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Role of GCR2 in Transcriptional Activation of Yeast Glycolytic Genes

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The *Saccharomyces cerevisiae GCR2* gene affects expression of most of the glycolytic genes. We report the nucleotide sequence of *GCR2*, which can potentially encode a 58,061-Da protein. There is a small cluster of asparagines near the center and a C-terminal region that would be highly charged but overall neutral. Fairly homologous regions were found between Gcr2 and Gcr1 proteins. To test potential interactions, the genetic method of S. Fields and O. Song (Nature [London] 340:245–246, 1989), which uses protein fusions of candidate gene products with, respectively, the N-terminal DNA-binding domain of Gal4 and the C-terminal activation domain II, assessing restoration of Gal4 function, was used. In a *Δgal4 Δgal80* strain, double transformation by plasmids containing, respectively, a Gal4 (transcription-activating region)/Gcr1 fusion and a Gal4 (DNA-binding domain)/Gcr2 fusion activated *lacZ* expression from an integrated *GAL1/lacZ* fusion, indicating reconstitution of functional Gal4 through the interaction of Gcr1 and Gcr2 proteins. The Gal4 (transcription-activating region)/Gcr1 fusion protein alone complemented the defects of both *gcr1* and *gcr2* strains. Furthermore, a Rap1/Gcr2 fusion protein partially complemented the defects of *gcr1* strains. These results suggest that Gcr2 has transcriptional activation activity and that the *GCR1* and *GCR2* gene products function together.

The glycolytic pathway is a major metabolic route in *Saccharomyces cerevisiae*, and most of the genes are highly expressed (16, 19). Their 5′ regions often have a Rap1 (37)-binding site (Rap1 is also known as GRF1 [6] or TUF [23]), and in some cases mutational analysis shows Rap1 binding to be essential for expression (7, 8, 10, 32). CTTCC motifs have also been implicated in expression (7, 9), and some of the genes also have ABF1-binding sites (5, 8). However, neither Rap1-binding sites (23, 29, 37) nor ABF1-binding sites (6) are restricted to glycolytic genes, and any special mechanism for glycolytic gene expression likely involves other elements.

One such element is Gcr1. *gcr1* mutants (1, 12, 13, 27) are severely reduced in expression of most glycolytic enzymes at the transcriptional level. The Gcr1 protein has been proposed as a transcriptional activator (1, 22), and very recently Baker indicated that it binds to the sequence containing a CTTCC motif (2). Recently we reported a new regulatory locus, *GCR2* (38). The levels of reduction of most glycolytic enzymes in *gcr2* mutants are very similar to those in *gcr1* mutants, but the growth defect is less. In this report, we present the *GCR2* sequence and also present genetic evidence indicating that there is physical interaction of Gcr2 and Gcr1 and that normal Gcr2 action likely contributes an activation domain to a Gcr1/Gcr2 complex.

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used are listed in Table 1. *Escherichia coli* DH5α [F⁻ *endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1* Δ(*lacU169* φ80 d*lacA*Δ*M15*)] was used to propagate all plasmids (18).

Matings, diploid selection, sporulation, and dissection were carried out by the usual methods (31). Yeast cells were transformed by the method of Ito et al. (24). Growth on different carbon sources was scored by measuring average colony size under a microscope.

Media. *E. coli* cells were grown in LB (14). Yeast cells were grown in rich medium (41) and synthetic complete medium (SC) (36) or SC dropout medium, depending on the selective pressure for plasmids; 2% 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 μg/ml.

DNA manipulation. Standard techniques for DNA manipulation used in this study are described by Sambrook et al. (35). *S. cerevisiae* chromosomal DNA was prepared by the method of Sherman et al. (36). Isolation of plasmid DNA from yeast cells was done by the method of Hoffman and Winston (21).

Plasmid constructions. Cloned *GCR2* DNA was derived from pGCR2 (38), a derivative of YCp50. pML16-2, pML17-1, pML18-1, and pML19-1 are dropout plasmids of pGCR2 with deletions of *Eco*RI, *Hin*dIII, *Bam*HI, and *Sal*I-*Sac*I fragments, respectively (Fig. 1b) (38). YEp351 (20), a multicopy plasmid with a *LEU2* selection marker, was used to construct a yeast genomic DNA library by cloning partially *Sau*3A-digested chromosomal DNA (average length of 10 to 20 kb) to its *Bam*HI site. PD206-1 and PD210-1 (Fig. 1b) were obtained from this DNA library. pL133-2 contains *GCR2* on YEp351, constructed by cloning a *Sal*I-*Xho*I fragment of pGCR2 into the *Sal*I site of YEp351.

pCL1 is a YCp50 derivative containing the *ADH1* promoter (P_{ADH1})-*GAL4*(1–881) gene (15). pCTC13 is a multicopy plasmid with a *LEU2* marker, kindly provided by Stanley Fields (State University of New York at Stony Brook). It encodes a fusion of a nuclear localization signal and Gal4(768–881) plus additional residues encoded by a *Bam*HI linker sequence under the control of the *ADH1* promoter and terminator. To construct pML77-8, the *Sac*I-

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TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Comment or reference
2845	α *leu2-3 leu2-112 ura3-52 his6*	38
YHU2012	a *leu2-3 leu2-112 ura3-52*	Isogenic with 2845,[a] (38)
MWGL29	α *gcr1-6 leu2-3 leu2-112 ura3-52 his6*	*gcr1* mutant of 2845 (38)
NW9-19-1	α *gcr2-1 leu2-3 leu2-112 ura3-52 his6*	*gcr2* mutant of 2845 (38)
DFY643	α Δ*gcr2*::*URA3 leu2-3 leu2-112 ura3-52 his6*	Δ*gcr2* mutant of 2845 (38)
YHU3002-8A	a Δ*gcr2*::*URA3*-big *leu2-3 leu2-112 ura3-52 his6*	See text
YHU3002-8B	a *leu2-3 leu2-112 ura3-52 GCR2*	See text
YHU3002-8C	α Δ*gcr2*::*URA3*-big *leu2-3 leu2-112 ura3-52 his6*	See text
YHU3002-8D	α *leu2-3 leu2-112 ura3-52 GCR2*	See text
GGY1::171	α Δ*gal4* Δ*gal80 leu2 his3*	*GAL1-lacZ* fusion gene integrated at the *URA3* locus (17)
DFY644	a Δ*gcr1*::*LEU2 leu2-3 leu2-112 ura3-52 his3*	38

[a] YHU2012 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.

