The E-box DNA binding protein Sgc1p suppresses the *gcr2* mutation, which is involved in transcriptional activation of glycolytic genes in *Saccharomyces cerevisiae*

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Abstract Glycolytic gene expression is mediated by the Gcr1p-Gcr2p transcriptional activation complex. A screen for multicopy suppressors of *gcr2* yielded *SGC1*. *SGC1*'s suppression activity was specific to *gcr2*, it did not extend to *gcr1*. Disruption of *SGC1* moderately affected glycolytic enzyme activities, although no growth defect was evident. Sgc1p exhibits a bHLH motif which is characteristic of E-box DNA-binding proteins. DNA footprinting experiments demonstrated Sgc1p's ability to bind at an E-box. However, its binding specificity was less than 10-fold, which is also characteristic of E-box binding proteins. LexA fusion experiments demonstrated that Sgc1p has weak intrinsic activating activity independent of *GCR1* and *GCR2*. We propose that Sgc1p binds at E-boxes of glycolytic genes and contributes to their activation.

Key words: Sgc1p; Gcr2p; Transcriptional activation; Glycolysis; Yeast; *Saccharomyces cerevisiae*

1. Introduction

The glycolytic pathway is a major metabolic route in *Saccharomyces cerevisiae*. Most of the genes encoding glycolytic enzymes are highly expressed [1]. The core UAS element of glycolytic enzyme genes contains binding sites for Rap1p (RPG-box) and Gcr1p (CT-box) [2]. The strong synergism between the binding sites for Rap1p and Gcr1p [2–9] suggests that both Rap1p and Gcr1p are intimately involved in glycolytic gene expression. Since Rap1p is capable of carrying out many diverse cellular functions such as activation and repression of transcription depending on the sequence context of its binding site, it has been suggested that its function may be determined by the interaction with other regulatory proteins [10,11]. The role of Rap1p at glycolytic enzyme gene UAS elements is to facilitate the binding of Gcr1p [9,12,13].

In addition to Rap1p, Gcr1p also acts with Gcr2p to mediate high level of glycolytic gene expression. Uemura and Jigami [14] presented genetic evidence for a physical interaction of Gcr2p and Gcr1p and postulated that the function of Gcr2p is to provide an activation domain to a Gcr1p-Gcr2p complex. Consistent with this view the drastic reduction of most glycolytic enzymes in *gcr1* mutants is mirrored in *gcr2* mutants [15]. Their further analysis provided genetic evidence for a potential transcriptional activation function in Gcr1p [16]. Hence, Gcr2p likely functions as a co-activator in the Gcr1p-Gcr2p complex.

To further understand the activation pathway from UAS elements to gene expressions, we sought to isolate suppressors of *gcr2* mutants. Here we report on the identification of *SGC1* as a multicopy suppressor of *gcr2* mutants.

2. Materials and methods

2.1. Strains and media

Yeast strain 2845 (α *leu2-3,112 ura3-52 his6*) [17] was used as the wild-type strain. MWGL29 (*gcr1-6*) [15], DFY643 (Δ*gcr2-2::URA3*) [15], and YHU3018 (Δ*gcr1::URA3*, Δ*gcr2-3F*) [12] are derivatives of 2845. Heterozygous diploid strains C221 (a/α *leu2-3,112/leu2-3,112 ura3-52/ura3-52 HIS6/his6 GCR1/*Δ*gcr1::URA3*) and C222 (a/α *leu-2-3,112/leu2-3,112 ura3-52/ura3-52 HIS6/his6 GCR2/*Δ*gcr2-3::URA3*) were used for the disruption of *SGC1*. CTY10-5d (a *ade2 trp1-901 leu2-3, 112 his3-200* Δ*gal4* Δ*gal80 URA3::lexAop-lacZ*), and its derivatives CTY10-5dΔgcr1 (Δ*gcr1::HIS3*) [18] and CTY10-5dΔgcr2 (Δ*gcr2-3::ADE2*) were used for LexA-system experiments. Yeast cells were transformed by the method of Ito et al. [19]. Plasmid DNA was isolated from yeast by the method of Hoffman and Winston [20]. Yeast strains were grown in rich medium [17] and synthetic complete (SC) or SC dropout medium [21] depending on the plasmid markers. Growth on plates was scored by measurements of colony size under a microscope fitted with an ocular micrometer.

