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Schizosaccharomyces pombe Git7p, a Member of the *Saccharomyces cerevisiae* Sgt1p Family, Is Required for Glucose and Cyclic AMP Signaling, Cell Wall Integrity, and Septation

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The *Schizosaccharomyces pombe fbp1* gene, encoding fructose-1,6-bisphosphatase, is transcriptionally repressed by glucose. Mutations that confer constitutive *fbp1* transcription identify *git* (glucose-insensitive transcription) genes that encode components of a cyclic AMP (cAMP) signaling pathway required for adenylate cyclase activation. Four of these genes encode the three subunits of a heterotrimeric G protein (*gpa2*, *git5*, and *git11*) and a G protein-coupled receptor (*git3*). Three additional genes, *git1*, *git7*, and *git10*, act in parallel to or downstream from the G protein genes. Here, we describe the cloning and characterization of the *git7* gene. The Git7p protein is a member of the *Saccharomyces cerevisiae* Sgt1p protein family. In budding yeast, Sgt1p associates with Skp1p and plays an essential role in kinetochore assembly, while in *Arabidopsis*, a pair of SGT1 proteins have been found to be involved in plant disease resistance through an interaction with RAR1. Like *S. cerevisiae* Sgt1p, Git7p is essential, but this requirement appears to be due to roles in septation and cell wall integrity, which are unrelated to cAMP signaling, as *S. pombe* cells lacking either adenylate cyclase or protein kinase A are viable. In addition, *git7* mutants are sensitive to the microtubule-destabilizing drug benomyl, although they do not display a chromosome stability defect. Two alleles of *git7* that are functional for cell growth and septation but defective for glucose-triggered cAMP signaling encode proteins that are altered in the highly conserved carboxy terminus. The *S. cerevisiae* and human *SGT1* genes both suppress *git7-93* but not *git7-235* for glucose repression of *fbp1* transcription and benomyl sensitivity. This allele-specific suppression indicates that the Git7p/Sgt1p proteins may act as multimers, such that Git7-93p but not Git7-235p can deliver the orthologous proteins to species-specific targets. Our studies suggest that members of the Git7p/Sgt1p protein family may play a conserved role in the regulation of adenylate cyclase activation in *S. pombe*, *S. cerevisiae*, and humans.

Transcription of the *Schizosaccharomyces pombe fbp1* gene is repressed by glucose (23, 48). Previous studies showed that glucose detection triggers a cyclic AMP (cAMP) signal responsible for the activation of cAMP-dependent protein kinase A, which acts to repress *fbp1* transcription (11, 20, 26). Glucose starvation leads to the activation of the Spc1p stress-activated mitogen-activated protein kinase pathway, which in turn activates the heterodimeric bZIP transcriptional activator Atf1p-Pcr1p (27, 44, 45, 51). These two signaling pathways regulate *fbp1* transcription via at least two distinct mechanisms, with Atf1p-Pcr1p acting both directly at one *cis*-acting element (UAS1) and indirectly at a second element (UAS2) (25). Transcriptional activation from UAS2 appears to be carried out by the Rst2p zinc finger protein (19). Additional regulators of *fbp1* transcription include Tup11p and Tup12p, homologs of the *Saccharomyces cerevisiae* Tup1p global corepressor, which are required for *fbp1* repression, and the CCAAT box binding factor, which is required in concert with Atf1p-Pcr1p for *fbp1* derepression (25).

The *S. pombe* adenylate cyclase (Git2p/Cyr1p) activation mechanism resembles that of the mammalian enzyme (46) in that it involves the activity of a heterotrimeric G protein coupled to a seven-transmembrane domain receptor protein. In *S. pombe*, the *gpa2*, *git5*, and *git11* genes encode the Gα, Gβ, and Gγ subunits, respectively, while the *git3* gene encodes the putative G protein-coupled receptor (GPCR) (13, 19, 20, 23, 36). The role of these four genes is to activate Gpa2p Gα, as mutational activation of Gpa2p or overexpression of the wild-type $gpa2^+$ gene bypasses the need for Git5p Gβ, Git11p Gγ, and Git3p GPCR (31, 32, 37, 52). The Git3p, Gpa2p, and Git2p/Cyr1p proteins also display sequence homology to *S. cerevisiae* Gpr1p GPCR, Gpa2p Gα, and Cyr1p adenylate cyclase, which act in the glucose-triggered cAMP signaling pathway (12, 30, 53), and are involved in the regulation of

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TABLE 1. Strain list

Strain	Genotype
CHP27	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his7-366 git7-27*
CHP93	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his7-366 git7-93*
CHP449	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M216 his7-366 git7-235*
CHP556	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M216 his3-D1 git7-93*
CHP558	*h*^{90} *fbp1*::*ura4 leu1–32 ade6-M216 git2-1*::*LEU2*$^+$
CHP578	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1–32 ade6-M210 his 3-D1*
CHP594	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his3-D1*
CHP595	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M216 his3-D1*
CHP718	*h*$^+$ *fbp1*::*ura4 ura4-D18 leu1–32 ade6-M210 his3-D1 git7-235*
CHP758	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1–32 ade6-M210 git7-GFP*::*kan*
CHP767	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1–32 ade6-M210 git7-GFP*::*kan skp1*::*ura4*$^+$ *ars1*(*Mlu*I)::pREP41-*skp1-3×HA LEU2*$^+$
CHP792	*h*^{90} *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1–32 ade6-M216 his7-366 git7-GFP*::*kan*
CHP795	*h*^{90} *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1–32 ade6-M216 his7-366*
CHP803	*h*$^-$ *ura4*::*fbp1-lacZ leu1–32 ade6-M210 skp1*::*ura4*$^+$ *ars1*(*Mlu*I)::pREP41-*skp1-3×HA LEU2*$^+$
FWP72	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32*
FWP101	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his7-366*
FWP145	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M216 git7-235*
KSP1	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his3-D1 git7-27*
KSP2	*h*$^-$ *ura4*::*fbp1-lacZ leu1-32 ade6-M210 git7-GFP*::*kan* (Ch16 *ade6-M216 m23*::*ura4*$^+$)
KSP5	*h*$^+$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 ade6-M210 his7-366 git7-V5*::int*LEU2*$^+$
PBP1	*h*$^-$ *fbp1*::*ura4 ura4*::*fbp1-lacZ leu1-32 git7-GFP*::*kan*

pseudohyphal growth (33, 40). Unlike *S. pombe*, *S. cerevisiae* does not appear to express a Gβγ dimer that functions in this signaling pathway. Related cAMP signaling pathways are also found in a number of fungal pathogens. For example, the human fungal pathogen *Cryptococcus neoformans* uses Gα (Gpa1) (2) and adenylate cyclase (Cac1) (3), which resemble *S. pombe* Gpa2p and Git2p, to detect nutrient conditions and to regulate aspects of differentiation and virulence.

Three additional *S. pombe* genes, *git1*, *git7*, and *git10*, required for glucose-stimulated adenylate cyclase activation (11, 21), are still required for glucose repression of *fbp1* transcription in a strain carrying an activated allele of *gpa2* or overexpressing *gpa2*$^+$ (37, 52). Thus, Git1p, Git7p, and Git10p presumably regulate adenylate cyclase activation either downstream from or in parallel to Gpa2p Gα. In addition, *git7-235* strains display a temperature-sensitive lethal growth phenotype (21), suggesting an essential role for this gene independent of cAMP signaling, as both adenylate cyclase and protein kinase A are dispensable in *S. pombe* (20, 34, 35).