*Xho*I fragment of pGCR8, which contains residues 68 to 844 of Gcr1, was blunted with T4 DNA polymerase and ligated to pCTC13 that had been digested with *Bam*HI and treated with Klenow fragment. This construction yielded an in-frame fusion between codon 881 of Gal4 and codon 68 of Gcr1 separated by five codons contributed by linker sequence.

pMA424 (30) is a multicopy plasmid with *HIS3* and encodes Gal4(1–147), plus additional residues encoded by a polylinker sequence, under the control of the *ADH1* promoter and terminator. pMA424E was constructed by digesting pMA424 with *Eco*RI, filling in the 5′ protruding end with Klenow fragment, and religation. Gal4(1–147)/Gcr1 fusion plasmid pML78-2 was constructed as follows. The same *Sac*I-*Xho*I fragment of pGCR8 used to construct pML77-8 was blunted with T4 DNA polymerase and ligated to pMA424E that had been digested with *Bam*HI and treated with Klenow fragment. This construction yielded an in-frame fusion between codon 147 of Gal4 and codon 68 of Gcr1 separated by seven codons contributed by the polylinker sequence. pL83-11 is a *LEU2* version of pML78-2, made by digestion of pML78-2 with *Bgl*II to delete a part of *HIS3* and replacement with the *LEU2*-containing *Bgl*II fragment of YEp13. Two kinds of Gal4(1–147)/Gcr2 fusion plasmids were constructed. For pL41-14, the *Hpa*I fragment of pGCR2, which contains residues 48 to 534 of Gcr2, was ligated to pMA424 that had been digested with *Bam*HI and treated with Klenow fragment. This construction yields an in-frame fusion between codon 147 of Gal4 and codon 48 of Gcr2 separated by six codons contributed by the polylinker sequence. To construct pL46-1, the *Sal*I-*Xho*I fragment of PD206-1 (the *Sal*I site being derived from the polylinker sequence of YEp351), which contains residues 223 to 534 of Gcr2, was ligated to pMA424E that had been digested with *Sal*I. This construction yielded an in-frame fusion between codon 147 of Gal4 and codon 223 of Gcr2 separated by 12 codons contributed by the linker sequence (Fig. 1d).

For *GCR1* and *GCR2* under P_{ENO1} control, first a multicopy plasmid with the *ENO1* promoter plus initiation codon, pML53-1, was constructed as follows. YEp352 (20), a multicopy plasmid with a *URA3* selection marker, was digested with *Eco*RI plus *Bam*HI, and the *Eco*RI-*Bam*HI fragment of pMC1403-ENO-C (39), which contains 724 bp of *ENO1* 5′ noncoding region and a *Bam*HI restriction site just downstream of ATG, was ligated. To express *GCR1*, pL58-11 was constructed. The same *Sac*I-*Xho*I fragment of pGCR8 was blunted by T4 DNA polymerase and ligated into pML53-1 that had been digested with *Bam*HI and treated with Klenow fragment. This construction made the 68th codon of Gcr1 the 6th codon of truncated Gcr1 as a result of the linker sequence (Fig. 1d). *GCR2* was expressed from pL45-3. The *Sac*I-*Xho*I fragment of PD206-1 was ligated into pML53-1 which had been digested with *Sal*I. This construction made 724 bp of 5′ *ENO1* noncoding region plus ATG fused in frame to codon 223 of Gcr2 separated by 10 codons contributed by the polylinker sequence (Fig. 1b).

pL88-1 contains *RAP1* on YEp352, constructed by cloning an *Eco*RI fragment of D56 into the *Eco*RI site of YEp352. D56 is a pUC19 derivative containing *RAP1*, and it was kindly provided by David Shore (Columbia University). Rap1/Gcr2 fusion plasmid pL87-6 was constructed as follows. First, a *Bgl*II fragment of D56, which contains the 5′ noncoding region and residues of 1 to 701 of Rap1, was ligated into the *Bam*HI site of YEp352. Then it was partially digested with *Sac*I, and the *Sac*I fragment of pML41-1, a plasmid containing *GCR2* on YEp352 (38), which contains residues 118 to 534 of Gcr2, was ligated. This construction yielded an in-frame fusion between codon 701 of Rap1 and codon 118 of Gcr2 separated by four codons contributed by linker sequence.

Nucleotide sequence analysis. Restriction fragments were cloned into M13mp18 and M13mp19 (33), and the sequence was determined by the dideoxy-chain termination method, using an Applied Biosystems model 373A DNA sequencer (Applied Biosystems, Foster City, Calif.) with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) and the 18-nucleotide sequencing primer −21M13 primer (Applied Biosystems).

Enzyme assays. For β-galactosidase assays, transformants were grown to mid-log phase in synthetic dropout medium (36) containing 2% each galactose, ethanol, and glycerol, and β-galactosidase activity was assayed as described previously (40).

For glycolytic enzyme assays, transformants were grown to mid-log phase in synthetic dropout medium containing 2% each glycerol and lactate. Cells extracts were prepared by vortexing cells with glass beads for 2 min (four times for 30 s each time) at 4°C and assayed as described previously (38).

Nucleotide sequence accession number. The sequence shown in Fig. 2 has been given GenBank/EMBL/DDBJ accession number D10104.