2.2. Plasmid constructions

A YEp13 base genomic DNA library (ATCC37323) was used to screen for multicopy suppressor plasmids of the Δ*gcr2* mutant. Plasmids (pD1420, pD1432 and pD1441) were independently isolated from the library. pL1141-1 (*SGC1*/YEp351) was prepared by subcloning a fragment from pD1420 into YEp351 [22]. It contains the *SGC1* gene from −654 bp 5′ to the initiation codon (*Sac*I site) to 98 bp downstream of the termination codon (*Sca*I site). pL1152-4 (*SGC1-1*/YEp351), an *SGC1-1* mutant (E^{189} to Q) version, was created by PCR based site-directed mutagenesis.

The following Sgc1p fusion plasmids were prepared for use in this study by standard methods. LexA fusion plasmids were constructed by using pBTM116 (LexA(1–202)), which resulted in the production of hybrid polypeptides between LexA and Sgc1p. The moieties of each

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Abbreviations: bHLH, basic/helix-loop-helix; IPTG, isopropyl-β-D-thiogalactopyranoside; Eno, enolase; Gpm, phosphoglycerate mutase; Pyk, pyruvate kinase; Zwf, glucose-6-P dehydrogenase

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protein are indicated; pL1117-1 (LexA(1–202)/Sgc1p(11–291)) and pL1179-54 (LexA(1–202)/Sgc1-1p(11–291)). Fusions of Sgc1p to the DNA binding domain of Gcr1p are pL1308-16 (Sgc1p(1–208)/Gcr1p(593–785)), pL1310-20 (Sgc1p(1–11)/Gcr1p(593–785)) and pL1401-3 (Sgc1p(1–208)). A His-tag and Sgc1p fusion plasmid pL1153-1 (His-tag/Sgc1p(11–291)) was constructed on pET-19b (Novagen).

For the disruption of *SGC1*, *Xho*I fragment (ca. 4 kb) of pD1420 was subcloned at the *Sal*I site of pUC18 (pL1113-1), and the region from *Bgl*II (at codon 11) to *Nco*I (at codon 208) was replaced with a *LEU2* marker (pL1119-1). To disrupt *SGC1*, pL1119-1 was digested with *Hin*dIII and *Bam*HI and the Δ*sgc1::LEU2* DNA fragment was transformed into the diploid strains C221 (*GCR1*/Δ*gcr1::URA3*) and C222 (*GCR2*/Δ*gcr2-3::URA3*). The proper disruptions were confirmed by PCR amplification of the integrated fragments. Detailed description of plasmid constructions is available upon request.

2.3. Induction of the His-tag/Sgc1p fusion protein and its purification

Escherichia coli strain BL21(DE3)pLysS harboring *His-tag/SGC1* fusion plasmid (pL1153-1) was grown at 37°C in 100 ml of LB with ampicillin and chloramphenicol to A_{600} of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and the culture was grown for an additional 2 h at 37°C. Cells were collected by centrifugation, resuspended in 3 ml of binding buffer (20 mM Tris–HCl, pH 7.9, 5 mM imidazol, 500 mM NaCl), and disrupted by passage through a French pressure cell. After removing the cell debris by centrifugation (17 000 rpm for 20 min), His-tag/Sgc1p(11–291) was purified over a His-bind resin column (Novagen) from the supernatant according to the protocol recommended by the manufacturer.

2.4. In vitro footprinting studies

DNaseI footprints were carried out as described [2] with 0.06 U of DNaseI (RQ1 DNase, Promega). The reaction mixtures were incubated at room temperature for 2 min and the reaction was stopped by adding an equal volume of stop solution (50% glycerol, 0.25 M EDTA, pH 8.0). The resulting mixtures were subjected to a denaturing sequencing gel electrophoresis after phenol extraction and ethanol precipitation. Probes used for electromobility-shift and DNaseI footprint experiments are described in Fig. 1.

3. Results and discussion

3.1. Isolation of a multicopy suppressor gene of a Δgcr2 mutant

To study the function of Gcr2p, we screened for multicopy suppressors of the Δ*gcr2* mutant. The *gcr2* mutants grow fairly well on glucose plates at 30°C (semi-permissive condition), but their growth is severely impaired on glucose at 37°C in the presence of antimycin A (1 μg/ml) (non-permissive condition) [15]. Thus, a multicopy yeast genomic library was transformed into the Δ*gcr2-2::URA3* mutant (DFY643), and Leu^+ transformants were selected under the non-permissive condition. Plasmid DNA was prepared from colonies with substantial growth and retransformed into the original host to confirm complementation.

