factors that bind adjacent to Gcr1p-binding sites facilitate or modulate Gcr1p binding *in vivo*. *GCR2* was reported as the second regulatory locus, which also may specifically regulate the expression of most glycolytic genes (UEMURA and FRAENKEL 1990). The levels of reduction of most glycolytic enzymes in *gcr2* mutants are very similar to those in *gcr1* mutants, but there is a lesser growth defect. Recently, we presented genetic evidence for a physical interaction of Gcr2p and Gcr1p as well as for normal Gcr2p action likely being a contributor somehow to an activation domain to a Gcr1p-Gcr2p complex (UEMURA and JIGAMI 1992a).

In this report we extend our analysis of Gcr1p and Gcr2p and provide genetic evidence for a potential transcriptional activation function in Gcr1p. Hence, Gcr2p likely functions normally as a coactivator in the Gcr1p-Gcr2p complex. We also present evidence for Gcr1p-Gcr1p interaction.

MATERIALS AND METHODS

Strains and genetic methods: The *S. cerevisiae* strains used in this study are listed in Table 1. *Escherichia coli* DH5 α [F⁻

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TABLE 1
S. cerevisiae strains

Strain	Genotype	Comments or source
2845	α <i>leu2-3 leu2-112 ura3-52 his6</i>	UEMURA and FRAENKEL (1990)
YHU2012	a <i>leu2-3 leu2-112 ura3-52</i>	Isogenic with 2845, ^a UEMURA and FRAENKEL (1990)
C110-1	a / α <i>leu2-3/leu2-3 leu2-112/leu2-112 ura3-52/ura3-52 his6/HIS6</i>	2845 $\times$ YHU2012
DFY67	a <i>gcr1-1 leu2-1 lys1 trp1-1</i>	CLIFTON <i>et al.</i> (1978)
MWGL29	α <i>gcr1-6 leu2-3 leu2-112 ura3-52 his6</i>	<i>gcr1</i> mutant of 2845, UEMURA and FRAENKEL (1990)
NW8-10-1	α <i>gcr1-7 leu2-3 leu2-112 ura3-52 his6</i>	<i>gcr1</i> mutant of 2845, UEMURA and FRAENKEL (1990)
NW9-19-1	α <i>gcr2-1 leu2-3 leu2-112 ura3-52 his6</i>	<i>gcr2</i> mutant of 2845, UEMURA and FRAENKEL (1990)
DFY643	α Δ <i>gcr2::URA3 leu2-3 leu2-112 ura3-52 his6</i>	Δ <i>gcr2</i> of 2845, UEMURA and FRAENKEL (1990)
YHU3002-8C	α Δ <i>gcr2::URA3-big leu2-3 leu2-112 ura3-52 his6</i>	UEMURA and JIGAMI (1992a)
YHU3016	α <i>gcr1-6 Δgcr2::URA3-big leu2-3 leu2-112 ura3-52 his6</i>	See text
C179-15C	α Δ <i>gcr1::URA3 leu2-3 leu2-112 ura3-52 his6</i>	See text
GGY1::171	α Δ <i>gal4 Δgal80 leu2 his3</i>	<i>GAL1-lacZ</i> fusion gene integrated at the <i>URA3</i> locus, GILL and PTASHNE (1987)

^aYHU2012 was constructed from a His⁺ revertant of 2845 by converting the mating type through transformation with a multicopy plasmid carrying the *HO* gene, curing of the plasmid from the diploid and segregation.

*endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1 Δ (lacUI69 ϕ 80 *dlacA* Δ M15)]* was used to propagate all plasmids (HANAHAN 1983).

Matings, diploid selection, sporulation and dissection were carried out by the usual methods (MORTIMER and HOWTHORNE 1975). Yeast cells were transformed by the method of ITO *et al.* (1983). Growth on various plates was scored by measuring average colony size under the microscope.

Media: *E. coli* cells were grown in Luria broth (LB) (DAVIS *et al.* 1980). Yeast cells were grown in rich medium (UEMURA and WICKNER 1988) and synthetic complete medium (SC) (SHERMAN *et al.* 1986) or SC dropout medium depending on the plasmid markers. Two percent of glucose or 2% glycerol plus 2% lactate was added as indicated. When necessary, the respiration inhibitor antimycin A was added to a final concentration of 1 μ g/ml.

DNA manipulation: Standard techniques of DNA manipulation used in this study are described in SAMBROOK *et al.* (1989). Isolation of plasmid DNA from yeast cells was done by the method of HOFFMAN and WINSTON (1987).

In vitro mutagenesis of plasmid DNA: Plasmid DNA was mutagenized according to the method of HASHIMOTO and SEKIGUCHI (1976). Briefly, closed circular pGCR8 DNA (100 μ g/ml) was incubated at 37° with 0.4 M hydroxylamine in 50 mM sodium phosphate buffer (pH 6.0) – 1 mM EDTA (total volume, 0.45 ml) for 30 hr. After the incubation the solution was diluted fivefold, and the plasmid DNA was suspended in 20 μ l of Tris-EDTA buffer after ethanol precipitation. The mutagenized DNA was used immediately to transform Δ *gcr2* mutant strain DFY643. DNA-transformed cells were plated on SC – Leu (Glu) plates containing antimycin A and incubated at 37°.