In this article, we describe the cloning and characterization of the *S. pombe git7* gene. Sequence analysis shows that Git7p is a member of the Sgt1p protein family found in many eukaryotes, including the budding yeast *S. cerevisiae*, plants, and mammals. *S. cerevisiae* Sgt1p associates with Skp1p and is required for kinetochore assembly (29), while *Arabidopsis* SGT1a and SGT1b associate with RAR1, playing a role in disease resistance (4, 5). Skp1p is a component of the SCF (Skp1p–Cullin–F-box) E3 ubiquitin ligase responsible for the polyubiquitination of proteins that are subsequently degraded by the proteasome (41); however, there is no evidence that the role of Skp1p and Sgt1p in kinetochore assembly involves ubiquitin ligase activity. Conservation of Sgt1p function in eukaryotes is suggested by the ability of the human ortholog HuSgt1p to restore viability to a strain lacking *S. cerevisiae* Sgt1p activity (29) and by the ability of the *Arabidopsis* orthologs to restore temperature-resistant growth to *S. cerevisiae sgt1-3* and *sgt1-5* mutant strains (5). Our work shows that, like *S. cerevisiae* Sgt1p, *S. pombe* Git7p is an essential protein, although cell death in *S. pombe* is associated with cell lysis along with cell division and septation defects. Two *git7* alleles that alter the carboxy terminus of Git7p confer a defect in *fbp1* regulation without affecting cell wall integrity and septation, suggesting that Git7p possesses discrete functional domains. Finally, we show that the expression of the human and *S. cerevisiae* Sgt1p proteins can restore *fbp1* transcriptional regulation in a *git7-93* mutant but not in a *git7-235* mutant. Such allele-specific suppression suggests that the Git7p/Sgt1p proteins form multimeric complexes that play conserved roles in a variety of cellular processes, including the regulation of adenylate cyclase activity, cell division, septation, and kinetochore assembly.

MATERIALS AND METHODS

***S. pombe* strains and growth media.** The *S. pombe* strains used in this study are listed in Table 1. The *fbp1*::*ura4*$^+$ and *ura4*::*fbp1-lacZ* reporters (21) are translational fusions integrated in single copies at the *fbp1* and *ura4* loci, respectively. Rich media YEA and YEL (16) were supplemented with 2% Casamino Acids. Defined medium PM (50) was supplemented with required nutrients at 75 mg/liter, except for L-leucine, which was used at 150 mg/liter. Solid medium SC containing 0.4 g of 5-fluoro-orotic acid (5-FOA)/liter and 8% glucose was used to determine 5-FOA resistance (21), which is a reflection of glucose repression of the *fbp1-ura4* reporter. Strains were grown at 30°C unless otherwise indicated.

Recombinant DNA Methods. All DNA manipulations were performed, unless otherwise stated, by using reagents and protocols from New England Biolabs. *Escherichia coli* transformations were done by using XL1-Blue electroporation-competent cells (Stratagene). The Failsafe PCR system (Epicentre Technologies) was used for PCR according to the manufacturer's instructions. Oligonucleotides were obtained from Integrated DNA Technologies. *S. pombe* plasmid transformations were performed by overnight incubation with polyethylene glycol–lithium acetate–Tris-EDTA buffer (13) or as described by Bähler et al. (6).

Cloning of the *git7* gene. A pBG2-based *S. pombe* genomic DNA library (38) was screened for clones that confer 5-FOA resistance (5-FOA^r) to host strain CHP556 (*git7-93*), indicating the restoration of glucose repression of the *fbp1-ura4* reporter. From 33,000 transformants, 37 5-FOA^r candidates were identified. Plasmids from these strains were rescued in *E. coli* (22) and screened for their

ability to restore growth at 37°C to *S. pombe* strain CHP718, which is temperature sensitive for growth due to the *git7-235* allele. Two of the 37 plasmid candidates conferred temperature-resistant growth to strain CHP718. These two plasmids, one of which was designated pHF1, contained identical inserts. Plasmid pHF1 was linearized in the insert with *Nhe*I and used to transform strain CHP718 (*his3-D1 git7-235*) to His^+. A stable integrant was identified and determined to be temperature resistant and 5-FOA^r, indicating that integrated plasmid pHF1 suppresses the *git7-235* mutation. This strain was then crossed with strain CHP595 (*his3-D1 git7*$^+$). All 13 tetrads examined displayed a parental ditype pattern (4 5-FOA^r:0 5-FOA^s [5-FOA sensitive] progeny), indicating that plasmid pHF1 had integrated in or near the *git7* locus. DNA sequence analysis of the two ends of the insert DNA from plasmid pHF1 revealed that the insert contains the sequence from positions 30,876 to 35,540 of cosmid c36 (GenBank accession number AL023589), which carries a portion of *S. pombe* chromosome 2, including two candidate open reading frames. Subcloning analyses demonstrated that the *git7* gene is open reading frame SPBC36.12c.

Disruption and green fluorescent protein (GFP) tagging of *git7*. The *git7* gene was disrupted by using a PCR-based approach as described by Bähler et al. (6). Oligonucleotides git7delta (5′-TCTGGCAAATAGTAATGCATTCGCGTAATGACGTCTTTGTTTCAAAATTTGCGAAGCACGCTTCGCATCGGATCCCCGGGTTAATTAA-3′) and git7revkan (5′-TTGGCACCAATTCCAGGAAGGCGACCCTTATCCATATCTTCGGATTTGTATAATGACTCGGAATTCGAGCTCGTTTAAAC-3′]) were used to PCR amplify a *kanMX6*-containing fragment from pFA6a-GFP-kanMX6. The amplified fragment was used to transform a diploid *S. pombe* strain, constructed by mating CHP556 (*git7-93*) with CHP594 (*git7*$^+$), to G418 resistance, replacing the wild-type *git7*$^+$ allele with a *kanMX6*-marked disruption allele. Homologous recombination at the *git7* locus was confirmed by Southern blot analysis. Azygotic asci, dissected on YEA medium, produced tetrads with only two viable progeny that were 5-FOA^s G418 sensitive, indicating that the *git7* deletion strain was nonviable. Progeny carrying the *git7* disruption could be rescued by transforming the diploid strain with plasmid pHF1 (*git7*$^+$) prior to tetrad dissection.

A *git7-GFP* fusion was created at the *git7* locus in strain FWP72 by homologous recombination with a PCR product made by PCR amplification of plasmid pFA6a-GFP-kanMX6 with primers git7GFPtag (5′-CAACTAACTGGAAAGATGTGAAAAGCAAAACATTTGAAACAAAGCCTCCACAGGGAATGGAACCAAAAAAAATTTCGGATCCCCGGGTTAATTAA-3′) and git7revkan (see above). The resulting strain, PBP1, was defective in glucose repression of *fbp1* transcription, as indicated by its 5-FOA^s phenotype and by elevated *fbp1-lacZ* expression in glucose-grown cells (see Results). Strain PBP1 was confirmed to have the homologous recombination event by Southern blot analysis and by a cross with strain CHP93 (*git7-93*), demonstrating that the *kanMX6* marker was linked to the *git7* locus (all 24 tetrads displayed the parental ditype pattern of 0 5-FOA^r:4 5-FOA^s progeny).