The transformants of DFY643 harboring pD1420, pD1432 and pD1441 grew equally well under the non-permissive condition. They contained different but overlapping inserts. The smallest region in common spanned from nucleotide 975882 to 980111 of chromosome XV. The only ORF in this region was *SGC1*. To further verify that the gene involved in the suppression activity was *SGC1*, a fragment ranging from 654 bp upstream of the initiation codon (ATG) of *SGC1* to 98 bp downstream of the termination codon was excised from pD1420 and subcloned into YEp351. The resulting plasmid, pL1141-1, suppressed the *gcr2* mutation (compare Table 1, line 4 with line 6), indicating *SGC1* encoded the suppression activity.

Since multicopy *SGC1* could suppress *gcr2* mutants, we were interested if it could also suppress *gcr1* mutation. Therefore, we introduced pL1141-1 (*SGC1*/YEp351) into the *gcr1* mutant MWGL29 and examined its growth on glucose plates. However, *SGC1* in multicopy (pL1141-1) was unable to suppress the *gcr1* mutation (Table 1 line 7). Thus, while wild-type

Table 1
Growth and glycolytic enzyme activities of transformants

Line	Host strain (genotype)	Plasmid	Relevant description	Growth (colony size (mm)) on plates[a]			Relative enzyme activity[b]			
				SC(Glu) (30°C)	SC(GL) (30°C)	SC(Glu)+A (37°C)	Gpm	Eno	Pyk	Zwf
1	2845	YEp351	Vector	1.2	0.6	1.1	(1.0)	(1.0)	(1.0)	(1.0)
2	(WT)	pL1141-1	*SGC1*	1.2	0.6	1.1	1.03	1.75	1.91	0.98
3		pL1152-4	*SGC1-1*	1.2	0.6	1.1	1.09	1.70	2.64	0.81
4	DFY643	pL1141-1	*SGC1*	0.8	0.3	0.8	0.36	0.42	0.31	1.14
5	(Δ*gcr2-2::URA3*)	pL1152-4	*SGC1-1*	0.8	0.3	1.0	0.33	1.02	0.69	0.97
6		YEp351	Vector	0.8	0.3	< 0.1	0.14	0.23	0.28	1.40
7	MWGL29	pL1141-1	*SGC1*	0.1	0.6	0.10	0.21	0.10	1.11	
8	(*gcr1-6*)	pL1152-4	*SGC1-1*	0.6	0.6	0.12	0.51	0.25	0.99	
9		pL1308-16	Sgc1p(1–208)/Gcr1p(593–785)	0.9	0.6					
10		pL1310-20	Sgc1p(1–11)/Gcr1p(593–785)	< 0.1	0.6					
11		p1401-3	Sgc1p(1–208)	< 0.1	0.6					
12		YEp351	Vector	0.1	0.6	0.074	0.14	0.16	1.14	
13	YHU3018	pL1141-1	*SGC1*	0.2	0.6	0	0.028	0.12	0.075	1.43
14	(Δ*gcr1::URA3*,	pL1152-4	*SGC1-1*	0.8	0.6	0.2	0.063	0.36	0.34	1.11
15	Δ*gcr2-3F*)	pL1308-16	Sgc1p(1–208)/Gcr1p(593–785)	1.0	0.6	0.5				
16		pL1310-20	Sgc1p(1–11)/Gcr1p(593–785)	< 0.1	0.6	< 0.1				
17		p1401-3	Sgc1p(1–208)	< 0.1	0.6	< 0.1				
18		YEp351	Vector	< 0.1	0.6	< 0.1	0.015	0.064	0.068	1.43

[a]The strains were streaked on synthetic drop-out plates (SC-Leu) containing 2% glucose (Glu), 2% glucose plus antimycin A (1 μg/ml) (Glu+A), or 2% glycerol plus 2% lactate (GL), and average colony size (mm) was measured after incubation at either 30°C or 37°C for 3 days.
[b]Transformants were grown to mid-log phase in synthetic medium containing 2% each of glycerol and lactate at 30°C and glycolytic enzyme assays were performed as described [15]. Values are normalized to those of the wild-type strain 2845. Specific activities (in μmol min^{-1} mg $protein^{-1}$) for the wild-type strains are 1.97 ± 0.18 for Gpm, 0.89 ± 0.11 for Eno, 0.57 ± 0.43 for Pyk and 0.19 ± 0.02 for Zwf.