Plasmid constructions: Cloned *GCR1* DNA was derived from pGCR8 (KAWASAKI and FRAENKEL 1982), which carries *GCR1* in YEp13 (BROACH *et al.* 1979). PD302-2, PD303-2, PD304-1, PD305-2, PD306-1, PD307-1 and PD308-1 are mutants of pGCR8, obtained through *in vitro* mutagenesis with hydroxylamine. pL205-5, pL206-5 and pL207-2 are chimeric plasmids of PD305-2 (*Gcr1mut1*) and PD302-2 (*Gcr1mut2*),

PD306-1 (*Gcr1mut3*) or PD307-1 (*Gcr1mut4*), respectively. To construct them, PD305-2 was digested with *NcoI*, and the fragment that contains the C-terminus half of *GCR1* (fragment F in Figure 1) was substituted by the same fragment from either PD302-2, PD306-1 or PD307-1. pL212-1 and pL213-1 are derivatives of PD307-1 (*Gcr1mut4*). pL212-1, which lacks the C-terminus half (after the codon 595) of *GCR1mut4*, was constructed by *SphI* digestion (to delete fragment G in Figure 1) and religation of PD307-1. Likewise, pL213-1, which lacks the N-terminus half (until codon 229) of *GCR1mut4*, was constructed by *HindIII* digestion (to delete fragment H in Figure 1) and religation of PD307-1.

pRS315, a YCp vector with *LEU2* (SIKORSKI and HIETER 1989), was used to construct single copy plasmids with various *GCR1* genes. *SalI* fragments that correspond to fragment I in Figure 1 were excised from pGCR8 (*GCR1* wild type), PD305-2 (*Gcr1mut1*), PD307-1 (*Gcr1mut4*), PD332-1 (*gcr1-6*) and PD366-1 (*gcr1-7*), and they were cloned in the *SalI* site of pRS315 to construct pL278-5, pL279-22, pL280-9, pL281-11 and pL282-19, respectively. Fragments were cloned in the same orientation in all plasmids.

pCTC13 is a multicopy plasmid with *LEU2* and encodes a fusion of nuclear localization signal and Gal4p (768–881) (transcription-activating region of Gal4p or Gal4A) plus additional residues encoded by *BamHI* linker sequence under the control of *ADHI* promoter and terminator (kindly provided by STANLEY FIELDS, State University of New York at Stony Brook). pMA424 (MA and PTASHNE 1987) is a multicopy plasmid with *HIS3* and encodes Gal4p (1–147) (DNA-binding domain of Gal4p or Gal4B), plus additional residues encoded by a polylinker sequence under the control of *ADHI* promoter and terminator. Construction of pML77-8: Gal4p (768–881)/*Gcr1p* (68–844) fusion plasmid; pML78-2: Gal4p (1–147)/*Gcr1p* (68–844) fusion plasmid; and pL41-14: Gal4p (1–147)/*Gcr2p* (48–534) fusion plasmid, were described previously (UEMURA and JIGAMI 1992a). pL527-2 and pL321-5: Gal4p (1–147)/*Gcr1p* (68–844) *mut1* and Gal4p (1–147)/*Gcr1p* (68–450) fusion plasmids, respectively, were constructed in the same way as pML78-2 by

FIGURE 1.—Plasmids. (a) Structure of pGCR8. pGCR8 contains the genomic DNA fragment (*Sau3A* partial digestion) at the unique *Bam*HI site of YEpl3, and the structure around the cloned fragments is shown. — and ~~~, the cloned genomic DNA fragment and a portion of YEpl3, respectively. □, the coding region of *GCR1*, and the arrow indicates the orientation of transcription. Mut1, mut2, mut3 and mut4 correspond to the mutation sites in plasmid PD305-2 (PD303-2, PD304-1, PD308-1), PD302-2, PD306-1 and PD307-1, respectively. *gcr1-6* and *gcr1-7* indicate the mutation sites of *gcr1-6* and *gcr1-7* alleles cloned in PD332-1 and PD366-1, respectively. □, the DNA-binding domain of *Gcr1p* determined by HUIE et al. (1992). Relevant restriction sites are indicated. B, *Bam*HI; E, *Eco*RI; H, *Hind*III; N, *Nco*I; P, *Pst*I; Sc, *Sac*I; S, *Sal*I; Sca, *Sca*I; Sp, *Sph*I; Ss, *Ssp*I. Fragments A–E are used to determine the mutation sites in mutant *GCR1* genes. Fragments F–J are used to construct plasmids described in the text. The figure is not drawn exactly to scale. (b) Structure of chimeric and drop-out mutant plasmids. pL205-5, pL206-5 and pL207-2 are chimeric mutant plasmids and contain *Gcr1mut2*, *Gcr1mut3* and *Gcr1mut4* in addition to *Gcr1mut1*, respectively. They were constructed by substituting the fragment F of PD305-2 (*Gcr1mut1*) with the same fragments of other plasmids. pL212-1 and pL213-1 are drop-out plasmids of PD307-1. (c) Truncated and fused genes. In pL113-8 *GCR1* is truncated at *Sau3A* site pointed out as *Gcr1'*. pML77-8, pML78-2, pL41-14, pL527-2 and pL321-5 contain *GAL4* hybrid genes.

using *GCR1* genes of PD305-2 (*gcr1mut1*) and PD307-1 (*gcr1mut4*). pL113-8, which derived from a library of *Sau3A* partially digested fragments of yeast genomic DNA, contains truncated *GCR1* in YEpl351 (HILL et al. 1986). pL113-8 was isolated as a clone that further enhanced the β -galactosidase activity of *GAL1-lacZ*, depending on the *Gal4B/Gcr2p* (48–534) fusion plasmid (pL41-14). DNA sequencing revealed that the *GCR1* gene is truncated at a GATC sequence (*Sau3A* site) at codon 628 and 629, with 23 additional amino acids (Gly Ser Leu Phe Leu Gly Leu Val Gly Tyr Leu His His Arg Ile Asp Ser Tyr Ile His His Pro Leu) coded in frame before termination.

Cloning of chromosomal *gcr1* mutant alleles: MWGL29

and NW8-10-1 are *gcr1* mutants. Isolation and characterization of MWGL29 were described previously (UEMURA and FRAENKEL 1990). NW8-10-1 was isolated as another *gcr1* mutant by the same screening that gave the *gcr1-6*, *gcr2-1*, *gcr3-1* and *gcr3-2* mutants (UEMURA and FRAENKEL 1990; UEMURA and JIGAMI 1992b). Briefly, yeast strain 2845 carrying an *ENO1-lacZ* fusion plasmid was mutagenized and screened for putative mutations decreasing *ENO1* expression by measuring *ENO1*-derived β -galactosidase activity. Like MWGL29, NW8-10-1 showed lower β -galactosidase activity and poor growth on glucose. The mutation is recessive with normal growth and β -galactosidase activity in diploids formed with the isogenic wild-type strain YHU2012. In the six tetrads examined, slow

growth on glucose and low β -galactosidase activity cosegregated 2:2, indicating single nuclear gene mutation. The mutation in NW8-10-1 is allelic to *gcr1*, because the diploid between NW8-10-1 and DFY67 (*gcr1-1*) grew poorly on glucose, but the impaired growth of NW8-10-1 was complemented by multicopy plasmid carrying *GCR1*, pGCR8. Furthermore, glycolytic enzyme profiles in NW8-10-1 were very similar to those of MWGL29 (data not shown). Based on these observations, the mutation in NW8-10-1 was named *gcr1-7*.