RESULTS

Sequence of the *GCR2* gene. Dropout plasmid pML16-2 complemented *gcr2*, but pML17-1, pML18-1, and pML19-1

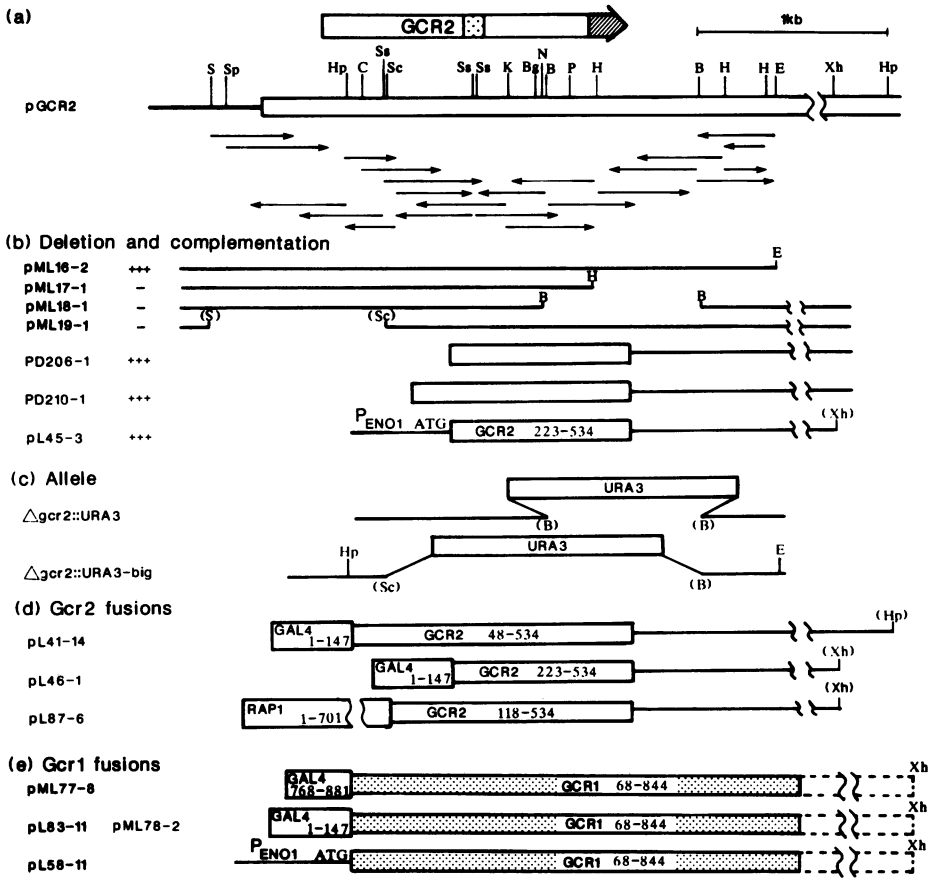

FIG. 1. DNA sequencing strategy and plasmids. Plasmids are described in the text. (a) DNA sequencing strategy. Open box and thin line indicate cloned yeast DNA and YCp50 DNA, respectively. The open arrow above the map denotes the long open reading frame proposed to encode the *GCR2* gene product. Dotted and hatched areas indicate the asparagine-rich region and the region containing 38% of charged residues. The arrows below the map represent the direction and extent of sequences obtained from a given M13 clone. (b) Deletion and complementation. Deleted regions in dropout plasmids are indicated by blanks. Complementation of a *gcr2* mutation was judged by the growth of transformants of NW9-19-1 on glucose-plus-antimycin A plates at 37°C. (c) Allele designations of chromosomal *gcr2* mutations constructed with plasmids. The *URA3* replacement Δ*gcr2*::*URA3* was described previously (38); Δ*gcr2*::*URA3*-big was constructed analogously but by replacing the *Sac*I-to-*Bam*HI region of *GCR2* with the *URA3* gene and integrating the *Hpa*I-*Eco*RI fragment into the genome of diploid strain at the *GCR2* locus, followed by dissection. (d) Fused *GCR2* genes. (e) Truncated and fused *GCR1* genes. Coding regions of Gcr1 (residues 68 to 844) are indicated by dotted bars. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Hp, *Hpa*I; K, *Kpn*I; N, *Nco*I; P, *Pst*I; Sc, *Sac*I; S, *Sal*I; Sp, *Sph*I; Ss, *Sst*I; Xh, *Xho*I. Restriction sites in parentheses are not conserved.

did not (Fig. 1b); thus, *GCR2* likely was in the ca. 2.9-kb *Eco*RI-*Sal*I fragment. The nucleotide sequence of this fragment was determined. The sequencing strategy is shown in Fig. 1a. A single open reading frame containing 534 codons was identified. The size and location of this open reading frame accorded well with the results of a complementation test. Figure 2 shows the nucleotide sequence and the amino acid sequence of the predicted 58,061-Da protein. The nucleotide sequence was compared with the sequences in GenBank, but no significant homology to other gene sequences was found. Comparison of the deduced amino acid sequence of Gcr2 with other sequences in EMBL protein data base showed no significant homology with any catalogued sequences. The codon usage of *GCR2* is not biased (codon bias index [3] of −0.036), which suggests that Gcr2 is a regulatory protein or is expressed at a low level.

We cite four features of the amino acid sequence. First, there is a cluster of 15 asparagines (52%) between amino acids 255 and 283. Second, the C-terminal region is highly charged, the segment from amino acids 474 to 534 containing 12 acidic residues and 11 basic residues (38%). According to the rules of Chou and Fasman (11), this sequence might be an α helix. Third, a possible nuclear localization signal was found at codons 281 to 288. Figure 3 shows a comparison of this sequence with some known nuclear localization sequences. Fourth, the most striking feature of the predicted Gcr2 protein is the considerable stretch of sequence similarity, based on the method of Lipman and Pearson (28), with the Gcr1 protein (Fig. 4). Between residues 250 and 483 of Gcr2, 17% identical (40 residues) and 52% conservative replacement (122 residues) were obtained when several gaps were introduced into the sequence. Within this region, residues 329 to 347 and 393 to 422 of Gcr2 had 58 and 37% identity, respectively, with stretches of Gcr1. Neither gene in multicopy suppressed a mutant of the other gene (38), arguing against similar functions. Rather, the protein similarity suggests the possibility that they function together (as discussed below).