SGC1 in multicopy could relieve the requirement for Gcr2p, it was unable to suppress *gcr1*.

Previously Nishi et al. [23] showed that a dominant mutation allele of *SGC1*, *SGC1-1*, is able to suppress *gcr1* mutation. Thus we examined if the dominant allele *SGC1-1* could bypass the requirement of both *gcr1* and *gcr2*. Accordingly we introduced *SGC1-1* (pL1152-4) into a *gcr1*, *gcr2* double mutant strain YHU3018 and the single mutants as well. In all cases *SGC1-1* suppressed the growth defects of the *gcr* mutants (lines 5, 8 and 14). As assessed by colony size on glucose plus antimycin A plates at 37°C, *SGC1-1* (pL1152-4) gives rise to more robust growth of the *gcr2* mutant than did *SGC1* (pL1141-1) itself (compare line 4 with line 5). The enhanced suppression activity was also confirmed in glycolytic enzyme assays as described later.

3.2. SGC1 in multicopy restores glycolytic enzyme activities in gcr2 mutants

To examine whether the suppression of the *gcr2* mutation by *SGC1* was due to the restoration of glycolytic enzyme activities, several glycolytic enzymes (phosphoglycerate mutase (Gpm), enolase (Eno) and pyruvate kinase (Pyk)) were assayed (Table 1). Transformants of *gcr2* mutants with the wild-type *SGC1* (pL1141-1) displayed increased glycolytic enzyme activities while the activity of the control, non-glycolytic enzyme glucose-6-P dehydrogenase (Zwf), which is unaffected in *gcr1* and *gcr2* mutants, was not affected (compare line 4 with line 6). Thus, the suppression is associated with partial restoration of the glycolytic enzyme activities. The suppression of *gcr1* and *gcr2* mutations by *SGC1-1* was also associated with the restoration of glycolytic enzyme activities. The effect of *SGC1-1* was more potent than that of *SGC1* (lines 5 and 8). No differences in growth of the wild-type were observed when these genes were present in multicopy, but interestingly, the wild-type strain 2845 harboring either *SGC1* or *SGC1-1* in multicopy had higher activities of Eno and Pyk, whereas the levels of Gpm were unaffected (lines 2 and 3). It is noteworthy that the recovery of Gpm activity was also small in the suppression of the *gcr1* mutant by *SGC1-1* (line 8).

3.3. Effect of SGC1 disruption on the glycolytic enzyme activities

To further address the role of *SGC1* in glycolytic gene expression, we disrupted *SGC1*. The *sgc1* disruption (Δ*sgc1::LEU2* DNA) was introduced into the diploid strains C221 (*GCR1*/Δ*gcr1::URA3*) and C222 (*GCR2*/Δ*gcr2-3::URA3*). The disruption of *SGC1* was recessive, because the diploid transformants grew normally. The diploid strains were sporulated and dissected. Three tetra-type tetrads from three independent transformants of each strain were analyzed. The *sgc1*, *GCR1* and *sgc1*, *GCR2* strains were viable and had a growth phenotype indistinguishable from the wild-type strain (Table 2, lines 1, 2, 5 and 6). No effect on the growth phenotype was observed on other carbon sources (2% each of fructose, mannose, sucrose, pyruvate, glycerol and 0.02% glucose) (data not shown).

The null mutation of *sgc1* moderately affected the glycolytic enzyme activities (Table 2, lines 2 and 6). The enzyme activities in *sgc1*, *gcr1* and *sgc1*, *gcr2* double mutants were lower than for the respective *gcr1* and *gcr2* single mutants (lines 4 and 8). The growth phenotype of the *sgc1*, *gcr2* double mu-