YEp351, a multicopy plasmid with a *LEU2* selection marker, was used to clone mutant *gcr1* alleles from MWGL29 (*gcr1-6*) and NW8-10-1 (*gcr1-7*) strains by the gap-repair method (ROTHSTEIN 1991). pGCR8 was digested with *Sall* and the ~8-kb fragment I (Figure 1) was recloned into the *Sall* site of a modified YEp351, in which the unique *HindIII* site had been destroyed by filling it in with the Klenow enzyme. Then the plasmid was digested with *HindIII* plus *NcoI* to delete the whole coding region of *GCR1* (fragment J, Figure 1), and the linearized fragment with a replication origin was transformed into MWGL29 (*gcr1-6*) and NW8-10-1 (*gcr1-7*). Plasmid DNA was recovered from *Leu*⁺ transformants, and the gap repair was confirmed by restriction enzyme analysis (plasmids PD332-1 and PD366-1, containing *gcr1-6* and *gcr1-7* alleles, respectively). Retransformation confirmed lack of complementation of the original mutants (Table 2, line 13 of MWGL29 column and line 14 of NW8-10-1 column). As with the suppressor mutations in *GCR1*, the entire genes, -300 of 5' noncoding region to +2652 (118 bp after the termination codon), were sequenced. Both mutations had single amber mutations at codons 358 (*gcr1-6*) and 735 (*gcr1-7*), respectively (Table 4).

Nucleotide sequence analysis: Restriction fragments were cloned into M13mp18 and M13mp19 (NORRANDE *et al.* 1983), and the sequence was determined by the dideoxy chain termination method using ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA) with Taq DNA polymerase (Ampli Taq; Cetus Corp., Norwalk, CT) and the 18-nucleotide sequencing primer "21M13 primer" (Applied Biosystems). All mutations were confirmed in both strands.

Enzyme assays: Activities of glycolytic enzymes and β -galactosidase were assayed as described previously (UEMURA and JIGAMI 1992a). For β -galactosidase assays transformants were grown to mid-log phase in synthetic dropout medium (SHERMAN *et al.* 1986) containing 2% each of galactose, ethanol and glycerol. Assays were done at least four times. For glycolytic enzyme assays transformants were grown to mid-log phase (Klett unit of 100–150) in synthetic dropout medium containing 2% each of glycerol and lactate.

RESULTS

Mutagenesis of *GCR1* gene and isolation of suppressor mutants: In the previous paper we indicated genetic evidence for a physical interaction of Gcr1p and Gcr2p as well as for their functioning together in a transcriptional activation complex. Based on Gal4p and Rap1p fusion experiments, together with BAKER's (1991) result that shows the specific DNA-binding activity of Gcr1p, we suggested that the normal Gcr2p likely provide an activation domain to a Gcr1p-Gcr2p complex (UEMURA and JIGAMI 1992a). However, because the β -galactosidase activity from *GAL1-lacZ* fusion induced by a Gal4p DNA binding domain (Gal4B)/Gcr2p fusion gene product was modest and the suppression of *gcr1* mutation by Rap1p/Gcr2p fusion was

partial, the possibility remained that Gcr1p not only tethers Gcr1p-Gcr2p complex to upstream activation sequences of glycolytic genes, but also is involved in activation. If Gcr1p had such an activation function, it might be possible to isolate Gcr1p mutants that are active in the absence of Gcr2p. Such suppressors would also provide additional evidence for a genetic interaction between Gcr1p and Gcr2p.

GCR1 plasmid pGCR8 was mutagenized with hydroxylamine as described in MATERIALS AND METHODS. Mutagenized DNA was transformed into DFY643 ($\Delta gcr2::URA3$), and the cells were incubated at 37° on SC -Leu (Glu) plates supplemented with antimycin A. In case part of Gcr2p was required, the screen for suppressors in *GCR1* deliberately employed a *gcr2* mutant strain DFY643 ($\Delta gcr2::URA3$), which showed the same phenotype as the original *gcr2* mutant NW9-19-1 in both enzyme profile and growth, but retained the N-terminal 75% of *GCR2* (UEMURA and JIGAMI 1992a). *gcr2* mutant strains grow very poorly on glucose plates at 37° in the presence of antimycin A, and this phenotype is not suppressed by multicopy *GCR1* (UEMURA and FRAENKEL 1990). Transformants were selected that formed colonies of substantial size under this condition. After single colony repurification candidates were chosen whose loss of plasmid (*Leu*⁻ colonies identified after growth on the permissive medium rich [GlyLac]) restored the original phenotype. Their plasmid DNA obtained by transformation of *E. coli* was retransformed into DFY643 to confirm their suppressing activity (Table 2, lines 1 to 7, DFY643 column as compared with line 8, the transformant with the wild-type *GCR1* plasmid, which hardly grew on glucose plus antimycin A plates at 37°; the growth of wild-type strain 2845 under the same conditions gave slightly larger colonies [1.1 mm]). Based on the size of colonies, they were classified into two groups. The growth was recovered fairly well by PD303-2, PD304-1, PD308-1 and PD305-2 (lines 1–4) (group 1), and it was partially recovered by PD302-2, PD306-1 and PD307-1 transformation (lines 5–7) (group 2). Assays of several glycolytic enzymes (phosphoglycerate kinase [Pkg], phosphoglycerate mutase [Pgm] and enolase [Eno]) confirmed that the growth recovery accompanied recovery of enzyme activities (Table 3, compare lines 2 to 5 with line 6, the *gcr2* host carrying *GCR1* itself).