Deletion of most of the *GCR2* coding sequence is not lethal. Previously, the chromosomal *GCR2* locus was disrupted by

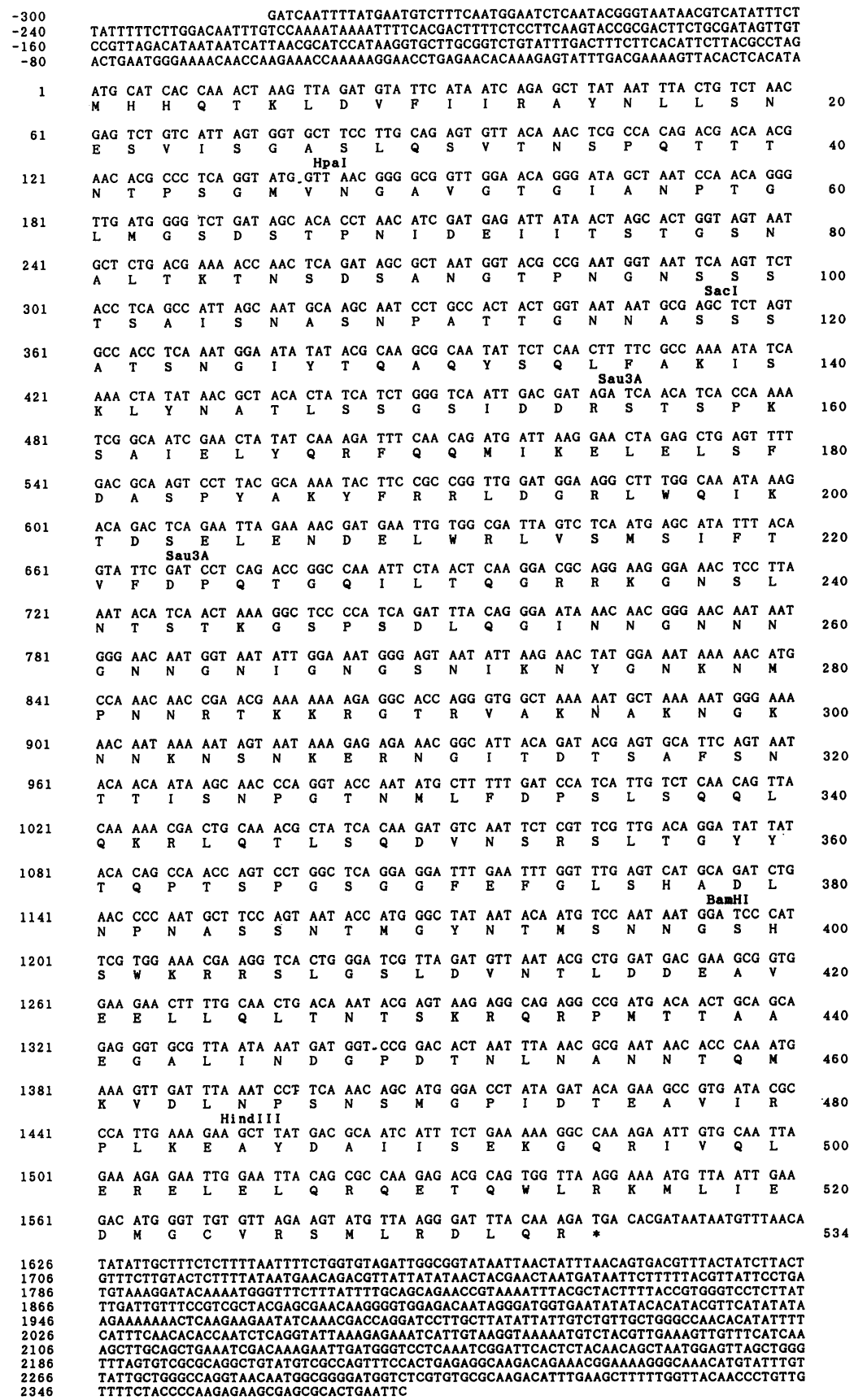

FIG. 2. Nucleotide sequence of the *GCR2* gene and predicted amino acid sequence. Nucleotides are numbered on the left, and amino acids are numbered on the right. The first ATG codon of the open reading frame was assigned the +1 position. Asterisks mark the termination codon. *Hpa*I, *Sac*I, *Sau*3A, *Bam*HI, and *Hin*dIII sites relevant to the construction of plasmids are marked.

SV40 large T	124	Thr Pro Pro Lys Lys Lys Arg Lys Val
SV40 VP1	1	Ala Pro Thr Lys Arg Lys Gly Ser
Polyoma large T	278	Thr Pro Pro Lys Lys Ala Arg Glu Asp
Polyoma large T	188	Pro Val Ser Arg Lys Arg Pro Arg Pro
Gcr2	281	Pro Asn Asn Arg Thr Lys Lys Arg Gly

FIG. 3. Homology with known nuclear localization signals. The Gcr2 sequence from amino acids 281 to 289 is compared with sequences that have been identified as nuclear localization signals in simian virus 40 (SV40) large T antigen (25, 26), simian virus 40 VP1 protein (42), and polyomavirus large T antigen (34). Numbers at the left indicate the position of the first residue shown.

substitution of a *Bam*HI fragment with the *URA3* gene, generating the Δ*gcr2*::*URA3* allele (38) (Fig. 1c). This disruption was not lethal, and its phenotype was the same as that of the original mutant in both enzyme profile and growth; i.e., there was major impairment of growth on glucose only at 37°C in the presence of an inhibitor of respiration, antimycin A (38). Since sequencing revealed that the Δ*gcr2*::*URA3* allele was deleted only for the C-terminal 25%, a larger deletion, Δ*gcr2*::*URA3*-big (entire substitution by *URA3* of the *Sac*I-*Bam*HI region, including 78% of the coding sequence) was also made (Fig. 1c). To construct this strain, the *URA3*-substituted *Hap*I-*Eco*RI fragment (Fig. 1c) was transformed to a diploid between strains 2845 and YHU2012 (*GCR2/GCR2 ura3/ura3*). The diploid transformants grew normally, and 26 tetrads examined showed 2:2 segregation of Ura^+ $Gcr2^-$ and Ura^- $Gcr2^+$. YHU3002-8A, YHU3002-8B, YHU3002-8C, and YHU3002-8D are one such tetrad. The large disruption caused the same growth impairment as did the smaller one (Table 2).

