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# Isolation and Characterization of an Invertase and Its Repressor Genes from *Schizosaccharomyces pombe*

Naotaka Tanaka,* Nobuhiro Ohuchi,* Yukio Mukai,† Yukio Osaka,* Yoshihiko Ohtani,* Mitsuaki Tabuchi,* M. Shah Alam Bhuiyan,* Hiroshi Fukui,* Satoshi Harashima,† and Kaoru Takegawa*,[1]

**Department of Life Sciences, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan, and †Department of Biotechnology, Graduate School of Engineering, Osaka University, Suita-shi, Osaka 565, Japan*

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**PCR was used to isolate an invertase homolog gene from the fission yeast *Schizosaccharomyces pombe.* The cloned *inv1*$^+$ gene encodes a protein of 581 amino acids with 16 potential asparagine-linked glycosylation sites, and has 39% and 38% identity to the *Schwanniomyces occidentalis* and *Saccharomyces cerevisiae SUC2* invertases. When the *inv1*$^+$ gene was disrupted, *S. pombe* strains lacked detectable invertase activity. This result showed that the *inv1*$^+$ gene encodes only one active invertase in *S. pombe* cells. The transcription of *inv1*$^+$ is repressed in the presence of glucose. The transcription of *inv1*$^+$ was not affected in *cyr1*Δ strain which lacks adenylate cyclase activity, unlike transcription of *S. pombe fbp1*$^+$ gene. We have identified an *S. pombe* gene (*scr1*$^+$) that encodes a homolog of the *Aspergillus nidulans* CREA which is required for glucose repression of the glyconeogenic pathway. Although the deletion of *scr1*$^+$ did not influence the transcription of *fbp1*$^+$ gene, glucose repression of the *inv1*$^+$ gene was severely affected. These results showed that glucose repression of *inv1*$^+$ gene is dependent on *scr1*$^+$ gene, and *S. pombe* cAMP signalling pathway may not be essential for glucose repression of *inv1*$^+$ gene.** 

The *SUC* gene family of *S. cerevisiae* includes six structural genes for invertase found at unlinked chromosomal loci (1-3). The expression of *S. cerevisiae SUC* genes is exclusively regulated by catabolite repression, mediated by glucose. A single structural gene, *SUC2*, which is not telomeric in location, codes for two different polypeptides in *S. cerevisiae* (4,5). The external form of the enzyme is highly glycosylated and transported to the periplasmic space, while internal invertase lacks the signal peptide, is retained in the cytoplasm, and is non-glycosylated. External invertase is regulated by glucose repression and the internal form is produced constitutively at low level. Catabolite repression of *S. cerevisiae SUC2* gene is a typical system for the regulation of sugar metabolism and has been thoroughly studied. Genetic selections in *S. cerevisiae* have resulted in the identification of a large number of the genes required for the transcription and catabolite repression of the *SUC2* gene (6-8).

Asparagine (N)-linked oligosaccharide chains in the *S. cerevisiae* invertase consisting of a core chain ($Man_{8}$-$GlcNAc_2$) formed in the endoplasmic reticulum, then elongate the resultant high mannose oligosaccharide in the Golgi to mannan which has a poly a 1,6-linked mannose backbone with a 1,2- and $\alpha$ 1,3-linked mannose side chains that may consist of 50 to 100 residues (9). The nature of the secretory pathway and glycosylation reactions associated with invertase biosynthesis in *S. cerevisiae* have been extensively studied (10). Thus, the invertase is also an excellent marker protein with which to study glycoprotein processing and the secretory pathway (11,12) in *S. cerevisiae.*

In the fission yeast, *Schizosaccharomyces pombe,* the existence of invertase activity has also been reported, and the synthesis of the enzyme is repressed in the presence of a high concentration of glucose in the culture medium (13,14). The invertase from *S. pombe* has been purified and characterized (15). The purified invertase was a glycoprotein containing 67% carbohydrates, most of which were mannose and galactose in a ratio of about 1: 1. Although the secreted invertase has served as a model glycoprotein for studies of oligosaccharide synthesis and structure (16) and for analysis of the secretory pathway (17) in *S. pombe,* the se-

[1] To whom correspondence should be addressed: Kaoru Takegawa, Dept. of Life Sciences, Fac. of Agriculture, Kagawa Univ., Mikicho, Kagawa 761-0795, Japan. Telefax:+81-87-898-7295; E-mail address: takegawa@ag.kagawau.ac.jp.

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quence and structural gene(s) for the *S. pombe* invertase have yet to be determined.