To examine if the truncated Gcr2p was necessary, the plasmids were also transformed in YHU3002-8C ($\Delta gcr2::URA3$ -big), which showed almost the same phenotype as that of DFY643, but retains only the N-terminal 22% of the gene. As summarized in Table 2 (lines 1–8, YHU3002-8C column), the mutant plasmids suppressed the growth defect of YHU3002-8C to a similar degree as that of DFY643 transformants, indicating Gcr2p was not required.

Identification of the suppressor mutation sites and their effect on Gcr1p function: First, the *GCR1* gene

TABLE 2
Growth on plates

Plasmid			Recipient strain ^b							
			DFY643 ($\Delta gcr2::URA3$) Gcr2p (1–398)			YHU3002-8C ($\Delta gcr2::URA3$ -big) Gcr2p (1–117)			MWGL29 (<i>gcr1-6</i>) Gcr1p (1–357)	
			Growth (colony size, mm) on ^c							
			G 30°	GL 30°	G + A 37°	G 30°	GL 30°	G + A 37°	G 30°	GL 30°
Line ^a	Name	Description								
1	PD303-2	Gcr1mut1	1.1	0.6	0.9	1.1	0.6	0.8		
2	PD304-1	Gcr1mut1	1.1	0.6	0.9	1.1	0.6	0.8		
3	PD308-1	Gcr1mut1	1.1	0.6	0.9	1.1	0.6	0.8		
4	PD305-2	Gcr1mut1	1.1	0.6	0.9	1.1	0.6	0.8	1.0	0.6
5	PD302-2	Gcr1mut2	1.1	0.6	0.6	1.0	0.5	0.5	1.0	0.6
6	PD306-1	Gcr1mut3	1.1	0.5	0.6	1.0	0.6	0.5	1.0	0.6
7	PD307-1	Gcr1mut4	1.1	0.6	0.7	1.0	0.6	0.4	<0.1	0.6
8	pGCR8	Gcr1	0.8	0.4	<0.1	0.6	0.6	<0.1	1.0	0.6
9	pL205-5	Gcr1mut1 + 2	1.1	0.7	1.0	1.2	0.6	1.0	1.1	0.7
10	pL206-5	Gcr1mut1 + 3	1.2	0.7	1.0	1.3	0.7	1.1	1.1	0.7
11	pL207-2	Gcr1mut1 + 4	1.1	0.7	1.0	1.2	0.7	1.1	0.1	0.7
12	pL113-8	Gcr1'	1.0	0.6	0.4	0.7	0.6	0.3	<0.1	0.7
13	PD332-1	<i>gcr1-6</i>	0.7	0.6	<0.1	0.7	0.6	<0.1	<0.1	0.7
14	PD366-1	<i>gcr1-7</i>	0.7	0.6	<0.1	0.7	0.6	<0.1	<0.1	0.7
15	pL212-1	PD307-1N-term	1.1	0.7	0.7	1.1	0.7	0.7	<0.1	0.7
16	pL213-1	PD307-1C-term	0.9	0.6	0.1	0.9	0.6	<0.1	<0.1	0.7
17	YEpl3	Vector	0.9	0.4	<0.1	0.9	0.6	<0.1	<0.1	0.6
18	pL279-22	Gcr1mut1				1.2	0.7	0.2		
19	pL280-9	Gcr1mut4				1.0	0.7	<0.1		
20	pL278-5	Gcr1				0.8	0.7	<0.1	1.1	0.6
21	pL281-11	<i>gcr1-6</i>							<0.1	0.6
22	pL282-19	<i>gcr1-7</i>							<0.1	0.6
23	pRS315	Vector							<0.1	0.6

of PD305-2 suppressor was divided into five fragments (fragments A–E in Figure 1), and the whole region of *GCR1* covering –300 of 5' noncoding region to +2652 (118 bp after the termination codon) was sequenced. As indicated in Table 4, a single C to T substitution was observed in the 150th codon (Gcr1mut1). Assuming this region is important to activate Gcr1p, fragments D of other mutants were also sequenced. The same mutation was found in PD303-2, PD304-1 and PD308-1, the other three group 1 suppressors, indicating that the mutation at the 150th codon (from His to Tyr) is very important to activate Gcr1p. On the other hand, no mutation was found in fragment D of group 2 mutants. Therefore, for PD302-2, PD306-1 and PD307-1 the entire 3-kb region was sequenced as for PD305-2. Mutation sites are summarized in Table 4 and Figure 1. All mutants had single base mutations (C to T, or G to A, as expected for hydroxylamine mutagenesis). Mutations in PD302-2 and PD306-1 were Pro to Ser (codon 471, Gcr1mut2) and Gly to Glu (codon 364, Gcr1mut3), respectively. The very interesting and unexpected observation was the mutation in PD307-1, which had an amber mutation in codon 451 (Gcr1mut4).

According to HUIE *et al.* (1992), the DNA-binding domain of Gcr1p is in the C-terminal region (from residue 690 to 844) of Gcr1p. Because the amber mutation site of PD307-1 is upstream of the DNA-binding domain, it is expected that the truncated Gcr1p of PD307-1 (Gcr1p [1–450]) has lost the DNA-binding activity. If the DNA binding activity of Gcr1p is essential for its function, this truncated Gcr1p should have lost the Gcr1 activity. To test this, plasmids with mutant *GCR1* genes were transformed into a *gcr1-6* strain, MWGL29 (as described below, it has an amber mutation at codon 358 of *GCR1*). MWGL29 (*gcr1-6*) can hardly grow on glucose (Table 2, line 17 of MWGL29 column), and, as expected, PD307-1 could not complement it (line 7 of MWGL29 column). The other suppressors tested retained Gcr1 activity and complemented the growth defect of MWGL29 (lines 4, 5, 6 and 8, MWGL29 column). Indeed, these suppressors were also effective in the double mutant strain YHU3016 (*gcr1-6*, $\Delta gcr2::URA3$ -big). As shown in Table 2, transformation of wild-type *GCR1* (line 8) complemented only the growth defect on glucose at 30°, whereas the transformation of suppressor mutants sup-