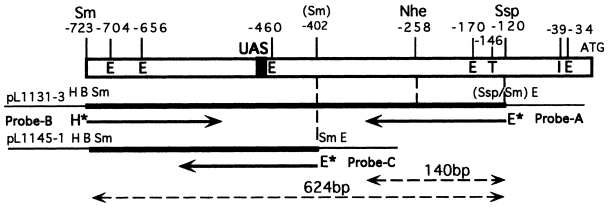

Fig. 1. Structure of the 5′-non-coding region of *ENO1*. The top bar indicates the 5′-non-coding region of *ENO1* from −723 to −1. Nucleotide positions are numbered relative to the translation initiation codon (ATG). Positions of the putative E-boxes (E), UAS, TATA sequence (T), mRNA initiation site (I), and relevant restriction enzyme sites are indicated. pL1131-3 and pL1145-1 were constructed by cloning the −120 to −723 (603 bp) and −402 to −723 (321 bp) regions of *ENO1* 5′ non-coding region into the *Sma*I site of pT7/T3α-19 (Gibco-BRL), respectively. The probes containing the *ENO1* UAS element were excised from pL1131-3 either as a *Bam*HI–*Eco*RI fragment (624 bp) or *Nhe*I–*Eco*RI (140 bp) fragment and were used as probes for electromobility-shift assays. The 772 bp *Nla*IV fragment of pUC18, which contains no putative E-box sequence (CANNTG), was used as a non-specific competitor DNA. The ^{32}P labeled ends are indicated by asterisks and the regions analyzed by in vitro footprinting experiments are indicated by arrows. Restriction enzyme sites are: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Nhe, *Nhe*I; Sm, *Sma*I; Ssp, *Ssp*I. Restriction sites in the parentheses are not conserved.

Table 2
Relative enzyme activities and growth characteristics of Δ*sgc1* single, Δ*sgc1*,Δ*gcr1* double and Δ*sgc1*,Δ*gcr2* double mutants

Line	Strain (genotype)	Growth (colony size (mm)) on plates[a]									Relative enzyme activity[b]			
		SC(Glu)			SC(GL)			SC(Glu)+A			Gpm	Eno	Pyk	Zwf
		23°C	30°C	37°C	23°C	30°C	37°C	23°C	30°C	37°C				
1	WT	1.0	1.25	1.0	0.25	0.55	0.25	1.0	1.25	1.0	(1.0)	(1.0)	(1.0)	(1.0)
2	Δ*sgc1::LEU2*	1.0	1.25	1.0	0.25	0.5	0.25	1.0	1.25	1.0	0.62	0.44	0.69	0.78
3	Δ*gcr1::URA3*	<0.1	<0.1	<0.1	0.2	0.5	0.1	0	0	0	0.066	0.073	0.13	1.44
4	Δ*gcr1::URA3*, Δ*sgc1::LEU2*	<0.1	<0.1	<0.1	0.2	0.4	0.1	0	0	0	0.011	0.047	0.071	1.20
5	WT	1.0	1.25	1.25	0.25	0.5	0.25	1.0	1.25	1.0	(1.0)	(1.0)	(1.0)	(1.0)
6	Δ*sgc1::LEU2*	1.0	1.25	1.25	0.2	0.5	0.25	0.7	1.25	1.0	0.71	0.52	0.87	1.08
7	Δ*gcr2-3::URA3*	0.7	1.15	0.9	0.1	0.3	0.1	0.5	0.7	0.1	0.11	0.055	0.074	1.12
8	Δ*gcr2-3::URA3*, Δ*sgc1::LEU2*	0.5	1.0	0.25	<0.1	0.3	<0.1	0.2	0.25	0	0.10	0.038	0.028	0.88

Data in lines 1–4 and lines 5–8 are average of three complete sets of tetrads with tetra-type segregation which are derived from three independent transformants of C221 and C222 with Δ*sgc1::LEU2* DNA, respectively.
[a]Same as in Table 2.
[b]Same as in Table 2. Specific activities for the wild-type strains are Gpm, 1.98 ± 0.28; Eno, 0.78 ± 0.10; Pyk, 2.10 ± 0.18 and Zwf, 0.25 ± 0.04 for line 1 and Gpm, 1.75 ± 0.26; Eno, 0.62 ± 0.16; Pyk, 1.28 ± 0.41 and Zwf, 0.26 ± 0.03 for line 5.