We now describe cloning of the *S. pombe* invertase gene, and that disruption of *inv1*$^+$ gene resulted in the complete loss of invertase activity. This result shows that *inv1*$^+$ gene encodes an active form of invertase in *S. pombe* cells. The transcription of *inv1*$^+$ is repressed in the presence of glucose. In the case of transcription of *fbp1*$^+$ (for fructose 1,6-bisphosphatase; fbpase) gene, catabolite repression occurs through a cAMP-signalling pathway. However, the *inv1*$^+$ gene was not constitutively expressed in *git2*Δ (*cyr1*Δ) strain which lacks adenylate cyclase activity. These results suggested that the *inv1*$^+$ transcription appears to be independent of the cAMP signalling pathway, unlike transcription of *S. pombe fbp1*$^+$ gene. In addition, we have identified an *S. pombe* CREA/MIG1 homolog (*scr1*$^+$) from a search of the *S. pombe* data base, and found that the *inv1*$^+$ expression on glucose is much higher in the scr1D strain than in the wild-type. This paper describes the first identification of a CREA homolog zinc finger protein, *S. pombe* Scr1p, in yeast. The *scr1*$^+$ gene may be a general regulatory gene which controls glucose repression of various genes in *S. pombe* like *Aspergillus CREA* and *Saccharomyces MIG1* repressor genes.

## MATERIALS AND METHODS

*Strains, media and yeast genetic methods.* *Escherichia coli* strain XL1-Blue (Stratagene) was used for all cloning procedures, and grown on standard media. The wild-type *S. pombe* strains TP4-1D (*h*$^+$ *leu1 his2 ura4 ade6-M216*) and TP4-5A (*h*$^-$ *leu1 ura4 ade6-M210*) were obtained from Dr. T. Toda (ICRF)(18). The *cyr1*Δ strain (*h*$^{90}$ *ura4 ade6-M210*) was kindly supplied by Dr. M. Kawamukai (Shimane Univ.). These strains were cultured in standard rich (YPD) and synthetic minimal (MM) media as described by Moreno et al. (19) at 28°C. Standard procedures for *S. pombe* manipulation were as described (20).

*Vectors and plasmid constructions.* *E. coli* plasmids pBluescript KS(+) and (−) were obtained from Stratagene. The *S. pombe* vector pAL-KS$^+$ was a gift from Dr. C. Shimoda (Osaka City Univ.). The *S. pombe ura4*$^+$ gene was kindly provided by Dr. P. Russell (Scripps). Plasmid DNA was propagated in *E. coli* XLI-Blue and prepared using Flexi-Prep Kit (Pharmacia). The chromosomal DNA from the wild-type TP4-1D cells was isolated (20), and the DNA was partially digested with *Sau*3AI and fragments of 8 to 15 kb were purified by Takara EASY Trap Kit, and ligated into *Bam*HI-digested λEMBL3 using Gigapack III packaging kit (Stratagene). This was packaged into phage particles and used to infect *E. coli* P2392. A 12-kb *Sau*3A fragment from the originally isolated PCR-positive phage was subcloned into pBluescript to generate pNO-B. The 2.6 kb *Bam*HI/*Hin*dIII fragment and 3.0 kb of *Hin*dIII fragment were ligated into *Bam*HI/*Hin*dIII digested pAL-KS to produce pNO1 plasmid.

*PCR amplification and cloning of the inv1*$^+$ *gene.* To amplify invertase-like sequences from cDNA libraries of *S. pombe*, the following oligonucleotides were synthesized: (1) 5′-GTTTGAATTCATGAA-T(C)GAT(C)GGA(T/G/C)AAT(C)GG-3′; (2) 5′-GTTTGAATTCT(C)-A(T)T(C)TGGGGA(T/G/C)CA-T(C)GC-3′; and (3) 5′-GTTTGAATT-CACT(C)TTA(T/G/C)GGA(G)TCA(T/G/C)CGA(G)AA-3′. Oligonucleotide (1) encodes amino acids MNDPNG; (2) encodes F(Y/H/T)WGHA; and (3) encodes FRDPKV. PCR was performed using 35 repetitions of the following temperature cycle: 94°C for 60 seconds, 49°C for 90 seconds, 72°C for 90 seconds. The reaction product was resolved by electrophoresis in a 1.5% agarose gel. A fragment of approximately 400 bp was recovered, and ligated into pMOS Blue vector (Amersham). All nucleotide sequence determination was performed using double-stranded plasmid DNA templates and an AutoRead Sequencing Kit (Pharmacia), according to the recommendations of the manufacturer. The entire invertase gene was isolated by plaque hybridizing the cloned PCR product.

*Disruption of the inv1*$^+$ *gene.* The *inv1*$^+$ locus was disrupted in the TP4-1D wild-type *S. pombe* strain by replacing an internal *inv1*$^+$ gene fragment with the *S. pombe ura4*$^+$ gene to produce strain NOD-1. A 1.4 kb *Bam*HI-*Bam*HI fragment was eliminated from the cloned *inv1*$^+$ open reading frame and a 1.6 kb *ura4*$^+$ cassette (21) was inserted. A linearized DNA fragment carrying this disrupted *inv1*$^+$ gene was used to transform a wild-type haploid strain, and *ura*$^+$ transformants were selected. To confirm that the *inv1*$^+$ gene had been disrupted, *ura*$^+$ transformants were analyzed by Southern blot to verify correct integration of the deletion constructs.