TABLE 2

Continued

Line ^a	Plasmid		Recipient strain ^b								
			NW8-10-1 (<i>gcr1-7</i>) Gcr1p (1-734)	YHU3016 (<i>gcr1-6</i> Δ <i>gcr2::URA3</i> -big) Gcr1p (1-357) Gcr2p (1-117)			C179-15C (Δ <i>gcr1::URA3</i>) Gcr1 (null)				
				Growth (colony size, mm) on ^c							
				G 30°	GL 30°	G 30°	GL 30°	G + A 37°	G 30°	GL 30°	G + A 37°
	Name	Description									
1	PD303-2	Gcr1mut1									
2	PD304-1	Gcr1mut1									
3	PD308-1	Gcr1mut1									
4	PD305-2	Gcr1mut1			1.2	0.6	0.8	1.2	0.7	1.2	
5	PD302-2	Gcr1mut2			1.1	0.6	0.6	1.2	0.7	1.2	
6	PD306-1	Gcr1mut3			1.2	0.6	0.6	1.2	0.7	1.2	
7	PD307-1	Gcr1mut4	1.1	0.6	0.1	0.6	<0.1	0.9	0.7	0.4	
8	pGCR8	Gcr1	1.1	0.6	1.0	0.3	<0.1	1.2	0.7	1.2	
9	pL205-5	Gcr1mut1 + 2									
10	pL206-5	Gcr1mut1 + 3									
11	pL207-2	Gcr1mut1 + 4									
12	pL113-8	Gcr1'	<0.1	0.6							
13	PD332-1	gcr1-6	0.6	0.6							
14	PD366-1	gcr1-7	<0.1	0.6							
15	pL212-1	PD307-1N-term	<0.1	0.6							
16	pL213-1	PD307-1C-term	<0.1	0.6							
17	YEp13	Vector	<0.1	0.6	<0.1	0.6	<0.1	<0.1	0.7	<0.1	
18	pL279-22	Gcr1mut1	1.0	0.6	1.2	0.6	0.4	1.2	0.7	1.2	
19	pL280-9	Gcr1mut4	0.8 ^d	0.6	0.1	0.5	<0.1	0.6	0.7	0.2 ^e	
20	pL278-5	Gcr1	1.0	0.6	0.9	0.2	<0.1	1.2	0.7	1.2	
21	pL281-11	gcr1-6	0.3	0.6							
22	pL282-19	gcr1-7	<0.1	0.6							
23	pRS315	Vector	<0.1	0.6	0.1	0.4	<0.1	<0.1	0.7	<0.1	

^a Entries 1-17 are YEp plasmids, and 18-23 are YCp plasmids.^b 2845 (α *leu2-3 leu2-112 ura3-52 his6*) was used as a wild-type strain. Other strains were derived from 2845 (UEMURA and FRAENKEL 1990).^c The strains were streaked on synthetic drop-out plates (SC -Leu) containing 2% glucose (G), 2% glycerol plus 2% lactate (GL) or 2% glucose plus antimycin A (1 μ g/ml) (G + A), and average colony size was measured after incubation at either 30° or 37° for 3 days. Growth of 2845 on complete synthetic plates was 1.2 mm (G, 30°), 0.7 mm (GL, 30°) and 1.1 mm (G + A, 37°).^d Colony size was heterogeneous (0.6-0.9).^e Colony size was heterogeneous (0.1-0.4).

pressed the growth defect on glucose plus antimycin A at 37°, too. And as expected, PD307-1 [Gcr1p (1-450)], which could not complement *gcr1-6*, did not suppress the double mutant.

Double mutants of *GCR1* were also constructed (MATERIALS AND METHODS); pL205-5, pL206-5 and pL207-2 have Gcr1mut2, Gcr1mut3 and Gcr1mut4 mutations together with Gcr1mut1. Suppression in DFY643 (Δ *gcr2::URA3*) and YHU3002-8C (Δ *gcr2::URA3*-big) was a little stronger for the double mutants than for Gcr1mut1 alone, as shown on glucose plates containing antimycin A at 37° (Table 2, lines 9-11), as well as by their slightly higher glycolytic enzyme levels (Table 3, lines 7-9). The chimeric genes also complemented the *gcr1-6* mutant strain MWGL29, except (as expected)

the one carrying amber mutation 4 (i.e., Gcr1mut1 + 4) (Table 2, lines 9-11).

Gcr1p (1-450), which cannot complement *gcr1-6* (Gcr1p [1-357]) mutation, can complement *gcr1-7* (Gcr1p [1-734]) and *gcr1*-null mutations: Although the above experiments with allele *gcr1-6* (Gcr1 [1-357]) suggested that Gcr1 function—at least—required complete Gcr1p, even this assumption proved to be incorrect. Gcr1mut4 (1-450), which (as described) did not suppress *gcr1-6*, did suppress (Table 2, line 7) another *gcr1* mutant strain NW8-10-1 (*gcr1-7*, see MATERIALS AND METHODS), and sequencing showed *gcr1-7* to be an amber mutation at codon 735. Complementation was confirmed by the assay of several glycolytic enzymes (Table 3, lines 13 and 14). The *gcr1-7* mutant strain