Construction of a transcriptionally regulated allele of *skp1*. The *S. pombe skp1*$^+$ gene (GenBank accession number AF071066) was precisely replaced with the *ura4*$^+$ marker in a diploid strain (*h*$^+$/*h*$^-$ *ura4-D18 ade6-210*/*ade6-216 leu1-32*) by transformation with a PCR-generated cassette bearing the 1.7-kb *ura4*$^+$ gene flanked by 84 and 58 bases of homology to the *skp1*$^+$ locus. This cassette was generated by PCR with oligonucleotides A (5′-CAGCATAACTAGAAATGCTAACAGCTTAACTTTCATTCATCCATTACTTACATACATCAACGCTTAGCTACAAATCCCACTGG-3′) and B (5′-GGCGCATGATGAGGTGGATGGGAAGATTCCCATTTTACACTGAAAACTACACTTAATGTCCAACACCAATGTTTATAACC-3′) to amplify *ura4*$^+$ with 58 bases of homology to the *skp1*$^+$ locus. Homology to the 5′ end of *skp1*$^+$ was extended by subsequent amplification of the primary product with oligonucleotides B and C (5′-GAGTTCTGCCACTGTAGGAATATCAGCATAACTAGAAATGC-3′). Transformants were selected by growth on medium lacking uracil, and the proper disruption was verified by PCR.

A transcriptionally regulated, hemagglutinin (HA)-tagged *skp1* allele (*nmt41-HA-skp1*) was generated by insertion of the *skp1*$^+$ open reading frame (residues 2 to 161) into the *Sal*I and *Bgl*II sites of pSLF173 (15) after PCR amplification by using oligonucleotides incorporating an *Xho*I site and a *Bgl*II site at the 5′ and 3′ends, respectively. PCR was performed by using a plasmid (c5) containing *S. pombe skp1* cDNA as a template. This HA-tagged gene was then shuttled into a lower-level expression construct that uses the *nmt41* promoter by release with *Xho*I and *Bgl*II from pSLF173 and cloning into the *Xho*I and *Bam*HI sites of pREP41x (14). This vector was linearized by digestion with *Mlu*I and integrated into the *ars1* locus of the *skp1*Δ::*ura4*$^+$/*skp1*$^+$diploid strain. The diploid strain was induced to sporulate by growth on minimal medium lacking thiamine, and *skp1*Δ::*ura4*$^+$ *ars1*(*Mlu*I)::pREP41-*skp1*$^+$*-3×HA LEU2*$^+$ spores were selected on minimal medium lacking uracil and leucine. Integration at *ars1* was confirmed by PCR. The sequence of the tagging vector was verified prior to integration.

β-Galactosidase assays. Strains were cultured overnight under repression conditions (8% glucose) in YEL or PM medium. Cultures were grown overnight to a final cell density of approximately 10^7 cells/ml. Protein lysates were prepared on ice and assayed for β-galactosidase activity as previously described (37).

Gap repair and DNA sequencing. Mutant alleles of *git7* were cloned by gap repair (39) by transforming strains KSP1 (*git7-27*), CHP556 (*git7-93*), and CHP718 (*git7-235*) to His^+ with *Eco*RV-linearized plasmid pHF5. Plasmids were rescued in *E. coli* (22), and the open reading frames of the rescued *git7* mutant alleles were sequenced by using custom oligonucleotides on one strand. Mutations were confirmed by sequencing of the complementary strand.

Construction of vectors expressing *S. pombe git7* and human and *S. cerevisiae SGT1* in *S. pombe*. The *S. pombe git7*, *S. cerevisiae SGT1*, and human *SGT1* genes were cloned into vector pNMT81-TOPO (Invitrogen Corp.) according to the manufacturer's instructions, placing the expression of these genes under the control of the thiamine-repressible low-level-expression *nmt81* promoter (14). The *git7* gene was amplified from plasmid pHF1 by PCR with primers git7ATG1-For (5′-TGATAAAATATTCACATGAAACCCATAGCC-3′) and git7-V5-Rev (5′-AAATTTTTTTGGTTCCATTCCCTGTGGAGG-3′) to produce an insert encoding the originally predicted 444-residue protein. This sequence was translationally fused to a carboxy-terminal V5 epitope tag (43) upon insertion into pNMT81-TOPO to create plasmid pKS1. A *git7* promoter-driven *git7-V5* fusion was constructed by homologous integration of a *Pac*I-linearized derivative of pKS1 (an *Sph*I fragment containing the *git7* 5′ coding sequence was removed to avoid having *git7*$^+$ expressed from the *nmt81* promoter) into the *git7*$^+$ genomic locus in strain FWP101 to create strain KSP5. A second, *nmt81*-driven *git7-V5* plasmid-borne construct was made by PCR amplification from the SPLE-2 cDNA library (25) with primers git7ATG3-For (5′-CCATAAAATGGGTGTAGATCTTTCTGA-3′) and git7-V5-Rev, resulting in plasmid pKS2, which expresses the 379-residue Git7p protein (which would be expressed from the third potential start codon in *git7*) fused to a carboxy-terminal V5 epitope tag (see Results). Plasmids expressing untagged forms of the human *SGT1* gene (pKS3) and the *S. cerevisiae SGT1* gene (pKS4) were constructed to carry PCR products amplified from plasmid BKK43 with primers HuSGT1-For (5′-ATGGCGGCGGCTGCAGCAGGA-3′) and HuSGT1-Rev (5′-TTAGTACTTTTTCCATTCCATATCATCAGG-3′) and from plasmid BKK9 with primers ScSGT1-For (5′-ATGCCTGTTGAAAAAGATTTAAAAACTGCCTAC-3′) and ScSGT1-Rev (5′-TTACCAATGTTTAGGTTCCATGCCTTC-3′), respectively (29).

Protein isolation and immunoblotting. Strains were cultured in PM liquid medium (lacking leucine for strains carrying autonomous plasmids) to an optical density at 600 nm of 0.6. Total protein extracts were prepared by trichloroacetic acid precipitation as described by Volland et al. (49). Protein extracts were electrophoresed on a sodium dodecyl sulfate–10% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. Immunodetection was carried out by using an anti-V5-horseradish peroxidase antibody (Invitrogen) according to the manufacturer's instructions, and visualization was carried out by using LumiGLO chemiluminescence (Kirkegaard & Perry Laboratories).

Immunofluorescence microscopy. Strains were cultured overnight in PM liquid medium and grown to a final cell density of approximately 10^7 cells/ml. Cells were fixed by using paraformaldehyde for 30 min and prepared as described by Hagan and Hyams (17). Git7p-V5 was localized by using an anti-V5 antibody (Invitrogen) according to the manufacturer's instructions and visualized by using Alexa Fluor 488-labeled immunoglobulin G as a secondary antibody (Molecular Probes) at 10 μg/ml for 1 h. Cells were resuspended in mounting medium containing 1 μg of Hoechst 33342/ml.