*Invertase assays.* For invertase assays, cells were grown to mid-logarithmic phase in minimal-MM media and washed with 10 mM $NaN_3$. After resuspension in 0.1 M sodium acetate (pH 6.0), the culture was mixed with 1% Triton X-100 and frozen on dry ice. The freeze-thaw lysed cells were assayed, yielding total invertase activity. Invertase assays were done as described previously (22). Plate assays for invertase were done as described by Paravicini et al. (23).

*Isolation and disruption of scr1*$^+$ *gene.* To amplify the DNA fragment of *scr1*$^+$ gene from chromosomal DNA of *S. pombe*, the following oligonucleotides were synthesized; *scr1*$^+$ sense 5′-GGCTCTCATCTG-TCCAACAA-3′ and antisense 5′-GCATAACACACAACCAAGGTG-3′. PCR was performed using 35 repetitions of the following temperature cycle: 94°C for 60 seconds, 49°C for 90 seconds, 72°C for 180 seconds. A fragment of 800 bp was recovered, and ligated into pGEM-T EASY vector (Stratagene). A null allele of *scr1*$^+$ was constructed as follows. The 800 bp fragment containing *scr1*$^+$ was digested with *Xba*I, and *Xba*I-digested *ura4*$^+$ gene was inserted. A linearized DNA fragment carrying this disrupted *scr1*$^+$ gene was used to transform a wild-type haploid strain, and *ura*$^+$ transformants were selected and confirmed by Southern hybridization.

*Isolation of RNA and Northern blot analysis.* *S. pombe* wild-type, *inv1*Δ, and *cyr1*Δ strains were grown in YPD supplemented with adenine and 8% glucose (repressing conditions). The cells were collected by centrifugation, and incubated for 3 h in YP media supplemented with 0.1% glucose +3% glycerol (derepressing conditions) as the carbon source to a concentration of $1 \times 10^7$ cells/ml. Total yeast RNA was prepared and separated on a formaldehyde-agarose gel, transferred to nylon membranes, and hybridized as described (24). The 2 kb *Hin*dIII *inv1*$^+$ fragment was used as a probe. The *fbp1*$^+$ and *leu1*$^+$ coding regions were amplified by PCR from chromosomal DNA of *S. pombe*, and used as a probe for the transcript as described (25).

## RESULTS

*Cloning of the S. pombe inv1*$^+$ *gene.* We attempted to isolate a gene for an invertase homolog from *S. pombe* by PCR using primers based on the three conserved amino acid sequences. PCR was performed with the *S. pombe* cDNA library as a template. A single DNA fragment of 400 bp was amplified. The sequencing of 10 individual clones revealed that all were identical and contained an open reading frame that showed strong homology to genes for the reported invertases.