Further evidence for the importance of the C-terminal region of Gcr2 came from the recovery from a library in multicopy vector YEp351 (see Materials and Methods) of clones complementing *gcr2-1*, Δ*gcr2*::*URA3*, and Δ*gcr2*::*URA3*-big but lacking the N-terminal coding sequences, e.g., plasmids PD206-1 and PD210-1 (see Table 4, lines 10 to 15). Restriction enzyme analysis and comparison with the *GCR2* sequence showed that they likely were derived from *Sau*3A fragments beginning at codons 223 and 156 and thus retaining the C-terminal 58 and 71% portions, respectively. (Intact *GCR2* had been obtained by the same selection but with a library in the centromere-based vector YCp50 [38].)

Interaction of Gcr1 and Gcr2. To test whether Gcr2 might interact with Gcr1, we employed the genetic method of Fields and Song, which uses protein fusions of candidate gene products with, respectively, the N-terminal DNA-binding domain of Gal4 (residues 1 to 147; Gal4B) and the C-terminal activation domain II (residues 768 to 881; Gal4A), assessing restoration of Gal4 function in Δ*gal4* Δ*gal80* *GAL1/lacZ* strain GGY1::171 (15). Since it was known that the C-terminal portion of Gcr2 sufficed for complementation of mutants, we made fusions of Gal4B with residues 48 to 538 of Gcr2 [i.e., Gal4B/Gcr2(48–534)] and with residues 223 to 534 [i.e., Gal4B/Gcr2(223–534)] (Fig. 1d). The Gcr1/Gal4A fusion contained at the C terminus residues 68 to 844 of Gcr1 fused in frame to Gal4A [i.e., Gal4A/Gcr1(68–844)] (Fig. 1e).

These plasmids were introduced into strain GGY1::171, which is deleted for both *GAL4* and *GAL80* and contains an integrated *GAL1/lacZ* fusion (17), and transformants were assessed for β-galactosidase activity (Table 3). Intact *GAL4* produced 3,806 U (line 1), whereas, as expected, double transformants of Gal4B and Gal4A portions alone showed no activity (line 3). Gal4B alone (line 2) did not activate *GAL1/lacZ*. Gcr1 (lines 6 and 8) and Gcr2 (lines 7 and 9) did not activate *GAL1/lacZ* with or without Gal4B. No activation was produced by Gal4B/Gcr1(68–844) (line 18). However, marginal activation was produced by Gal4B/Gcr2(48–534) (line 10), and this level was tripled with the shorter fusion Gal4B/Gcr2(223–534) (line 14); thus, a portion of Gcr2 could weakly substitute for the Gal4 activation domain. Gal4A/Gcr1(68–844) alone did not activate (lines 4 and 5), but it caused a substantial increase in the low-level activation by Gal4B/Gcr2(48–534) (line 13) or Gal4B/Gcr2(223–534) alone (line 17). Since neither Gal4A nor intact Gcr1 enhanced the activation from Gal4B/Gcr2(48–534) (lines 11 and 12) or from Gal4B/Gcr2(223–534) (lines 15 and 16), the most direct

TABLE 2. Growth of *GCR2* disruptants on plates

Strain	Relevant genotype[a]	Growth (colony size [mm]) on[b]: YPD 30°C	YPD 37°C	YPGL, 30°C	SC (Glu) + anti 37°C
2845	Wild type[c]	2.3	2.3	0.8	1.5
NW9-19-1	*gcr2-1*	1.5	0.4	0.7	0.2
DFY643	Δ*gcr2*::*URA3*	1.5	0.4	0.7	<0.1
YHU3002-8C	Δ*gcr2*::*URA3*-big[c]	1.2	0.4	0.6	<0.1

[a] 2845 (α *leu2-3 leu2-112 ura3-52 his6*) was used as a wild-type strain. Other strains were derived from 2845 (38).

[b] The strains were streaked on rich plates containing either 2% glucose (YPD) or 2% glycerol plus 2% lactate (YPGL) and synthetic plates containing 2% glucose plus antimycin A (1 μg/ml) [SC(Glu) + anti), and average colony size was measured after incubation at either 30 or 37°C for 3 days.

[c] Two wild-type spores (YHU3002-8B and YHU3002-8D) from one complete tetrad were identical in growth to strain 2845. YHU3002-8C (shown) and YHU3002-8A (identical in growth to YHU3002-8C) were the two Δ*gcr2*::*URA3*-big spores from the same tetrad.