TABLE 3
Relative enzyme activities in transformants

Line	Strain	Plasmid		Relative enzyme activity ^a			
		Name	Description	Pgk	Gpm	Eno	Zwf
1	2845 (wild type)	None		1.0	1.0	1.0	1.0
2	YHU3002-8C	PD305-2	Gcr1mut1	0.44	0.49	0.33	1.15
3	($\Delta gcr2::URA3-big$)	PD302-2	Gcr1mut2	0.70	0.29	0.43	1.63
4	(Gcr2p [1-117])	PD306-1	Gcr1mut3	0.40	0.35	0.49	1.12
5		PD307-1	Gcr1mut4	0.37	0.34	0.24	1.13
6		pGCR8	Gcr1	0.19	0.16	0.18	0.93
7		pL205-5	Gcr1mut1 + 2	0.54	0.54	0.50	1.07
8		pL206-5	Gcr1mut1 + 3	0.80	0.62	0.82	1.09
9		pL207-2	Gcr1mut1 + 4	0.51	0.38	0.33	1.29
10		pL113-8	Gcr1'	0.53	0.38	0.32	1.11
11		pL212-1	PD307-1N-term	0.23	0.33	0.34	0.88
12		pL213-1	PD307-1C-term	0.14	0.15	0.16	0.92
13	NW8-10-1	PD307-1	Gcr1mut4	0.65	0.22	0.42	1.34
14	(<i>gcr1-7</i>)	pGCR8	Gcr1	0.66	0.72	0.96	1.10
15	(Gcr1p [1-734])	pL113-8	Gcr1'	0.22	0.07	0.14	1.24
16		PD332-1	<i>gcr1-6</i>	0.26	0.11	0.22	1.31
17		PD366-1	<i>gcr1-7</i>	0.15	0.05	0.08	1.20
18		pL212-1	PD307-1N-term	0.18	0.06	0.08	1.28
19		pL213-1	PD307-1C-term	0.24	0.05	0.09	1.29
20		YEpl3	Vector	0.24	0.10	0.12	1.29
21	C179-15C	PD305-2	Gcr1mut1	0.49	0.37	0.57	1.03
22	($\Delta gcr1::URA3$)	PD307-1	Gcr1mut4	0.42	0.23	0.33	0.84
23	(Gcr1 null)	pGCR8	Gcr1	0.43	0.41	0.42	1.00
24		YEpl3	Vector	0.25	0.07	0.12	1.27
25		pL279-22	Gcr1mut1	0.47	0.50	0.71	0.80
26		pL280-9	Gcr1mut4	0.40	0.20	0.70	1.04
27		pL278-5	Gcr1	0.48	0.51	0.70	0.89
28		pRS315	Vector	0.20	0.04	0.15	0.93

^a Enzyme activities in 2845 were 2.602 μ mol/min mg (phosphoglycerate kinase, Pgk), 1.316 μ mol/min mg (phosphoglycerate mutase, Gpm), 0.146 μ mol/min mg (enolase, Eno) and 0.302 μ mol/min mg (glucose 6-phosphate dehydrogenase, Zwf).

was also suppressed, although incompletely, by the *gcr1-6* plasmid (amber mutation at codon 358) (Table 2, line 13 and Table 3, line 16); the reciprocal complementation was negative, *i.e.*, *gcr1-7* on a plasmid and *gcr1-6* chromosomal (Table 2, line 14). Neither plasmid suppressed *gcr2* mutations (Table 2, lines 13 and 14). Another plasmid, pL113-8, that specifies a truncated Gcr1p (1-629) fused to 23 amino acids from the vector (see MATERIALS AND METHODS) weakly suppressed *gcr2* mutations (Table 2, line 12 of DFY643 and YHU3002-8C columns, and Table 3, line 10), but it did not suppress *gcr1-7* (Table 2, line 12, and Table 3, line 15).

Certain of the mutations were also assessed on centromere-based constructions (MATERIALS AND METHODS) (lines 18-22, Table 2). Complementation of *gcr2* in single copy plasmid was feeble (group 1 mutation Gcr1mut1) or absent (group 2 mutation Gcr1mut4), although both complemented *gcr1-7* (Table 2, lines 18 and 19). *gcr1-6* in single copy plasmid weakly complemented *gcr1-7* (Table 2, line 21).

A surprising result was the complementation of C179-15C. C179-15C is a null mutant of *GCR1*. To construct

it, the whole coding region of *GCR1* (from -669 *HindIII* site to *NcoI* site at ~1.9 kb downstream of open reading frame, corresponds to the region J of Figure 1) was substituted with *URA3*. In contrast to the complementation of *gcr1-6* strain (amber mutation at codon 358), C179-15C was partially complemented by PD307-1 (Gcr1p [1-450]) (Table 2, line 7). Lower but significant complementation was observed in single copy construct, too (line 19). Complementation was also confirmed in the assay of several glycolytic enzymes (Table 3, lines 22 and 26).

The mechanism of suppression of *gcr2* by *gcr1* alleles: Regarding suppression of *gcr2*, perhaps the most striking result was for Gcr1mut4, which, with an amber mutation at codon 451, presumably makes only that fragment of the protein. Another plasmid, pL212-1, which is deleted for material 3' to codon 594, also suppressed *gcr2*, and a construction for the C-terminal portion pL213-1 did not suppress (Table 2, lines 15 and 16 and Table 3, lines 18 and 19). These results show that C-terminal material beyond codon 594 is not required for suppression and, likely, that fragment 1-450 is sufficient.

TABLE 4
Mutation sites

Plasmid		Mutation	
Name	Description	Position (residues at)	Base (amino acid) changed ^a
PD303-2	Gcr1mut1	150	CAC (His) → TAC (Tyr) ^b
PD304-1	Gcr1mut1	150	CAC (His) → TAC (Tyr) ^b
PD305-2	Gcr1mut1	150	CAC (His) → TAC (Tyr)
PD308-1	Gcr1mut1	150	CAC (His) → TAC (Tyr) ^b
PD302-2	Gcr1mut2	471	CCG (Pro) → TCG (Ser)
PD306-1	Gcr1mut3	364	GGA (Gly) → GAA (Glu)
PD307-1	Gcr1mut4	451	CAG (Gln) → TAG (Amber)
PD332-1	gcr1-6	358	CAG (Gln) → TAG (Amber)
PD366-1	gcr1-7	735	TGG (Trp) → TAG (Amber)
pL113-8	Gcr1'	629	GAT (Asp)/C (Pro) fused to vector DNA ^c

^a Changed nucleotides are underlined.

^b For PD303-2, PD304-1 and PD308-1 only fragment D in Figure 1 were sequenced.

^c Based on the DNA sequencing, 23 amino acids (GlySerLeuPheLeuGlyLeuValGlyTyrLeuHisHisArgIleAsp-SerTyrIleHisHisProLeu) would be added after codon 629 (Pro).