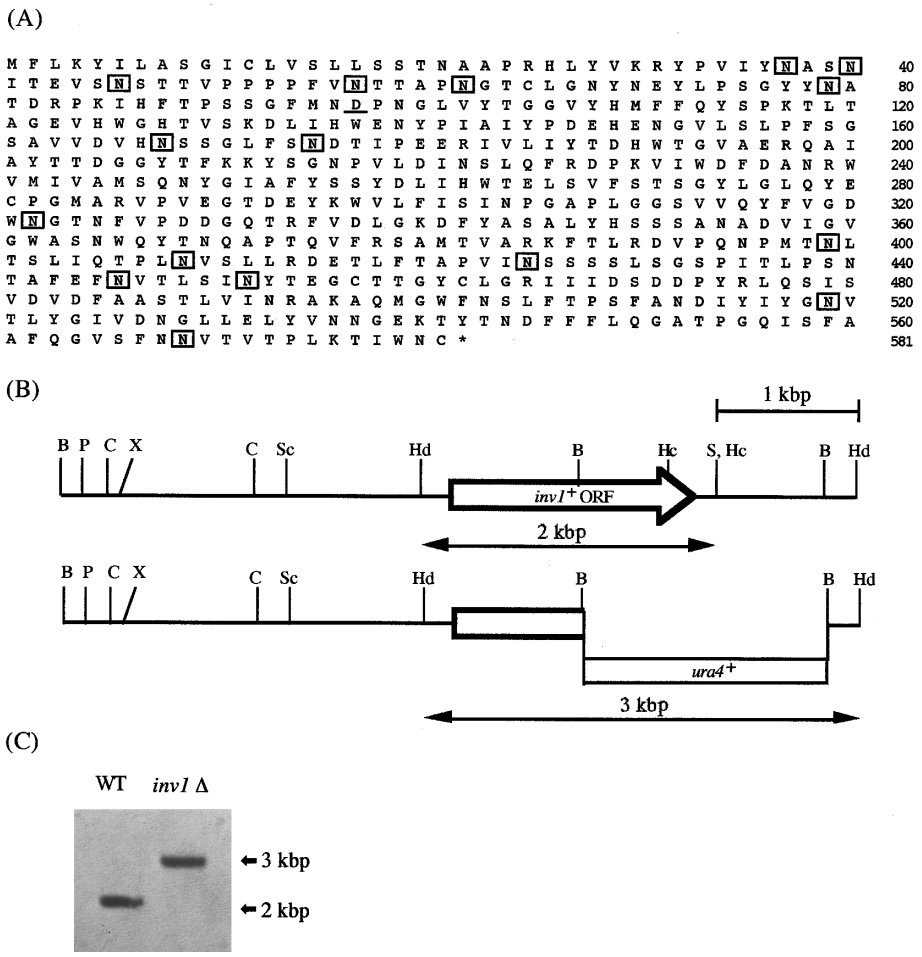


**FIG. 1.** (A) The deduced amino acid sequence of Inv1p. The boxed asparagine residues show potential N-glycosylation sites. The putative active-site Asp is underlined. (B) Restriction map and gene disruption of the *inv1*$^+$ locus. Restriction sites are abbreviated as follows: B, *Bam*HI; Hd, *Hin*dIII; P, *Pst*I; C, *Cla*I; X, *Xba*I; Sc, *Sac*I; Hc, *Hin*cII; amd S, *Sal*I. (C) Southern blot analysis of the *inv1*$^+$ gene disruption mutant. Genomic DNA was isolated from *S. pombe* wild-type strain TP4-1D and disruption mutant strain NOD-1, which was digested with *Hin*dIII and *Sal*I. The digested DNA (5 $\mu$g) was separated on a 1.0% agarose gel. Following denaturation, the DNA was transferred onto Hybond N+ and the blot hybridized. The 2.0 kb *Hin*dII-*Sal*I fragment was used as the probe.

We designated this gene *inv1*$^+$. With the cloned PCR fragment as a probe, a genomic library of *S. pombe* λEMBL3-*Sau*3A was screened by plaque hybridization. λEMBL3 DNA was fragmented by restriction enzymes, ligated into pBluescript vector, and the 5 kb fragment containing the invertase gene was sequenced. The nucleotide sequence of the *inv1*$^+$ gene contained a single open reading frame of 1,743 bp encoding a protein of 581 amino acids with 16 potential N-glycosylation sites (Fig. 1A). We compared the *inv1*$^+$ gene with the known sequences of invertases from other yeasts. Comparison of the amino acid sequence of the Inv1p with those from other origins reveals striking homologies. The protein shows 39 % identity to invertases from *Schwanniomyces occidentalis* (26), 38 % to *SUC2* gene from *S. cerevisiae* (27). Reddy and Maley (28) demonstrated that the aspartate residue, Asp42, is essential for the *S. cerevisiae* invertase activity, by site-directed mutagenesis and chemical modification analyses. The *S. pombe* Inv1p is well conserved around the putative active site-residue Asp97 (Fig. 1A).

*Disruption of the inv1*$^+$ *gene.* To examine the phenotypic consequences of a null allele of *inv1*$^+$, we performed a gene deletion-disruption of this locus. A linear fragment of the *inv1*$^+$ gene in which the 1.4 kb *Bam*HI-*Bam*HI internal fragment had been replaced with the *S. pombe ura4*$^+$ gene (Fig. 1B) was used to transform TP4-1D haploid cells. Several *ura*$^+$ transformants were isolated and the structure of the disrupted allele was verified by Southern blot analysis (Fig. 1C). We then examined the characterization of invertase activities in wild-type and *inv1*Δ strains. Invertase activity was measured in cell extracts from wild-type and *inv1*Δ cells using sucrose as the substrate. There was signifi-