RESULTS

Cloning of the *S. pombe git7* gene. The *git* genes, including *git7*, were originally identified in a genetic screen for mutations that confer a defect in the glucose repression of both *fbp1-ura4* and *fbp1-lacZ* reporters in *S. pombe* (21). Thus, while wild-type (*git*$^+$) strains carrying these reporters are 5-FOA^r in a glucose-rich medium, *git* mutant strains are 5-FOA^s. By screening plasmid libraries for clones that restore a 5-FOA^r phenotype to *git* mutant hosts, we have been able to clone *git* genes as well as multicopy suppressors (13, 20, 26, 32). To clone *git7*$^+$, strain CHP556 (*git7-93*) was transformed to His^+ with a pBG2-based *S. pombe* genomic DNA library (38). From 33,000 transformants, we identified 37 5-FOA^r candidates. These candidate plasmids were subsequently screened for their ability to sup-

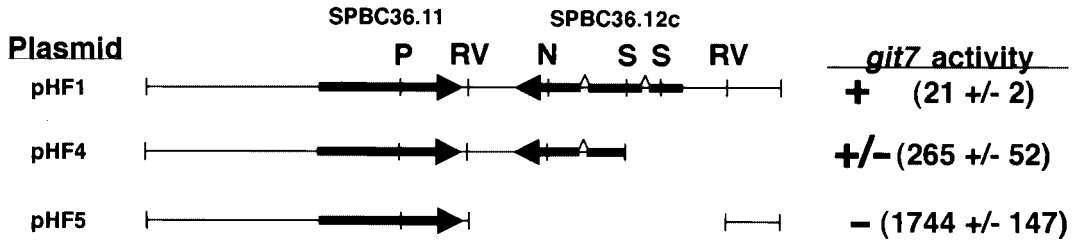

FIG. 1. Restriction map of insert DNA and *git7* suppression analysis for plasmid pHF1 and related plasmids. Restriction sites for *Pst*I (P), *Eco*RV (RV), *Nhe*I (N), and *Spe*I (S) are shown. *git7* activity is based on the ability of the plasmids to restore glucose repression of *fbp1-lacZ* expression when introduced into strain CHP556 (*git7-93*) and to restore growth at 37°C to strain CHP718 (*git7-235*). β-Galactosidase activity (in parentheses) in CHP556 transformants represents the average specific activity and standard error for three independent transformants grown under glucose repression conditions and assayed twice each. Under the same conditions, strain CHP578 ($git7^+$) carrying plasmid pHF5 possessed 5 ± 1 U of activity.

press the temperature-sensitive growth of strain CHP718 conferred by the *git7-235* allele. The original library screening was not carried out with this strain due to its poor transformation efficiency. Two of the 37 rescued plasmid candidates conferred temperature-resistant growth to strain CHP718. These two plasmids possess identical inserts, and one was designated pHF1 (Fig. 1). Sequence analysis across the two ends of the insert showed that pHF1 carries a portion of chromosome 2 from bp 30,876 to 35,540 of cosmid c36. Subclones from pHF1 demonstrated that the *git7*-complementing activity is encoded by ORF SPBC36.12c (GenPept accession number CAA19060; Fig. 1), which is divided into three exons and encodes a 379-residue protein. Integration of plasmid pHF1 into the *S. pombe* genome by homologous recombination followed by genetic linkage analysis indicated that pHF1 carries the *git7* gene and not a multicopy suppressor (see Materials and Methods). In addition, plasmid pHF1 failed to act as a multicopy suppressor when introduced into strains carrying mutations in any of five *git* genes required for adenylate cyclase activation: *git1*, *git3* (GPCR), *git5* (Gβ), *gpa2* (Gα), and *git10* (data not shown).

Git7p is a member of the *S. cerevisiae* Sgt1p protein family. A BLASTP analysis shows that Git7p is a member of a small family of proteins related to the *S. cerevisiae* Sgt1p protein (Fig. 2). Members of this family possess three distinct domains: a weakly conserved amino-terminal domain that appears to be a tetratricopeptide domain (5); a moderately conserved central domain referred to as the CS domain, due to its presence in some cysteine- and histidine-rich domain-containing proteins (42); and a highly conserved carboxy-terminal domain (Fig. 2). During our subcloning analysis of *git7*, we constructed plasmid pHF4, which lacks exon 1 and part of exon 2 and presumably expresses only the carboxyl-terminal 216 residues of Git7p. This plasmid partially restores both *fbp1* regulation in a *git7-93* strain (Fig. 1, plasmid pHF4) and 37°C growth in a *git7-235* strain (data not shown).

The Git7p protein was originally predicted to be 444 residues long due to the fact that exon 1 potentially includes 150 codons. However, a Clustal W alignment (47) of the putative 444-residue Git7p protein and other members of the family suggested that Git7p possesses a 50-residue amino-terminal domain not found in the other proteins (data not shown). The identity of the *git7* translational start site was brought into question when we cloned this presumed open reading frame into an *nmt81*-driven expression vector (*nmt* stands for "no message on thiamine"; see Materials and Methods). Plasmid pKS1 expresses a smaller-than-expected Git7p-V5 product whose activity is not repressed by thiamine (Fig. 3; CHP93/pKS1 cells express 12 ± 7 U of β-galactosidase activity after 48 h of thiamine repression, indicating plasmid suppression of the host *git7-93* mutation). Thus, it appears that the 5′ end of the cloned sequence includes the *git7* promoter. The most likely candidate TATA box is immediately upstream of the second putative ATG start codon, too close to allow transcription of this ATG. As translation from the third available start codon would produce a 379-residue protein, a size similar to those of other members of the Sgt1p/Git7p family (Fig. 2), we cloned the *git7* open reading frame starting with this third potential start codon into the *nmt81*-driven vector. Plasmid pKS2 expresses a Git7p-V5 protein with the same mobility as that of plasmid pKS1; however, this activity is now thiamine repressible (Fig. 3; CHP93/pKS2 cells express 1,884 ± 184 U of β-galactosidase activity after 48 h of thiamine repression, indicating a loss of suppression of the host *git7-93* mutation). As cells expressing Git7p-V5 from the *git7* genomic locus (see Materials and Methods) produce a protein with the same mobility as those of the plasmid-expressed constructs (Fig. 3), the authentic Git7p protein appears to be 379 residues long.

***S. pombe* Skp1p does not appear to regulate *fbp1-lacZ* expression.** It has been shown that the *S. cerevisiae* Sgt1p protein interacts with Skp1p of the SCF E3 ubiquitin ligase. We therefore set out to determine whether the *S. pombe* homolog of Skp1p plays a role in *fbp1* regulation and cAMP signaling, as does Git7p. Surprisingly, thiamine repression of *nmt41-HA-skp1* expression in a $git7^+$ strain (CHP803) had almost no effect on *fbp1-lacZ* expression. Cells continued to display glucose-repressed β-galactosidase levels (25 ± 8 U) after 16 h in the presence of thiamine. After 48 h of thiamine repression, cells showed arrested growth, yet β-galactosidase levels were only 207 ± 64 U. As we have observed a higher level of derepression of *fbp1-lacZ* expression in cells grown to stationary phase under glucose-rich conditions, this small degree of derepression does not suggest a role for *S. pombe* Skp1p in *fbp1* regulation. Therefore, it appears that the role of Git7p in cAMP signaling is Skp1p independent.