Fig. 2. In vitro binding of Sgc1p to the 5′-non-coding region of *ENO1*. Electromobility-shift analysis was carried out with radiolabeled 5′-non-coding region (−723 to −121) of *ENO1*. The probe containing the 5′-non-coding region of *ENO1* was incubated with increasing amount of His-tag/Sgc1p(11–291). Standard DNA binding reaction was carried out with two-fold dilution series of an *E. coli* produced and purified Sgc1p preparation (lanes 1–6). Lane 7 does not contain Sgc1p.

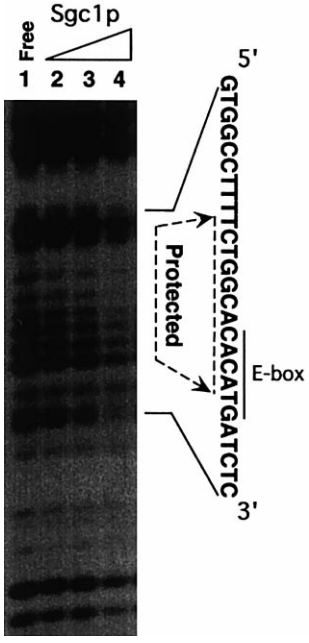

Fig. 3. Sgc1p protects the E-box sequence motif in the *ENO1* 5′-non-coding region from DNaseI cleavage. DNaseI protection analysis was carried out with purified His-tag/Sgc1p(11–291) and a radiolabeled 624 bp fragment carrying −723 to −121 of the 5′-non-coding region of *ENO1*. Lanes 1–4: DNA fragments treated with 0, 1, 2 and 5 μl of purified Sgc1p to form nucleoprotein complex were treated with 0.06 U of DNaseI. The sequences of the protected area and the position of the E-box are denoted on the right based on the product of dideoxy sequencing reaction of pUC19 used as a molecular weight standard.

tant was more impaired than the *gcr2* mutant on glucose at 37°C and on glucose at 30°C in the presence of antimycin A (lines 7 and 8). In contrast, we did not detect an additional impairment in the *sgc1*, *gcr1* double mutant to that associated with *gcr1* mutants (lines 3 and 4).

The phenotype of *sgc1* disruptants is consistent with the hypothesis that there is collaboration between Sgc1p and the Gcr proteins. Since the effect of the *sgc1* disruption on glycolytic gene expression was relatively small, it is quite likely that Sgc1p functions in conjunction with the Gcr1p-Gcr2p complex, but the possibility that Sgc1p works in a parallel pathway still remains.

3.4. Intrinsic transcriptional function of Sgc1p

In light of our knowledge of Gcr2p function, we hypothesized that Sgc1p may have a role in transcriptional activation. Therefore, we used the LexA system to investigate this possibility. We transformed plasmids encoding LexA/Sgc1p hybrids into the reporter strains CTY10-5d. *lacZ* Gene expression was weakly activated in transformants expressing the LexA/Sgc1p fusion (pL1117-1), whereas Sgc1p without the LexA moiety was not able to activate the reporter gene (pL1141-1) (Table 3).

Does the intrinsic activating activity of Sgc1p require Gcr1p or Gcr2p? To address this question, we introduced LexA/Sgc1p hybrids into gcr$^-$ derivatives of CTY10-5d. The *gcr1* and *gcr2* disruptions did not affect the activity of the LexA/Sgc1p fusion protein, indicating the transcriptional activity of Sgc1p is independent of Gcr1p and Gcr2p. Unfortunately, we could not examine the activation potential of the dominant mutant Sgc1-1p in the LexA system, because we were unable to obtain the desired transformant of plasmid pL1179-54 (LexA/Sgc1-1p). We hypothesized that the inability to isolate the desired transformant was due to toxicity of Sgc1-1p when overexpressed. Actually, we confirmed that the overexpression of basic/helix-loop-helix (bHLH) region of Sgc1p was toxic (data not shown).

Deletion mapping experiments suggested that function of N-terminal region of Sgc1p is essential for its activation function (data not shown). To further address this point, we examined the complementation activity of Sgc1p/Gcr1p fusions.