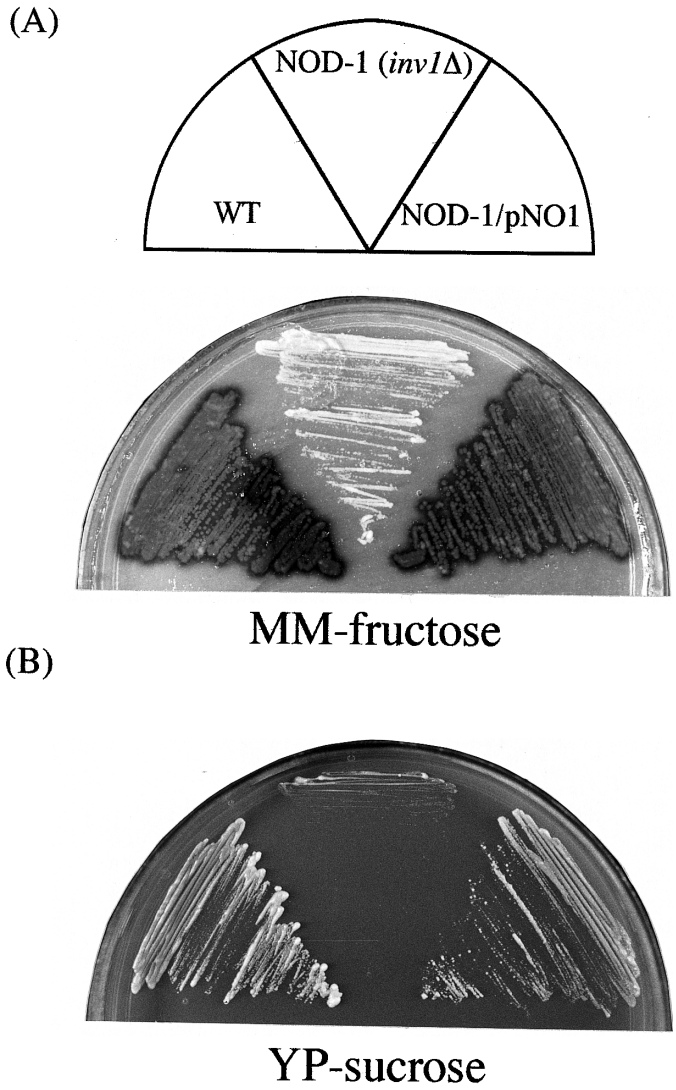


**FIG. 2.** Characterization of the invertase-defective and growth phenotype of NOD-1 (*invΔ*) strain. (A) The parental strain TP4-1D, the deletion strain NOD-1, and NOD-1 strain carrying plasmid pNO1 were streaked on MM-Leu plates. After incubation at 30°C for 72 h, the cells were replica plated onto MM-fructose. The replica plates were then overlaid with an invertase assay solution containing 1.2% agar. Colonies secreting invertase turned red. (B) The cells were streaked onto 26°C YP-sucrose plates supplemented with 10 $\mu$g/ml antimycin A and 20 $\mu$g/ml bromocresol purple, and incubated for 6 days. WT, TP4-1D (wild-type); NOD-1 (*inv1Δ*); and NOD-1/pNO1, NOD-1 carrying plasmid pNO1.

cant invertase activity in the wild-type strain and no detectable invertase activity in *inv1Δ* cells by plate assays (Fig. 2A). We also examined the invertase activity found in extracts of cells where *inv1*$^+$ is expressed using pAL-KS$^+$ plasmid (strain NOD-1/pAL-inv1$^+$)(Fig. 2A). Moreover, *inv1Δ* strains did not grow on MM-sucrose plates (Fig. 2B). These results showed that the disruption of *inv1*$^+$ gene completely eliminated their capacity to synthesize invertase and the *inv1*$^+$ gene encodes only one active invertase in *S. pombe* cells.

*Isolation and characterization of S. pombe CREA homolog gene.* An examination of the *S. pombe* Genome Database revealed that several gene products are predicted to encode proteins homologous to the $C_2H_2$-zinc finger family. One of these candidates, a gene in chromosome 2 (SPBC1D7_2), revealed a single contiguous open reading frame potentially encoding a 565 amino acid protein. This gene product has two $C_2H_2$ zinc finger motifs in the amino terminal region, and possesses overall amino acid identity of 32% to CREAp from *Aspergillus nidulans* (29) and 27% to Mig1p from *S. cerevisiae* (30)(Fig. 3A). Although the amino acid sequence of this protein revealed a relatively low overall level of similarity with CREAp and Mig1p, the zinc finger motifs are highly conserved with CREAp (86 %) and Mig1p (73 %), which are required for glucose repression of the glyconeogenic pathway, and Rsv1p (54 %), a zinc finger protein required for stationary viability in *S. pombe* (31) (Fig. 3B). Therefore we designated this gene *scr1*$^+$ (for *S. pombe* CREA homolog gene). The Scr1p also contains an alanine rich region which contains CREAs from *A. nidulans* (29) and *A. niger* (32) near the zinc-finger domains. Interestingly, the Scr1p contains a stretch of 59 amino acids that frequently appears as an SPXX or TPXX motif (33) which is common in transcriptional regulatory proteins (34), and this motif is highly homologous to those of CREAp, *Trichoderma* Cre1p (35) and *Metarhizium* Crr1p (36)(Fig. 3C). Although the roles of SPXX and TPXX motif of the Scr1p are not known, this highly conserved region may be required for *scr1*$^+$ function.

To examine the phenotypic consequences of a null allele of *scr1*$^+$, we performed a gene deletion-disruption of this locus. A linear fragment of the *scr1*$^+$ gene inserted with *S. pombe ura4*$^+$ gene (Fig. 4A) was used to transform haploid strain TP4-1D. Consistent with the observation that the MIG1 gene is not essential in *S. cerevisiae*, many candidate *ura4*$^+$-disrupted *scr1*$^+$ transformants were obtained. Several transformants were isolated, and the structure of the disrupted allele was verified by Southern blot analysis. The *scr1Δ* cells grew well at 26°C and 36°C, and did not exhibit a temperature-sensitive growth defect. Wild-type and *scr1Δ* strains were streaked onto raffinose media containing 2-deoxyglucose (Fig. 4B). The *scr1Δ* strains permitted growth on raffinose in the presence of glucose analog. These results suggested that the *inv1*$^+$ repression of glucose is relieved in the *scr1Δ* strains.