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Gcr2    250' DLQGINNGNNNGNNGNIGNGSNIKNYGNKNMPNNRTKKRGTRV-AKNAKNGKNNKNSNKE
             * * .. .....*   ..*..*   ..... .*  ..   .. ....* . .... ...
Gcr1    361" LLSGNQAIGSKSENIVSSTGGGILILDKNSINSNVLSNLVQSIDPNHSKPNGQAQTHQRG

Gcr2    309' RNGITDTSAFSNTTISNPGTNMLFDPSLSQQLQKRLQTLSQDVNSRSLTGYYTQPTS--P
             ..* ... . *... . . .** * ****. .* .* **... ..    .   . *.* .
Gcr1    421" PKGQSHAQVQSTNSPALAPINM-F-PSLSNSIQPMLGTLAPQPQDIVQKRKLPLPGSIAS

Gcr2    367' GSGGFEFGLSHADLNPNASSNTMGYN-TMSNNGSHSWKRRSLGS--LDVNTLDDEAVEEL
             ...*  *. *... .* ... ... . * *... .*   .*..*  * ..**...**.*
Gcr1    479" AATGSPFSPSPVGESPYSKRFKLDDKPTPSQTALDSLLTKSISSPRLPLSTLANTAVTES

Gcr2    424' LQLTNTSKRQRPMTTAAEGALINDGPDTNLNANNTQMKVDLNPSNSMGPIDTEAVIRPLK
             .. ..  .. . ........ .... ....*....  . .*. . ..   *... .
Gcr1    539" FRSPQQFQHSPDFVVGGSSSSTTENNSKKVNEDSPSSSSKLAERPRLPNNDSTTSMPESP
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FIG. 4. Comparison of Gcr2 with Gcr1. The optimized alignment based on the method of Lipman and Pearson (28) is denoted by an asterisk for an identity and a dot for a conservative replacement. Insertions made during optimization are marked with dashes.

TABLE 3. Transcriptional activation by hybrid Gal4 proteins[a]

Line	1st plasmid	2nd plasmid	β-Galactosidase activity[b] (U)	Growth (colony size [mm]) on SC(Glu)-His and/or -Leu[c]
1	Gal4(1–881) (pCL1)	None	3,806	NT
2	Gal4B (pMA424)	None	<1	1.0
3	Gal4B (pMA424)	Gal4A (pCTC13)	<1	1.0
4	None	Gal4A/Gcr1(68–844) (pML77-8)	<1	1.0
5	Gal4B (pMA424)	Gal4A/Gcr1(68–844) (pML77-8)	<1	1.0
6	None	Gcr1 (pGCR8)	<1	1.0
7	None	Gcr2 (pL133-2)	<1	1.0
8	Gal4B (pMA424)	Gcr1 (pGCR8)	<1	1.0
9	Gal4B (pMA424)	Gcr2 (pL133-2)	<1	1.0
10	Gal4B/Gcr2(48–534) (pL41-14)	None	13	1.0
11	Gal4B/Gcr2(48–534) (pL41-14)	Gal4A (pCTC13)	10	1.0
12	Gal4B/Gcr2(48–534) (pL41-14)	Gcr1 (pGCR8)	4	1.0
13	Gal4B/Gcr2(48–534) (pL41-14)	Gal4A/Gcr1(68–844) (pML77-8)	63	1.0
14	Gal4B/Gcr2(223–534) (pL46-1)	None	38	0.5
15	Gal4B/Gcr2(223–534) (pL46-1)	Gal4A (pCTC13)	35	0.5
16	Gal4B/Gcr2(223–534) (pL46-1)	Gcr1 (pGCR8)	11	1.0
17	Gal4B/Gcr2(223–534) (pL46-1)	Gal4A/Gcr1(68–844) (pML77-8)	147	1.0
18	Gal4B/Gcr1(68–844) (pML78-2)	None	2	0.5

[a] GGY1::171 (α *Δgal4 Δgal80 leu2 his3* with *GAL1-lacZ* integrated at the *URA3* locus) was used as the host strain (17). pCL1 is the YCp50 derivative containing the P_{ADH1}-*GAL4*(1–881) gene (15). Other plasmids are depicted in Fig. 1. Gal4B and Gal4A are the DNA-binding domain (residues 1 to 147) and transcription activation region II (residues 768 to 881), respectively, of Gal4 protein.

[b] Transformants were grown in 2% galactose–2% ethanol–2% glycerol and assayed in tetraplicate for β-galactosidase activity.

[c] Transformants were streaked on synthetic dropout plates with 2% glucose [SC(Glu)], and the average colony size was measured after 3 days of incubation at 30°C. NT, not tested.

interpretation of the latter results is that they reflect an interaction of Gcr1 and Gcr2 domains and hence reassociation of Gal4B and Gal4A in a complex. Furthermore, the decrease of β-galactosidase activity by the introduction of multicopy *GCR1* to Gal4B/Gcr2(48–534) and Gal4B/Gcr2 (223–534) transformants (lines 12 and 16) might likewise reflect the interaction of Gcr1 and Gcr2, overexpressed Gcr1 titrating the Gal4B/Gcr2 fusion protein. Indeed, it might be that the β-galactosidase levels in the strain carrying two fusion plasmids (lines 13 and 17) are themselves somewhat attenuated because of the presence of intact Gcr1 in the indicator strain GGY1::171.

Table 3 also shows that the various plasmids did not affect growth, other than for Gal4B/Gcr2(223–534), which was somewhat inhibitory in the absence of Gal4A/Gcr1(68–844). The titration of inhibition by the latter plasmid might likewise reflect an interaction of Gcr1 and Gcr2 domains and hence prevention of an inhibitory action of the Gcr2 domain alone.

Effect of truncations and fusions on *gcr* mutant phenotypes. Complementation of *gcr1* was examined on glucose plates at 30°C, and that of *gcr2* was examined on glucose plates with antimycin A at 37°C (Table 4). The slightly (N-terminal) truncated version of Gcr1 [Gcr1(68–844)], under P_{ENO1} control, complemented a *gcr1* mutant (line 8), and several truncated versions of Gcr2 (e.g., PD206-1 and PD210-1, derived from a gene bank as described above) complemented *gcr2* mutants (lines 10 to 15), as did the constructed fragment of Gcr2 [Gcr2(223–534)] under P_{ENO1} control (line 18). When tested for complementation of the other gene, just as P_{GCR1} and P_{GCR2} in multicopy under normal control did not complement *gcr2* and *gcr1* mutants, respectively, neither did the truncated versions (lines 9 and 17).