To test whether this latter fragment—by contrast with intact Gcr1p—is conferring transcriptional activation, the two-hybrid system of FIELDS and SONG (1989) was employed. As previously shown (UEMURA and JIGAMI 1992a), the low activation of *GAL1-lacZ* by Gal4B/Gcr2p (48–534) alone was decreased by pGCR8 but increased with a Gal4A/Gcr1p (68–844) fusion (Table 5, lines 10–12). Interestingly, Gcr1mut4, a truncated Gcr1p (lacking Gal4A), gave a much higher increase (Table 5, line 14). The missense mutant Gcr1mut1 gave a threefold increase (Table 5, compare line 12 and line 13). Low activation was also observed for pL113-8 (Gcr1p [1–629]) (line 15) but none with PD332-1 (*gcr1-6*) and PD366-1 (*gcr1-7*), i.e., Gcr1p (1–357) and Gcr1p (1–734), respectively (lines 16 and 17). As shown above, *gcr2* suppression by these three plasmids was moderate (pL113-8) or absent (PD332-1 and PD366-1). All the positive activations of *GAL1-lacZ* required Gal4B/Gcr2p (48–534) (Table 5, lines 1–9). The simplest interpretation of these results is that Gcr1p (1–450) is interacting with Gcr2p and conferring activation without a requirement for Gal4A. The higher β -galactosidase activity derived from Gal4B/Gcr1p (68–450) ([pL321-5], 230 units) than that of Gal4B/Gcr1p (68–844) ([pML78-2], 3 units) also supported this interpretation (Table 6, lines 15 and 17).

Recalling that Gcr1p (1–450) does not have Gcr1 activity alone (because it did not suppress *gcr1-6*), it is likely that its suppression of *gcr2* requires Gcr1p and, hence, that Gcr1p acts at least as a dimer. This possibility was tested with the two-hybrid system using a Gal4B/Gcr1p (68–844) fusion as the first plasmid. Substantial activation was observed with Gcr1mut4 (Table 6, line 13) but not with Gcr1p or missense mutant Gcr1mut1 (line 10 and 12), and the activation was dependent on

the Gal4B/Gcr1p first plasmid (see line 6). Significant, but low, activation was also observed with Gal4A/Gcr1p (68–884) and with Gcr1p (1–629) (lines 11 and 14).

DISCUSSION

For the expression of glycolytic genes, Gcr1p and Gcr2p are required as specific regulatory factors. In the previous paper we provided evidence that suggested Gcr1p and Gcr2p work as a complex, with specific DNA-binding activity in Gcr1p and transcriptional activation contributed by Gcr2p. The present results, of specific mutations in *GCR1* suppressing *gcr2*, are additional genetic evidence for Gcr1p/Gcr2p interaction. They also show, through use of the two-hybrid system, a potent activating function residing in the N-terminal half of Gcr1p itself, as seen with the amber mutant Gcr1mut4 (Gcr1p [1–450]) (Table 6, compare lines 15 and 17 and Table 5, line 14). This latter result also points to the Gcr1p/Gcr2p interaction involving the N-terminal half of Gcr1p.

Evidence for an activation domain in Gcr1p has also been presented using LexA/Gcr1p fusions (SCOTT and BAKER, 1993; TORNOW *et al.*, 1993). Although these results differ somewhat from ours in the extent of activation observed with (near) full-length Gcr1p (being low in Table 6, line 15), the units employed (U/OD₆₀₀) differ from the units (U/mg protein) by a factor of ~5. Also, the constructions are quite different, *lexA* vs. Gal4B fusions as well as the reporter constructs (*lexA_{op}-lacZ* or *GAL1-lacZ*), and GOLEMIS and BRENT (1992) have shown that the heterologous fusion domains greatly affect the DNA binding by the LexA moiety. In both TORNOW *et al.* (1993) and the present work, activation is increased by C-terminal truncation of Gcr1p (Table 6, lines 13 and 17).

TABLE 5
Gcr1p/Gcr2p interaction in the two-hybrid system

Line	1st plasmid		2nd plasmid		β -galactosidase activity ^a (U)
	Name	Description	Name	Description	
1	pMA424	Gal4B	None		<1
2	None		pGCR8	Gcr1	<1
3	pMA424	Gal4B	pGCR8	Gcr1	<1
4	pMA424	Gal4B	pML77-8	Gal4A/Gcr1p (68-844)	<1
5	pMA424	Gal4B	PD305-1	Gcr1mut1	<1
6	pMA424	Gal4B	PD307-1	Gcr1mut4	<1
7	pMA424	Gal4B	pL113-8	Gcr1'	<1
8	pMA424	Gal4B	PD332-1	gcr1-6	<1
9	pMA424	Gal4B	PD366-1	gcr1-7	<1
10	pL41-14	Gal4B/Gcr2p (48-534)	None		13
11	pL41-14	Gal4B/Gcr2p (48-534)	pML77-8	Gal4A/Gcr1p (68-844)	63
12	pL41-14	Gal4B/Gcr2p (48-534)	pGCR8	Gcr1	4
13	pL41-14	Gal4B/Gcr2p (48-534)	PD305-1	Gcr1mut1	13
14	pL41-14	Gal4B/Gcr2p (48-534)	PD307-1	Gcr1mut4	282
15	pL41-14	Gal4B/Gcr2p (48-534)	pL113-8	Gcr1'	29
16	pL41-14	Gal4B/Gcr2p (48-534)	PD332-1	gcr1-6	3
17	pL41-14	Gal4B/Gcr2p (48-534)	PD366-1	gcr1-7	4

GGY1::171 (α $\Delta gal4 \Delta gal80 leu2 his3$ with *GAL1-lacZ* integrated at *URA3* locus) was used as the host strain (GILL and PTASNE 1987). Plasmids are described in MATERIALS AND METHODS. Gal4B and Gal4A are DNA-binding domain (residues 1–147) and transcription activation region II (residues 768–881) of Gal4p, respectively. Gcr1' is the same as described in Table 4.

^a Transformants were grown in 2% galactose, 2% ethanol and 2% glycerol and assayed for β -galactosidase activity as described.