*The transcription of inv1*$^+$ *gene is controlled by scr1*$^+$ *gene.* To determine if the deletion of the *scr1*$^+$ gene altered transcript levels for the *inv1*$^+$ gene, we assayed invertase activity in *scr1Δ* strains under repressing conditions (8% glucose). The cells were collected by centrifu-

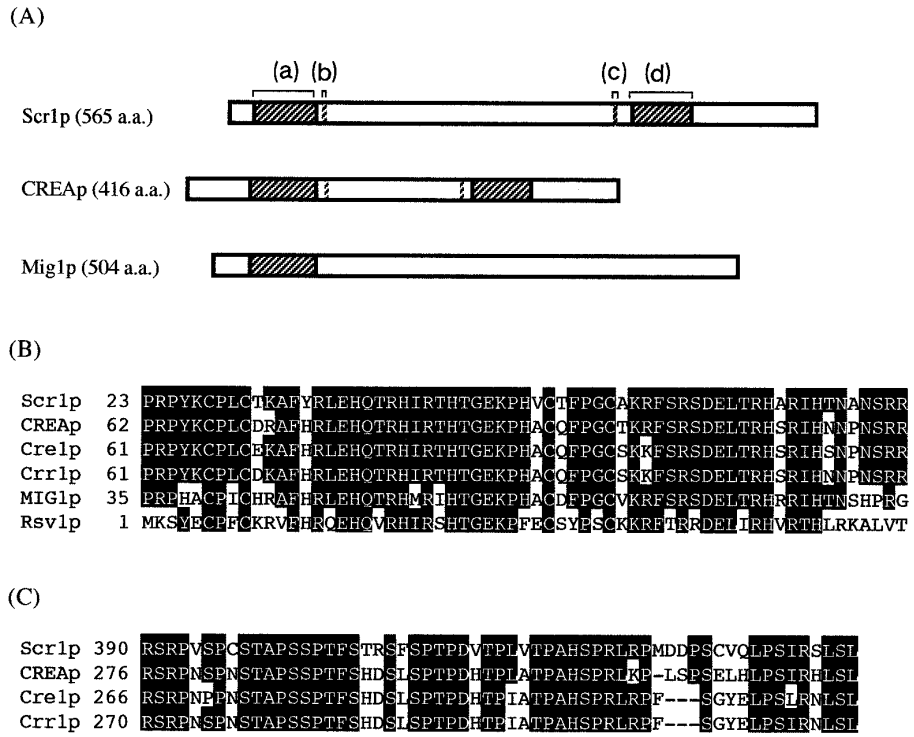


**FIG. 3.** (A) Schematic view of homology between Scr1p and related zinc finger proteins. (a), Zinc finger domain; (b), Ala-rich region; (c), acidic region; and (d), region with frequent SPXX and TPXX motifs. (B) Alignment of zinc finger motifs in CREAp (*A. nidulans*), Cre1p (*T. reesei*), Crr1p (*M. anisopliae*), Mig1p (*S. cerevisiae*), and Rsv1p (*S. pombe*), with Scr1p. The residues which are conserved between the different zinc finger proteins are shown in black boxes. Amino acid numbering is indicated at the beginning and end of each sequence. (C) The most highly conserved domains are aligned between Scr1p and other related proteins.

gation, and incubated for 3 h in YP media supplemented with 0.1% glucose +3% glycerol (derepressing conditions) as the carbon source. The invertase activity and transcription of the *inv1*$^+$ gene in wild-type strain (TP4-1D) was significantly repressed in the presence of glucose (Fig. 5A). In contrast, the *scr1*$\Delta$ strains have elevated levels of invertase activity and the transcription of the *inv1*$^+$ gene when grown under repressing conditions (Fig. 5A). These results show that the production of invertase in *S. pombe* is dependent on *scr1*$^+$ gene.

Transcription of the *fbp1*$^+$ gene of *S. pombe* is also controlled by glucose (37). Hoffman and Winston (25) have isolated many mutants that constitutively express fbpase in the presence of glucose. Genetic and molecular analyses of these mutants have led to the identification of eight genes that appear to encode components of a cAMP signal transduction pathway. Mutations in the *git2*$^+$ gene cause high levels of production of fbpase in the presence of glucose (38), and the *git2*$^+$ gene was identical to previously identified *cyr1*$^+$ gene which encodes adenylate cyclase (39). To determine whether *inv1*$^+$ is affected by the cAMP signalling pathway, we assayed invertase activity and examined *inv1*$^+$ expression in *cyr1*$\Delta$ strain. We measured invertase activity in *cyr1*$\Delta$ strain grown under repressing and derepressing conditions (Fig. 5A). The invertase activity and transcription of *inv1*$^+$ were still repressed in *cyr1*$\Delta$ strain in the presence of glucose (Fig. 5B-A, lane 5 and 6). In contrast, the transcription of *fbp1*$^+$ in *cyr1*$\Delta$ strain was constitutively expressed in the presence of glucose (Fig. 5B-B, lane 5 and 6) as reported previously (25, 38). Interestingly, the transcription of *fbp1*$^+$ gene is still repressed in *scr1*$\Delta$ strains (Fig. 5B-B). These results demonstrate that *S. pombe* cAMP signalling pathway may not be essential for glucose repression of *inv1*$^+$ gene, and glucose repression of *fbp1*$^+$ and *inv1*$^+$ in *S. pombe* is regulated by a different system.