As expected, Gcr1(68–844) fused C terminal to Gal4B or Gal4A under *ADH1* control still functionally complemented a *gcr1* mutant (lines 20 and 24). The unexpected finding, however, was that Gal4A/Gcr1(68–844) also complemented *gcr2* mutations (lines 25 to 27). The cognate Gal4B/Gcr1(68–844) plasmid did not (lines 21 and 22), nor did plasmids carrying intact Gal4 or only Gal4A (lines 30, 31, and 34 to 36). Thus, when fused to Gcr1, but not alone, Gal4A bypasses the normal requirement of Gcr2, suggesting that the normal function of Gcr2 in association with Gcr1 might be to provide an activation domain or to constitute a component of an activator. The complementation result was confirmed by assay of key enzymes (Table 5).

A Rap1/Gcr2 fusion can partially suppress the growth defect of *gcr1* mutants. The data described above suggested that the normal function of Gcr2 might be to provide an activation domain to the Gcr1/Gcr2 complex. However, since it is now known that Gcr1 has the ability to bind the upstream regulatory region of glycolytic genes (2), complementation of *gcr2* by Gal4A/Gcr1(68–844) could be simply explained by the fact that the fusion of a strong activation domain (Gal4A) to a DNA-binding protein can activate transcription of glycolytic genes by a mechanism which bypasses the regulatory machinery that normally modulates glycolytic gene expression. Since Rap1 binds to regulatory regions of many glycolytic genes (18a), we also studied a Rap1(1–701)/Gcr2(118–534) fusion (Table 6). The fusion retained Gcr2 function (line 5). It also partially complemented the growth and enzyme defects of *gcr1* mutants (lines 8 and 11). Since multicopy *RAP1* alone did not complement *gcr1* or *gcr2* (lines 6, 9, and 12), nor did multicopy *GCR2* or *GCR1* (38), the effect must be caused by the Rap1/Gcr2 fusion.

DISCUSSION

GCR1 and *GCR2* specify positive elements needed for expression of most glycolytic genes in *S. cerevisiae*. This work concerns the role of *GCR2* and suggests that it provides a transcriptional activation domain to a Gcr1/Gcr2 complex. This model depends on four findings. First, the predicted amino acid sequence is for a protein with a nuclear

TABLE 4. Growth on plates

Line	Plasmid[a]		Recipient strain	Relevant genotype[b]	Growth (colony size [mm]) on[c]:		
	Name	Description			Glu, 30°C	GlyLac, 30°C	Glu + anti, 37°C
1	pML53-1	Vector	2845	Wild type	1.1	0.5	0.8
2			MWGL29	*gcr1-6*	<0.1	0.5	
3			NW9-19-1	*gcr2-1*	0.9	0.4	<0.1
4	YEp351	Vector	NW9-19-1	*gcr2-1*	0.9		<0.1
5			DFY643	Δ*gcr2*::*URA3*	0.9		<0.1
6			YHU3002-8C	Δ*gcr2*::*URA3*-big	0.9		<0.1
7	pL58-11	Gcr1(68–844)	2845	Wild type	1.0	0.5	1.0
8			MWGL29	*gcr1-6*	1.0	0.5	
9			NW9-19-1	*gcr2-1*	0.5	0.4	0.1[d]
10	PD206-1	Gcr2(223–534)	NW9-19-1	*gcr2-1*	1.2		0.8
11			DFY643	Δ*gcr2*::*URA3*	1.2		0.8
12			YHU3002-8C	Δ*gcr2*::*URA3*-big	1.2		0.9
13	PD210-1	Gcr2(156–534)	NW9-19-1	*gcr2-1*	1.2		0.8
14			DFY643	Δ*gcr2*::*URA3*	1.2		0.8
15			YHU3002-8C	Δ*gcr2*::*URA3*-big	1.2		0.8
16	pL45-3	Gcr2(223–534)	2845	Wild type	1.2	0.7	1.0
17			MWGL29	*gcr1-6*	<0.1	0.5	
18			NW9-19-1	*gcr2-1*	1.2	0.7	0.9
19	pL83-11	Gal4B/Gcr1(68–844)	2845	Wild type	1.1	0.6	0.7
20			MWGL29	*gcr1-6*	1.1	0.6	
21			NW9-19-1	*gcr2-1*	0.5	0.3	<0.1
22			DFY643	Δ*gcr2*::*URA3*	0.5	0.3	<0.1
23	pML77-8	Gal4A/Gcr1(68–884)	2845	Wild type	1.2	0.5	1.0
24			MWGL29	*gcr1-6*	1.2	0.5	
25			NW9-19-1	*gcr2-1*	1.2	0.5	0.9
26			DFY643	Δ*gcr2*::*URA3*	1.2	0.5	1.1
27			YHU3002-8C	Δ*gcr2*::*URA3*-big	1.3	0.5	1.0
28	pCL1	Gal4(1–881)	2845	Wild type	1.2	0.6	1.1
29			MWGL29	*gcr1-6*	0.1	0.5	
30			NW9-19-1	*gcr2-1*	0.8	0.5	<0.1
31			DFY643	Δ*gcr2*::*URA3*	0.8	0.5	<0.1
32	pCTC13	Gal4A	2845	Wild type	1.2	0.5	0.7
33			MWGL29	*gcr1-6*	0.1	0.5	
34			NW9-19-1	*gcr2-1*	0.8	0.5	<0.1
35			DFY643	Δ*gcr2*::*URA3*	0.9	0.5	<0.1
36			YHU3002-8C	Δ*gcr2*::*URA3*-big	0.9	0.5	<0.1

[a] Plasmid constructions are described in Materials and Methods. Gal4B and Gal4A are as indicated in Table 3, footnote *a*.
[b] 2845 (α *leu2-3 leu2-112 ura3-52 his6*) was used as a wild-type strain (38). Other strains were derived from 2845, and they are isogenic except for the genotypes described in the table.
[c] Transformants were streaked on synthetic dropout plates containing 2% glucose (Glu), 2% glycerol plus 2% lactate, (GlyLac), or 2% glucose plus antimycin A (1 μg/ml) (Glu + anti), and the average colony size was measured after 3 days of incubation at either 30 or 37°C.
[d] The size of colonies was heterogeneous (0.05 to 0.2 mm).

localization sequence (residues 281 to 288) and a highly charged C-terminal domain. Many eukaryotic transcription factors contain significant charge clusters (4). It should be noted that a plasmid with only the C-terminal portion of the gene (from residue 223), PD206-1, retained *GCR2* function, while a construction lacking the final 116 residues, the original Δ*gcr2*::*URA3* mutant (38), conferred the same mutant phenotype as did the original *gcr2-1* mutation or the larger substitution, Δ*gcr2*::*URA3*-big. Consistent with Gcr2 having a potential activating domain is the fact that in the genetic system of Fields and Song (15), which assesses Gal4 function, a Gal4B/Gcr2 fusion alone gave some, albeit low, activity, whereas a Gal4B/Gcr1 fusion did not activate *GAL1-lacZ*.