The *git7* gene is essential for cell wall integrity and septa-

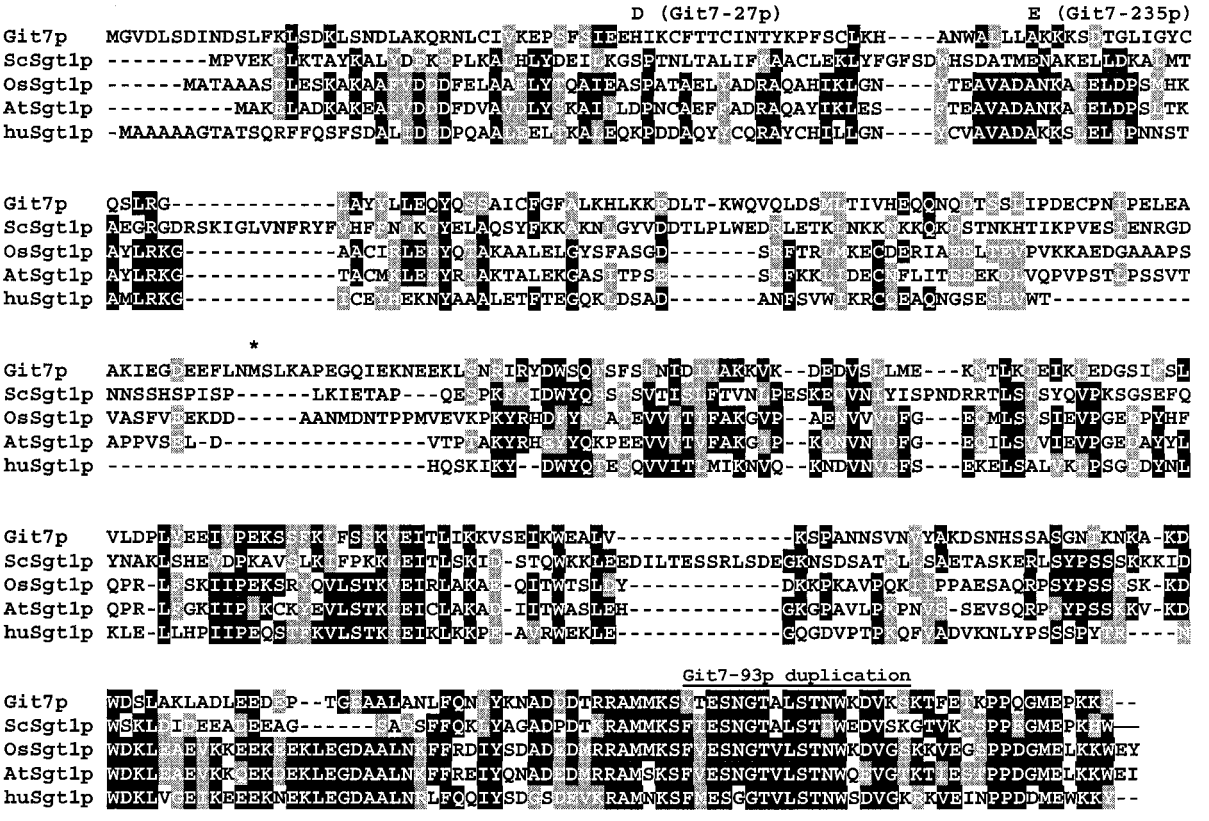

FIG. 2. Amino acid sequence alignment of *S. pombe* Git7p and related proteins. The Git7p protein (GenPept accession number CAA19060) was aligned with the *S. cerevisiae* Sgt1p protein (ScSgt1p) (GenPept accession number NP_014700), the *Oryza sativa* Sgt1p protein (OsSgt1p) (GenPept accession number BAB19060), the *Arabidopsis thaliana* Sgt1p protein (AtSgt1p) (GenPept accession number T05589), and the human Sgt1p protein (huSgt1p) (GenBank accession number AF132856) by using the Clustal W (version 1.8) sequence alignment program (47); the alignment was displayed by using BOXSHADE. Identical residues are shown as white letters shaded in black, while conserved residues are shown as white letters shaded in gray. The methionine encoded by the putative start codon used by the partially functional, truncated *git7* open reading frame carried on plasmid pHF4 (Fig. 1) is indicated by an asterisk. Alterations to the Git7p protein conferred by the *git7-27*, *git7-93*, and *git7-235* alleles are also indicated.

tion. We constructed a *git7*-null allele (*git7*Δ) by homologous recombination (6) in an effort to study the role of *git7* in cAMP signaling and other processes. The sporulation of a diploid strain carrying the *git7*Δ allele showed that *git7* is essential for viability. *git7*Δ spores germinated to produce microcolonies of approximately 200 to 300 cells, at which point all the cells appeared to undergo lysis (data not shown). *git7*Δ spore viability was rescued by either plasmid pHF1 or plasmid pHF4 (Fig. 1). Microscopic examination of cells from such transformants revealed the presence of both lysed cells and multinucleate cells, which likely resulted from plasmid loss (data not shown). Thus, Git7p seems to be required for both cell wall integrity and septation.

The original collection of *git* mutant strains included three independent *git7* mutants (21). At the time, it was noted that *git7-235* strains were temperature sensitive for growth. We therefore reexamined strains carrying the *git7-27*, *git7-93*, or *git7-235* allele for defects in growth and septation at 30 and 37°C. As shown in Fig. 4, most *git7-235* cells underwent lysis or failed to septate after 24 h at 37°C on solid medium. Even at 30°C, these defects were evident in *git7-235* cells. Strains carrying the *git7-27* allele displayed lysis and septation defects similar to those of *git7-235* strains (Fig. 4), although to a lesser degree. Strains carrying the *git7-93* allele appeared to have no growth defects, although they resembled other cAMP pathway mutants in that they divided at a slightly reduced cell length (26). Surprisingly, of the three original mutant alleles, the *git7-93* allele conferred the most severe defect with regard to glucose repression of an *fbp1-lacZ* reporter (Table 2). Thus, Git7p has distinct roles in cAMP signaling and in cell wall integrity and septation.

To better characterize the growth defects conferred by the *git7-235* allele, a temperature shift experiment was performed with two *git7-235* strains growing in liquid medium. Strains FWP145 and CHP449 differ by the presence of a *his7-366* allele in the latter strain. We have noted in the past that the *his7-366* mutant allele enhances growth defects that are conferred by mutations in other genes (unpublished data). Consistent with this observation, a greater percentage of cells in the CHP449 culture than in the FWP145 culture exhibited one of three growth defects at all time points (Table 3). These included multinucleate cells (cells with four or more nuclei; this phenotype was more common in cells growing on solid medium than in liquid medium), binucleate cells that appeared to be arrested during cell division, and lysed cells (Table 3 and Fig. 5). In addition, some cells that did form septa appeared to

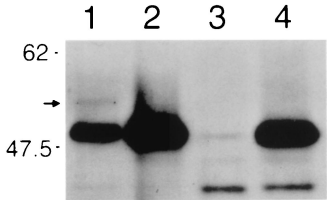

FIG. 3. Immunoblot of Git7p-V5 proteins. Protein extracts were prepared from CHP93 (*git7-93*) cells carrying either plasmid pKS1 (including 444 potential codons of *git7*) or plasmid pKS2 (including 379 potential codons of *git7*) and grown in the absence of thiamine (to promote expression from the plasmid-borne *nmt81* promoter). Additional extracts were made from strains FWP101 (untagged *git7*$^+$) and KSP5 (*git7-V5* integrated at the *git7* locus). Extracts were subjected to a Western blot analysis to detect V5-tagged (43) proteins. Lane 1, 20 μg of extract from CHP93/pKS1; lane 2, 7 μg of extract from CHP93/pKS2; lane 3, 60 μg of extract from FWP101; lane 4, 60 μg of extract from KSP5. Note that the CHP93/pKS1 extract in lane 1 contains a 54-kDa protein (indicated by an arrow) which was not seen in the other lanes and which may represent the protein translated from the first available start codon in pKS1.

fail to complete cytokinesis (Fig. 5C). Further examination of the CHP449 cells failed to show any actin delocalization (data not shown). These results confirm a role for Git7p in cell wall integrity, cell division, and septation.