## DISCUSSION

We have isolated the *inv1*$^+$ gene which encodes an active invertase in *S. pombe.* The striking feature of the Inv1p is the high levels of N-linked glycosylation sites, which constitute 16 Asn-X-Ser/Thr sites. The amino acid sequence of S. cerevisiae invertase includes 14 N-linked glycosylation sites (27), and 13 are wholly or partially glycosylated to give an average of 9-10 oligosaccharides per subunit invertase (40). The carbohydrate content of the *S. pombe* invertase (67% of the total mass) is higher than that of S. cerevisiae enzyme

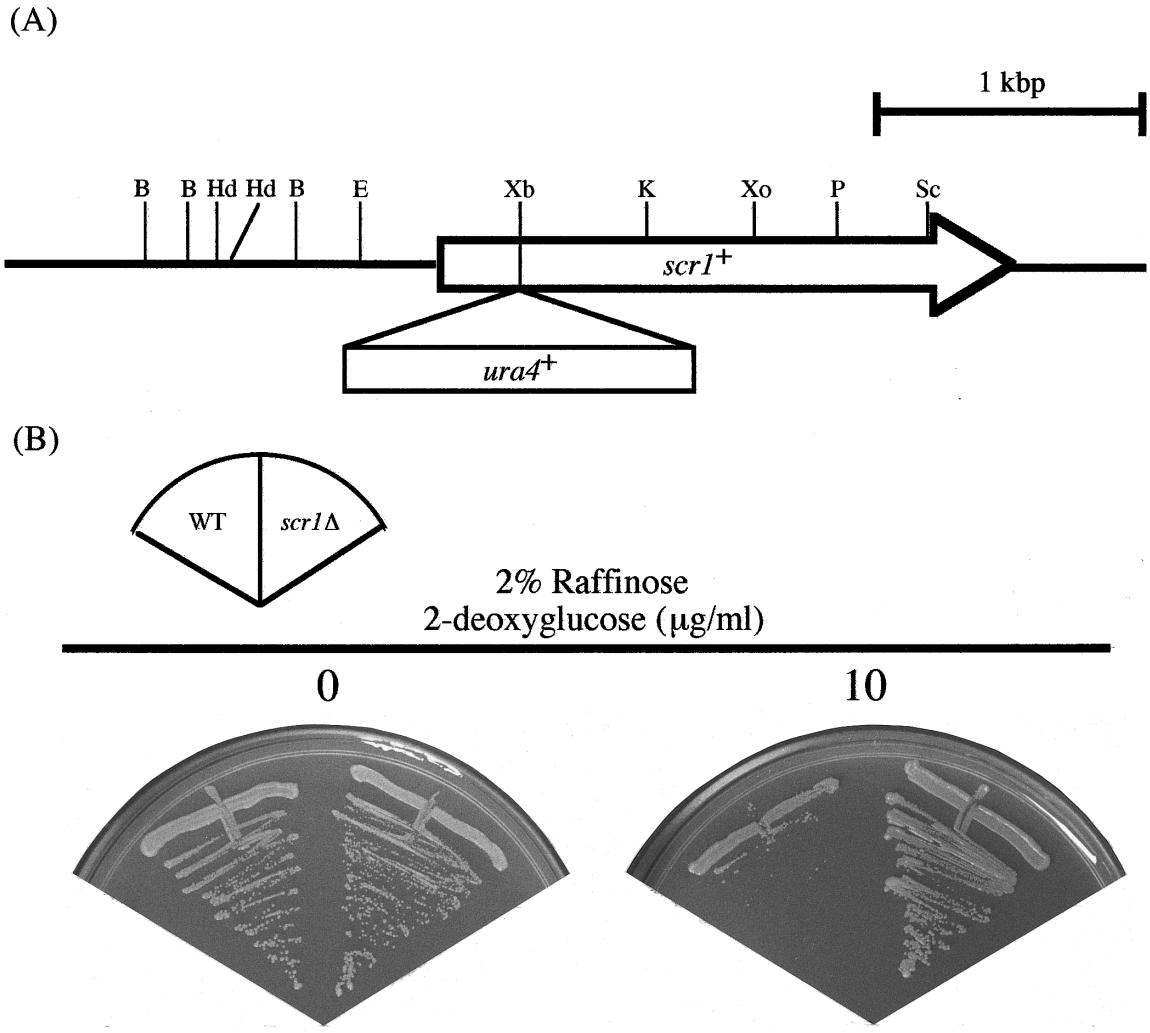


**FIG. 4.** Disruption of *scr1*$^+$ gene. (A) Restriction map and gene disruption of the *scr1* gene. Restriction sites are abbreviated as follows: B, *Bam*HI; Hd, *Hin*dIII; E, *Eco*RI; Xb, *Xba*I; K, *Kpn*I; Xo, *Xho*I; P, *Pst*I; and Sc, *Sac*I. (B) Disruption of *scr1*$^+$ makes the *S. pombe* invertase gene resistant to glucose repression. The wild-type and *scr1*Δ strains were grown on raffinose plates containing 0 or 10 μg/ml of 2-deoxyglucose, and incubated for 4 days at 26°C.