TABLE 5. Relative enzyme activities in transformants

Strain	Plasmid		Relative activity[a]		
	Name	Description	Eno	Gpm	Zwf
NW9-19-1 (*gcr2-1*)	pML77-8	Gal4A/Gcr1(68–844)	1.16	1.14	1.16
	pL83-11	Gal4B/Gcr1(68–844)	0.10	0.17	1.17
	pCTC13	Gal4A	0.17	0.29	1.28
DFY643 (Δ*gcr2*::*URA3*)	pML77-8	Gal4A/Gcr1(68–844)	0.75	0.66	0.85
	pL83-11	Gal4B/Gcr1(68–844)	0.13	0.19	1.24
	pCTC13	Gal4A	0.20	0.27	1.14

[a] Relative to the activity of 2845(pCTC13), which was assigned a value of 1.0. Enzyme activities in 2845(pCTC13) were 0.303 (enolase [Eno]), 0.999 (phosphoglycerate mutase [Gpm]), and (glucose 6-phosphate dehydrogenase [Zwf]) 0.201 μmol/min/mg.

TABLE 6. Growth and relative enzyme activities of transformants

Line	Strain	Plasmid		Growth (colony size [mm]) on[a]:			Relative enzyme activity[b]		
		Name	Description	SC-Ura(Glu), 30°C	SC-Ura(GlyLac), 30°C	SC-Ura(Glu) + anti, 37°C	Eno	Pyk	Zwf
1	2845 (wild type)	YEp352	Vector	1.0	0.7	1.0	1.0	1.0	1.0
2		pL87-6	Rap1/Gcr2[c]	1.0	0.7	1.0	0.90	0.81	0.87
3		pL88-1	Rap1	1.0	0.7	1.0	0.93	0.63	1.09
4	NW9-19-1 (*gcr2-1*)	YEp352	Vector	0.8	0.6	<0.1	0.30	0.21	1.19
5		pL87-6	Rap1/Gcr2	0.8	0.6	0.8	0.81	0.47	1.10
6		pL88-1	Rap1	0.8	0.4	<0.1	0.21	0.14	1.25
7	MWGL29-1 (*gcr1-6*)	YEp352	Vector	<0.1	0.6		0.22	0.05	1.04
8		pL87-6	Rap1/Gcr2	0.3	0.5		0.56	0.35	1.19
9		pL88-1	Rap1	<0.1	0.6		0.29	0.17	1.19
10	DFY644 (Δ*gcr1*::*LEU2*)	YEp352	Vector	0.1	0.7		0.11	0.20	1.00
11		pL87-6	Rap1/Gcr2	0.5	0.6		0.56	0.34	1.13
12		pL88-1	Rap1	0.1	0.7		0.17	0.20	1.19

[a] Transformants were streaked on synthetic dropout plates containing 2% glucose [SC-Ura(Glu)], 2% glycerol plus 2% lactate [SC-Ura(GlyLac)], or 2% glucose plus antimycin A (1 μg/ml) [SC-Ura(Glu) + anti], and the average colony size was measured after 3 days of incubation at either 30 or 37°C.

[b] Enzyme activities in 2845(YEp352) were 0.242 (enolase [Eno]), 1.072 (pyruvate kinase [Pyk]), and 0.236 (glucose 6-phosphate dehydrogenase [Zwf]) μmol/min/mg.

[c] Rap1(1–701)/Gcr2(118–534) fusion.

Second, with the Gal4 system, the increase in β-galactosidase activity to a level above that produced by Gal4B/Gcr2 alone, conferred by the additional presence of Gal4A/Gcr1, suggests reconstitution of Gal4 activity through interaction of Gcr1 and Gcr2. This result is analogous to the demonstration of Snf1/Snf4 interaction by the same technique (15).

Third, complementation of *gcr2* mutants by Gal4A/Gcr1, but not by Gcr1, Gal4, or Gal4A alone, suggests that Gcr2 may normally provide a primary transcriptional activation domain.

Fourth, the partial complementation of *gcr1* mutants by a Rap1/Gcr2 fusion also suggests that Gcr2 functions to provide a transcriptional activation domain to a Gcr1/Gcr2 complex. The simplest conclusion is that the normal Gcr2 function is to provide a transcriptional activation domain for glycolytic gene expression, this domain being effective only in a Gcr1/Gcr2 complex. Most transcriptional activators are composed of a single polypeptide having both DNA-binding and transcriptional activation functions, but transcriptional activator complexes composed of two or three different subunits have recently been reported. In yeast cells, a Hap2/3/4 complex is the typical example of this type of activator. Hap2, Hap3, and Hap4 are involved in expression of the *CYC1* gene through interaction with a CCAAT sequence located in the UAS2UP1 element, and a model whereby Hap2 and Hap3 are primarily responsible for site-specific DNA binding and Hap4 provides the primary transcriptional activation domain has been proposed (33a).

Since Gcr1 has specific DNA-binding activity for an essential element of glycolysis gene promoters, our model proposes that Gcr1 and Gcr2 function as a transcriptional activation complex, Gcr1 providing the specific DNA-binding function and Gcr2 providing the activation function.

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