A Git7p-GFP fusion is defective in cAMP signaling. In an effort to study the localization of Git7p in the cell, we constructed a *git7-GFP* fusion at the *git7* locus by homologous recombination (see Materials and Methods) (6). Cells expressing this fusion as the sole source of Git7p activity were viable and showed no growth defects (data not shown); however, these cells were defective in the glucose repression of *fbp1-lacZ*, indicating a defect in cAMP signaling (Table 2). Thus, like the *git7-93* allele, the *git7-GFP* fusion allele confers a defect in the glucose/cAMP signaling pathway but has no effect on cell wall integrity or on septation.

Git7p is required for nutrient regulation of mating. *S. pombe* cells normally require either a glucose or a nitrogen starvation signal to initiate mating and meiotic entry (44). Therefore, cells carrying mutations in genes required for the glucose-triggered cAMP signal will mate and sporulate in nutrient-rich media (24, 31, 32, 34, 52). Consistent with the defect in the glucose repression of *fbp1-lacZ* expression (Table 2), the *git7-GFP* allele allows homothallic (h^{90}) cells to mate in a glucose-rich medium (Fig. 6). This starvation-independent mating is similar to that conferred by a deletion of the *git2* adenylate cyclase gene (*git2*Δ), as the addition of 5 mM cAMP to the medium prevented conjugation in both *git7-GFP* and *git2*Δ cells (Fig. 6). Thus, *git7* is required for the cAMP-dependent regulation of conjugation as well as *fbp1* transcriptional regulation.

The carboxy-terminal domain of Git7p is specifically involved in cAMP signaling. Both the *git7-GFP* and the *git7-93* alleles confer a defect in *fbp1* regulation but not in cell growth and septation (Table 2, Fig. 4, and data not shown). We therefore cloned all three spontaneous *git7* alleles by gap repair of *Eco*RV-linearized plasmid pHF5 (Fig. 1). The *git7-93* allele contains a 54-bp duplication of codons 345 to 363, a sequence that is flanked in the wild-type allele by an 8-bp direct repeat. This leads to a duplication of 18 amino acids (YTESNGTALSTNWKDVKS; Fig. 2) in the carboxy-terminal domain of Git7p; this domain is highly conserved in other members of this protein family. Thus, both the *git7-93* and the *git7-GFP* alleles encode proteins that are altered at their carboxy termini. In contrast, both the *git7-27* and the *git7-235* alleles contain single missense mutations in the region encoding the amino terminus of Git7p. The mutation in *git7-27* changes a histidine to an aspartic acid at residue 42, while the mutation in *git7-235* changes an alanine to a glutamic acid at residue 69 (Fig. 2). As these two mutant alleles confer temperature-sensitive lethality, it is possible that these mutations result in a general instability of the Git7p protein. Alternatively, they may identify a domain required for all Git7p functions, including cell wall integrity and septation.

***git7* mutants do not display a kinetochore assembly defect.** As *S. cerevisiae* Sgt1p is required for kinetochore assembly (29), we tested whether any phenotypes associated with *git7* mutations would suggest a similar role for Git7p in *S. pombe*. We therefore examined the effect of the microtubule-destabilizing drug benomyl on strains carrying various *git7* alleles. Strains carrying any of the three spontaneous mutant alleles or the *git7-GFP* allele were benomyl sensitive (Fig. 7). The intro-

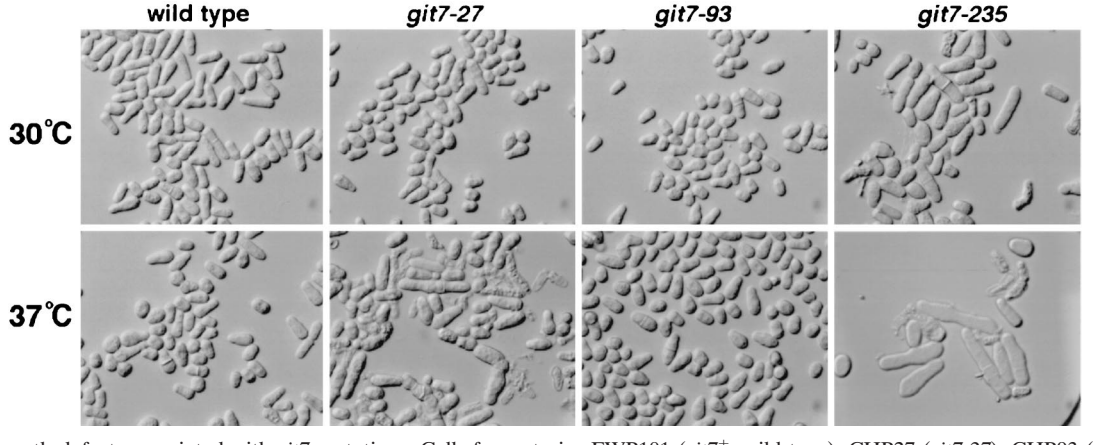

FIG. 4. Growth defects associated with *git7* mutations. Cells from strains FWP101 (*git7*$^+$; wild type), CHP27 (*git7-27*), CHP93 (*git7-93*), and CHP449 (*git7-235*) were grown for 24 h on YEA plates at either 30 or 37°C before being photographed.

TABLE 2. Defect in glucose repression of *fbp1-lacZ* expression in *git7* mutant strains[a]

Strain	Relevant genotype	β-Galactosidase activity
FWP101	*git7*$^+$	5 ± 0
CHP27	*git7-27*	1,124 ± 149
CHP93	*git7-93*	2,130 ± 301
CHP449	*git7-235*	1,600 ± 63
PBP1	*git7-GFP*	1,288 ± 61

[a] Cells were grown in YEL medium to exponential phase under repression conditions (8% glucose) and assayed as described in Materials and Methods. The values given represent mean specific activities and standard errors from two to four independent cultures of each strain.

duction of plasmid pKS1 (*git7-V5*) into a *git7-93* strain restored benomyl-resistant growth, providing further evidence that Git7p-V5 is fully functional for all Git7p activities of which we are aware, unlike Git7p-GFP. However, when assayed for mitotic stability of the Ch16 minichromosome (1), strain KSP2 (*git7-GFP*) did not display a chromosome stability defect (data not shown). Thus, the benomyl sensitivity is not likely due to a defect in kinetochore assembly, as is seen in *S. cerevisiae sgt1* mutants (29), but may reflect other interactions with spindle or cytoplasmic microtubules. To test whether Git7p plays a role in spindle assembly, we constructed a double mutant carrying *git7-235* and a deletion of the *mad2* spindle checkpoint gene (*mad2*Δ) (18). The *git7-235 mad2*Δ double mutant failed to display any synthetic growth defects that would be expected if Git7p were required for spindle assembly (data not shown).