(50%)(15). Therefore the difference of molecular weight between *S. pombe* and S. cerevisiae is caused mainly by the oligosaccharide moiety (41, 42). It remains to be determined which of the Asn residues in *S. pombe* invertase are actually glycosylated. The carbohydrate moiety of the *S. pombe* invertase is composed of mannose and galactose in a ratio of about 1 : 1 (15). Recently we have isolated an *S. pombe* mutant (*gms1*) defective in protein galactosylation (43), and the *gms1*Δ strains lacked galactose residues in cell surface glycoproteins (44). Inv1p as a marker protein will be a valuable tool for studying oligosaccharide synthesis in *S. pombe* (16). We are attempting to elucidate the oligosaccharide structure of Inv1p in *gms1*Δ cells.

In this paper, we described that the transcription of *inv1*$^+$ gene is repressed in the presence of glucose. In contrast to the zinc finger proteins of other yeasts which are Mig1 homologs (45), *S. pombe scr1*$^+$ encodes a homolog of the *A. nidulans* CREA which is required for glucose repression of the glyconeogenic pathway. This study is the first to identify a CREA homolog zinc finger protein, *S. pombe* Scr1p, in yeast. The *A. nidulans creA* gene has been cloned and characterized by Dowzer and Kelly (29, 46). DNase I footprint experiments revealed that CREA specifically binds to a (G/C)(C/T)GG(G/A)G motif (GC box)(47), identical to that for Mig1p (48). However, not all the possible sequences derived from the consensus sequence are binding sites. Interestingly, the *S. cerevisiae SUC2* gene was expressed in *S. pombe*, and the *S. pombe* cells were able to produce active *SUC2* invertase in a constitutive fashion (41, 42). At present we do not know why the tran-

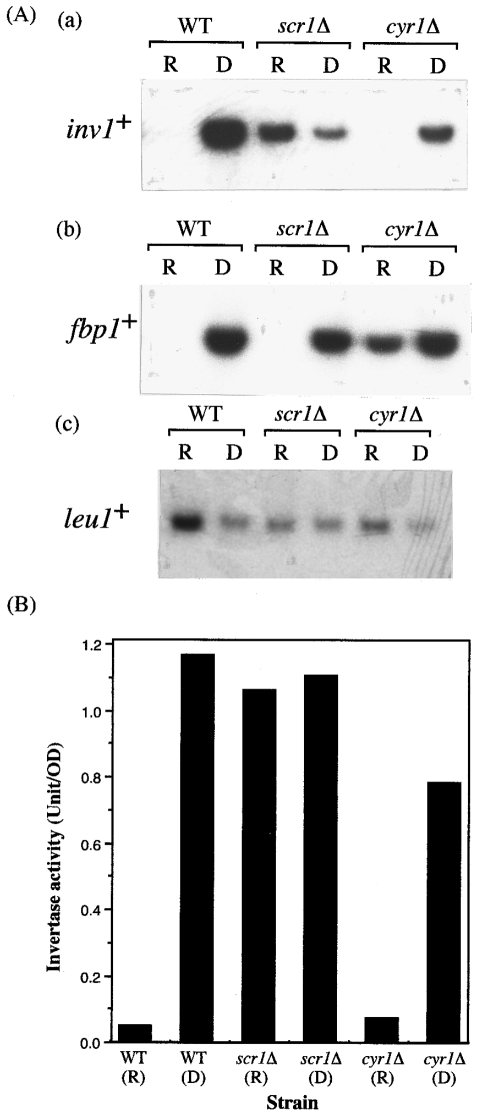


**FIG. 5.** (A) Northern blot analysis of *inv1*$^+$ mRNA. Total RNA was extracted from the wild-type (lanes 1 and 2), scr1D (lanes 3 and 4), and *cyr1*Δ (lanes 5 and 6) strains grown either under repressing (8% glucose; lanes 1,3 and 5) or derepressing (0.1% glucose and 3% glycerol; lanes 2, 4, and 6) conditions. Each RNA sample (2 $\mu$g) was separated on an agarose gel in the presence of formamide, blotted onto a nylon membrane and hybridized to *inv1*$^+$ (A), *fbp1*$^+$ (B), and *leu1*$^+$ (C) probes. R, Glucose repressed; D, derepressed. (B) Invertase activity in wild-type, *scr1*Δ, and *cyr1*Δ strains.

scription of *S. cerevisiae SUC2* gene is not repressed by glucose in *S. pombe* cells, but this may be caused by the binding specificities between the *S. pombe* Scr1p and *S. cerevisiae* Mig1p zinc finger proteins. We surveyed the 6-bp sites (GC-box) identical to the consensus sequence for CREA/Mig1 binding in the upstream of *inv1*$^+$ gene, and identified two consensus sequences (5′-CTCCG(A)C-3′) (unpublished results). Efforts are underway to determine whether that the Scr1p actually interacts with these sites in the upstream of *inv1*$^+$ gene. Characterization of genes involved in the regulation of *inv1*$^+$ will reveal not only a mechanism for glucose repression in *S. pombe* but also new insights into the molecular mechanisms governing transcription mechanisms in eukaryotic cells.

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