The importance of residue 150 is shown by multiple isolations of the same suppressor of *gcr2*, *gcr1mut1*. Interestingly, this missense mutation contributes slightly to activation in the two-hybrid system, and Gcr1mut4, which has an amber mutation in codon 451 and is less active than Gcr1mut1 with respect to the suppression of *gcr2* mutation, showed higher β -galactosidase activity in the two hybrid system (Table 5, lines 13 and 14 and Table 6, lines 12 and 13). Possibly the removal of the Gcr1p DNA-binding domain might result in increased levels or activity of the protein within the cell, and that interpretation fits with the *gcr1mut1*+4 double mutant being more active in suppression than either single gene mutant. Another plausible explanation would be that the absence of the Gcr1p DNA-binding domain in such constructions increases the probability of interactions with UAS_{GAL} sites rather than Gcr1p-binding sites (CT-box) and would thus be more active than the Gal4B/Gcr1p hybrid that conserved the DNA-binding domain of Gcr1p. The intermediate β -galactosidase level by Gcr1p (1–629), which is less active than Gcr1mut4 in Gcr1 function but has also lost the DNA-binding domain, (Table 5, line 15 and Table 6, line 14), can be likewise explained. The much higher activation by Gal4B/Gcr1mut4 (nonsense mutant) than Gal4B/Gcr1p or Gal4B/Gcr1mut1 (missense mutant) also supports this possibility (Table 6, lines 15–17).

The observation that the overexpressed (multicopy) intact Gcr1p was inhibitory in the two-hybrid system

with both Gal4B/Gcr2p (Table 5, line 12) and Gal4B/Gcr1p (Table 6, line 10) can be explained the same way. Probably the hybrid will interact more with CT-box of many glycolytic genes rather than the UAS_{GAL} site because of the overexpression of intact Gcr1p. Therefore, the recovery of β -galactosidase activity in the transformant of Gcr1mut1 might be the compensatory effect of activation mutation of Gcr1p and the inhibitory effect by the DNA binding activity of Gcr1p.

In the context of the new results, we would speculate that rather than transcriptional activation residing only in Gcr2p, that the activation by the complex at least in part resides in exposure of an otherwise cryptic activation domain of Gcr1p. Several findings reported here also point to Gcr1p/Gcr1p interaction, *i.e.*, its acting as a dimer or an oligomer. One is that the suppressor *gcr1mut4* (Gcr1p [1–450]) does not have Gcr1 activity alone (Table 2). Another is the evidence for such interaction observed with the two-hybrid system (Table 6). A third is the several cases of complementation of *gcr1* mutations (Table 2). These results do not allow explanation of important details, such as the strength of complementation, effect of gene dose and nonreciprocity of complementation of *gcr1-6* and *gcr1-7* even in low copy. However, overall they fit with the necessity of at least the DNA-binding domain on one partner; the data of HUIE *et al.* (1992), which demonstrated that the C-terminal 155 amino acids (residues 690–844) retained DNA binding activity but that the

TABLE 6
Gcr1p/Gcr1p interaction in the two-hybrid system

Line	1st plasmid		2nd plasmid		β -galactosidase activity (U)
	Name	Description	Name	Description	
1	pMA424	Gal4B	YEpl3	Vector	<1
2	pMA424	Gal4B	pCTC13	Gal4A	<1
3	pMA424	Gal4B	pGCR8	Gcr1	<1
4	pMA424	Gal4B	pML77-8	Gal4A/Gcr1	<1
5	pMA424	Gal4B	PD305-1	Gcr1mut1	<1
6	pMA424	Gal4B	PD307-1	Gcr1mut4	<1
7	pMA424	Gal4B	pL113-8	Gcr1'	<1
8	pML78-2	Gal4B/Gcr1	YEpl3	Vector	5
9	pML78-2	Gal4B/Gcr1	pCTC13	Gal4A	4
10	pML78-2	Gal4B/Gcr1	pGCR8	Gcr1	<1
11	pML78-2	Gal4B/Gcr1	pML77-8	Gal4A/Gcr1	15
12	pML78-2	Gal4B/Gcr1	PD305-1	Gcr1mut1	2
13	pML78-2	Gal4B/Gcr1	PD307-1	Gcr1mut4	86
14	pML78-2	Gal4B/Gcr1	pL113-8	Gcr1'	14
15	pML78-2	Gal4B/Gcr1	None		3
16	pL527-2	Gal4B/Gcr1mut1	None		10
17	pL321-5	Gal4B/Gcr1mut4	None		230

The host strain and the assay condition are the same as described in Table 5. Gal4B/Gcr1 and Gal4A/Gcr1 are fusions of Gcr1p (68–844) to the DNA-binding domain and activation region of Gal4p, respectively.

139 amino acids (residues 706–844) could not bind DNA, indicated that the 16 amino acids between residues 690 and 706 are essential for DNA binding of Gcr1p. This region is retained in *gcr1-7* product (Gcr1p [1–734]). The other partner would have to provide elements from the N-terminal half, *i.e.*, for interaction with Gcr2p and transcriptional activation. The complexities of Gcr1p/Gcr1p interaction are illustrated by the fact that Gcr1p (1–734) does not carry complete Gcr1 function—implying the need for C-terminal material—and yet is complemented (albeit in multicopy) by Gcr1p (1–450).

TORNOW *et al.* (1993) have recently reported the unexpected finding that plasmid-carried Gcr1p (1–443) suppressed the virtual null mutation Gcr1p (1–65), demonstrating Gcr1 function (albeit in multicopy) in the absence of its known (HUIE *et al.* 1992) C-terminal DNA-binding domain. It should be noted, however, that Gcr1p (1–734), which does not complement Gcr1p (1–375) as mentioned, probably contains the DNA-binding domain, and that Gcr1p (1–734), like Gcr1p (1–450) and other *gcr1* alleles, itself alone confers a typical *gcr1* phenotype. Thus, the Gcr1p DNA-binding domain and other C-terminal functions are needed for function of the normal protein.

We are grateful to STANLEY FIELDS for generous gifts of plasmids pCTC13 and pMA424 and to MARK PTASHNE for yeast strain GGY1::171. We also thank DAN FRAENKEL for his comments and critical review of the manuscript.

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Communicating editor: M. CARLSON