Localization of a Git7p-V5 fusion. Because the Git7p-GFP fusion protein is not functional in the glucose-cAMP pathway, we examined the localization of the fully functional Git7p-V5 protein (Fig. 3 and Table 4) expressed from the *git7* promoter in a fusion integrated in a single copy (see Materials and Methods). The Git7p-V5 protein is seen as punctate staining in both the nucleus and the cytoplasm of cells (Fig. 8), while little or no staining is seen in untagged cells. A similar localization pattern has been observed for *S. cerevisiae* Sgt1p (C. Dubacq and C. Mann, personal communication).

Complementation of *git7-93* by plasmid-expressed *S. cerevisiae* and human Sgt1p proteins. We tested whether the human Sgt1p (Sgt1p) or *S. cerevisiae* Sgt1p proteins could restore transcriptional regulation of the *fbp1-lacZ* reporter when expressed in *git7* mutant strains. Indeed, the expression of either the human or the *S. cerevisiae SGT1* gene from the *nmt81* promoter completely suppressed the constitutive *fbp1-lacZ* expression observed in a *git7-93* mutant strain (Table 4). However, the expression of these genes in a *git7-235* strain had little or no effect on the constitutive expression of the *fbp1-lacZ* reporter. As expected, the expression of the Git7p-V5 fusion from the same vector suppressed both *git7-93* and *git7-235* mutations (Table 4). Consistent with this allele-specific suppression, we observed that the *git7-93* transformants were benomyl resistant, while the *git7-235* transformants remained benomyl sensitive (data not shown). Therefore, the ability of the human and budding yeast proteins to restore Git7p functions to *S. pombe* depends upon the expression of the Git7-93p mutant protein.

TABLE 3. Effect of growth at 36°C on *git7-235* cells[a]

h at 36°C	% of cells (CHP449/FWP145) with the following morphology:			
	Wild type	Multinucleate	Cell division	Lysed
0	90/100	10/0	0/0	0/0
4	70/89	13/5	14/6	3/0
6	68/81	12/4	16/14	4/1
8	36/65	14/2	33/24	17/9
27	12/27	15/6	37/46	36/22

[a] Cells were grown in YEL medium, stained with Hoechst 33342 to detect nuclei and Calcofluor to detect septa, and examined by microscopy. Two hundred cells were examined for each time point.

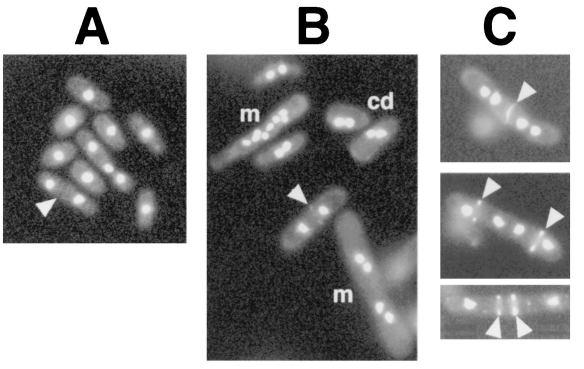

FIG. 5. Various morphologies associated with the *git7-235* allele. CHP449 (*git7-235*) cells were grown for 24 h at 37°C, stained with Hoechst 33342 and Calcofluor, and photographed. Septa are indicated by arrowheads. (A) Cells displaying the wild-type phenotype, being either uninucleate or binucleate with a septum. (B) Multinucleate (m) and cell division-arrested (cd) binucleate cells. (C) Cells containing septa but failing to complete cytokinesis.

DISCUSSION

In this study, we have cloned and carried out an initial characterization of the *S. pombe git7* gene, which is required for the glucose repression of *fbp1* transcription as part of the glucose-triggered cAMP signaling pathway. In previous studies, it was shown that *git7* is required for both the maintenance of basal cAMP levels and the generation of the glucose-stim-

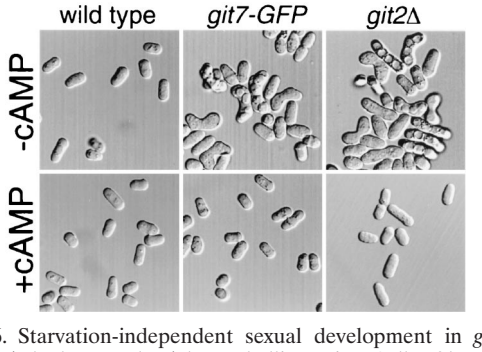

FIG. 6. Starvation-independent sexual development in *git7-GFP* and *git2*Δ (adenlyate cyclase) homothallic strains. Cells of homothallic strains CHP795 (wild type), CHP792 (*git7-GFP*), and CHP558 (*git2*Δ) were pregrown at 37°C (to inhibit conjugation) in PM medium (8% glucose) to a concentration of 10^7 cells/ml, diluted to 10^6 cells/ml in the presence or absence of 5 mM cAMP, and incubated overnight at 30°C without shaking before being photographed.

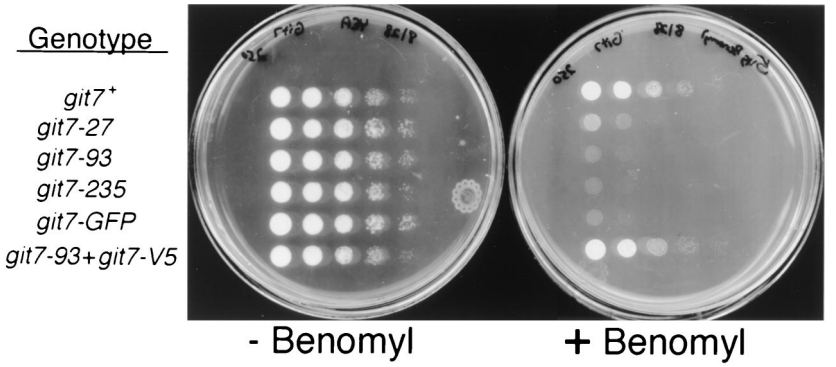

FIG. 7. Benomyl sensitivity in *git7* mutant strains. Serial dilutions (1:5) of strains FWP101 (*git7*$^+$), CHP27 (*git7-27*), CHP93 (*git7-93*), CHP449 (*git7-235*), CHP758 (*git7-GFP*), and CHP93 carrying plasmid pKS1 (*git7-93* + *git7-V5*) were spotted on YEA plates containing 75 μg of adenine/ml and either 0 or 5 μg of benomyl/ml. Growth was recorded after 3 days at 25°C.

ulated cAMP signal (11). It was also shown that while mutational activation of Gpa2p Gα bypasses the requirement for the Git3p GPCR or Git5p-Git11p Gβγ, it fails to suppress mutations in *git1*, *git7*, or *git10* (52). Therefore, while the role of the Git3p GPCR and Git5p-Git11p Gβγ is to activate Gpa2p Gα, Git1p, Git7p, and Git10p appear to function either downstream from or in parallel to Gpa2p Gα.