Table 3
Transcriptional activation by the LexA/Sgc1p hybrid protein

Line	Plasmid	Description	β-galactosidase activity (U)[a] in					
			CTY10-5d		CTY10-5dΔgcr1		CTY10-5dΔgcr2	
			Glu	GL	Glu	GL	Glu	GL
1	pL1117-1[b]	LexA/Sgc1p(11–291)[b]	1.63 ± 0.57	3.82 ± 0.92	2.06 ± 0.09	3.08 ± 0.64	0.99 ± 0.07	3.76 ± 0.24
2	pL1141-1	Sgc1p(1–291)	0.05 ± 0.01	0.07 ± 0.01				
3	pBTM116	LexA Vector	0.10 ± 0.02	0.40 ± 0.14	0.32 ± 0.01	0.42 ± 0.13	0.06 ± 0.01	0.20 ± 0.01

[a]Transformants were grown in 2% glucose (Glu), 2% lactate and 2% glycerol (GL) and assayed for β-galactosidase activity as described [14]. The values are average of three independent experiments.
[b]Transformants of LexA/Sgc1p(11–291) were weakly toxic.

The truncated Sgc1p (pL1401-3, Sgc1p(1–208)), which lacks HLH region, did not suppress Δ*gcr1* single and Δ*gcr1*, Δ*gcr2* double mutant strains (Table 1, lines 11 and 17). However its fusion to the DNA binding domain of Gcr1p (pL1308-16, Sgc1p(1–208)/Gcr1p(593–785)) restored complementation activity in both Δ*gcr1* single and Δ*gcr1*, Δ*gcr2* double mutant strains (lines 9 and 15), whereas the DNA binding domain of Gcr1p itself (pL1310-20, Sgc1p(1–11)/Gcr1p(593–785)) did not (lines 10 and 16). This is an indication that the bHLH region of Sgc1p provides DNA binding function and the essential transcriptional activation function of Sgc1p resides in the N-terminal region and once it is tethered to DNA, it can provide activation function in Gcr1p and Gcr2p independent manner.

3.5. Sgc1p is an E-box binding protein

The C-terminal region of Sgc1p exhibits strong similarity to bHLH DNA binding motif of E-box binding proteins [23,24]. To assess Sgc1p's DNA binding ability, we carried out DNA electromobility-shift assays. Sgc1p was purified from *E. coli* as a His-tag fusion protein, and the 5′ non-coding region of *ENO1* (Fig. 1) was used as a probe. Fig. 2 shows the appearance of a single major shifted band as an increasing amount of Sgc1p was added to the binding reaction. To determine the exact position where Sgc1p bound, we performed in vitro DNaseI protection assays. Weak protection was observed around the E-box region immediately upstream of the TATA sequence by using a probe-A of Fig. 1 (Fig. 3). The measurement of intensities of bands around the E-box confirmed the protection (data not shown). No other protections were observed in this region by using probes -B and -C of Fig. 1 (data not shown).

Sgc1p gave rise to a sequence specific footprint albeit weakly. Such observations are not uncommon for E-box proteins. We carried out a series of DNA competition experiments to determine the degree to which Sgc1p displays specificity for its binding site. In a gel-shift assay with a probe containing the footprinted E-box near the TATA sequence of *ENO1* (140 bp probe in Fig. 1), there was only an about eight-fold difference in competition between the specific and non-specific competitors (3.5 nM (specific) vs. 30 nM (non-specific)) (data not shown). This finding was not unexpected, since as a class, E-box binding proteins tend to bind DNA with a low specificity. For example, the DNA binding specificity of rat MASH-1 has a DNA binding specificity of less than 10 [25] which is similar to the binding specificity we measured for Sgc1p.

3.6. Conclusion

We identified *SGC1* as a multicopy suppressor of *gcr2* mutants, and we showed that the suppression activity of *SGC1* in multicopy did not extend to *gcr1* mutants. Previous work identified a dominant mutant of *SGC1*, *SGC1-1*, as a suppressor of *gcr1* mutants [23]. We tested the prediction that Sgc1p was an E-box DNA binding protein and showed that it was capable of binding to an E-box in a sequence specific manner, but with a low degree of sequence specificity which is characteristic of E-box binding proteins. We further showed that Sgc1p has intrinsic transcriptional activation activity which is independent of Gcr1p and Gcr2p, although the contribution of Sgc1p to the overall level of glycolytic gene expression is small when compared to that of Gcr1p or Gcr2p.

Considering the suppression characteristics of *SGC1* and *SGC1-1* in relation to *gcr1* and *gcr2* mutants, we hypothesize that the Gcr1p-Gcr2p activation complex at UAS elements coordinates the binding of Sgc1p to E-box elements where Sgc1p then contributes to the overall activation process of the cognate genes.

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