It was surprising to discover that *git7* is homologous to the *S. cerevisiae SGT1* gene, whose best-characterized role had been in kinetochore assembly (29). However, Sgt1p must play multiple roles in *S. cerevisiae*, as different temperature-sensitive *sgt1* alleles result in either G_1 or G_2 arrest. In addition, while Sgt1p physically interacts with Skp1p of the SCF E3 ubiquitin ligase, Sgt1p may carry out at least one Skp1p-independent function in G_1. The *S. cerevisiae skp1-11* allele causes G_1 arrest due to the accumulation of the Sic1p inhibitor of the Cdc28p-Clb kinase required for G_1 exit (7). As such, the arrest point for *skp1-11* cells is after the pheromone arrest point in G_1. Meanwhile, the arrest point for *sgt1-5* cells in G_1 precedes the pheromone arrest point (29), indicating a role for Sgt1p distinct from that of Skp1p in G_1. Our studies show little or no connection between Git7p and Skp1p in *S. pombe*. While both Git7p and Skp1p are essential in *S. pombe*, cells depleted of Skp1p do not show the same phenotypes as *git7* mutants. Thiamine repression of *nmt41-HA-skp1* cells causes cell growth arrest but not a defect in septation or an increase in cell lysis (data not shown). In addition, *fbp1-lacZ* expression remains glucose repressed upon Skp1p depletion, with only partial derepression as cell growth is arrested, indicating that *S. pombe* Skp1p is not involved in the glucose-triggered cAMP signaling pathway.

Our characterization of a *git7* deletion allele and other mutant alleles revealed a large number of mutant phenotypes, including constitutive *fbp1* transcription, starvation-independent conjugation and sporulation, cell lysis, a septation defect, a cell division defect, and sensitivity to the microtubule-destabilizing drug benomyl. These phenotypes suggest at least three distinct Git7p functions. The inability to repress *fbp1* transcription and conjugation is due to a defect in the cAMP signaling pathway, as has been shown for other *git* genes (26, 31, 32, 34, 52). The septation and lysis defects are most likely related, since lysis appears to occur at the septum during cytokinesis (data not shown). Along with a failure to form septa, *git7-27* and *git7-235* cells also form defective septa that lead to either cell lysis during cytokinesis or a failure to undergo cytokinesis, as shown in Fig. 5C. Finally, the cell division defect and benomyl sensitivity may be related, since microtubules are involved in the migration and positioning of the nuclei during mitosis in *S. pombe* (17). The lack of a mitotic chromosome stability defect in *git7* mutants or synthetic growth defects in a *git7-235*

TABLE 4. Suppression of constitutive *fbp1-lacZ* expression by human Sgt1p and *S. cerevisiae* Sgt1p in *git7-93* but not *git7-235* cells[a]

Plasmid-expressed protein	β-Galactosidase activity in the following host:	
	git7-93	*git7-235*
None	2,428 ± 488	1,581 ± 198
Git7p-V5	21 ± 3	17 ± 4
Human Sgt1p	21 ± 9	1,919 ± 214
S. cerevisiae Sgt1p	11 ± 3	1,176 ± 37

[a] β-Galactosidase activity was assayed in CHP93 (*git7-93*) and CHP449 (*git7-235*) transformants grown in PM medium lacking thiamine (to derepress expression from the *nmt81* promoter in the vector) under glucose-rich conditions. The plasmids used were pNMT81-TOPO (none; empty vector control), pKS2 (Git7p-V5), pKS3 (human Sgt1p), and pKS4 (*S. cerevisiae* Sgt1p). Values represent mean specific activities and standard errors from two to four independent cultures.

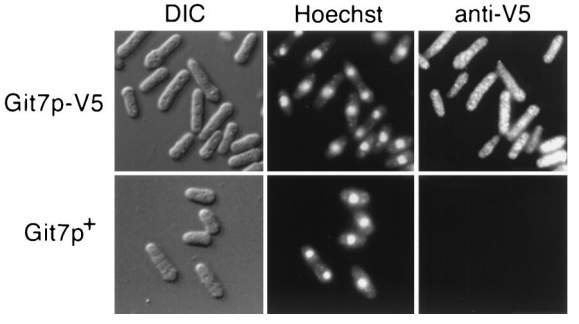

FIG. 8. Localization of Git7p-V5. Indirect immunofluorescence was used to detect Git7p-V5 localization in KSP5 cells (carrying an integrated *git7-V5* fusion) and in FWP101 cells (untagged *git7*$^+$). Cells were stained with Hoechst 33342 to detect DNA prior to microscopy. KSP5 cells display a punctate signal throughout the nucleus and the cytoplasm. DIC, differential interference contrast microscopy.

*mad2*Δ strain indicates that, unlike *S. cerevisiae* Sgt1p (29), Git7p plays no role in kinetochore assembly. However, as only a subset of *S. cerevisiae sgt1* mutants display a kinetochore assembly defect, our failure to observe a similar defect in *git7* mutants does not rule out a similar role for Git7p. The characterizations described here represent an initial investigation of a protein that is involved in at least three distinct processes, and further detailed studies are required in each area.

Protein modeling work carried out on members of the Sgt1p protein family by Azevedo et al. (5) suggests that there are three distict domains, with the poorly conserved amino-terminal domain acting as a tetratricopeptide domain (9). Our studies show that alteration of the highly conserved carboxy terminus by a short duplication in the *git7-93* allele (Fig. 2) or by the addition of a large GFP tag to the carboxy terminus leads to defects in the glucose-triggered cAMP signaling pathway (Table 2) and benomyl sensitivity (Fig. 7) but has no effect on either septation or cell wall integrity (Fig. 4). Meanwhile, the Git7-27p and Git7-235p proteins both have alterations in the amino-terminal domain and share similar phenotypes that include cell lysis and septation defects when grown at 37°C (Fig. 4 and 5), in addition to the cAMP signaling and benomyl sensitivity phenotypes. These results indicate that Git7p is composed of distinct functional domains that act in a modular fashion such that the *git7-93* mutation that interferes with cAMP signaling does not affect other essential Git7p functions. In support of this notion, we observed allele-specific suppression of the *git7-93* mutation by the expression of human or budding yeast *SGT1* genes (Table 4). Because such expression fails to suppress the same constitutive *fbp1-lacZ* expression or benomyl-sensitive growth conferred by a *git7-235* allele, it appears that Git7-93p provides a function that is defective in Git7-235p and that is needed to allow human or *S. cerevisiae* Sgt1p to act in *S. pombe*. Thus, Git7-235p may be defective in forming complexes with either Sgt1p or other components of a protein complex, while Git7-93p can interact with Sgt1p, which then provides a functional carboxy-terminal domain to the complex. However, since we have not observed intragenic complementation between *git7-93* and *git7-235*, it is possible that Git7-235p cannot interact with human or budding yeast Sgt1p because it is unstable.

The ability of human and *S. cerevisiae SGT1* genes to suppress a *git7-93* mutation suggests that this gene family may have a conserved role in cAMP signaling, although it need not be as a function of glucose detection. Indeed, *S. cerevisiae* Sgt1p has also been shown to interact both physically and genetically with adenylate cyclase in budding yeast (Dubacq and Mann, personal communication), supporting the idea that Sgt1p is involved in cAMP signaling. However, it remains to be seen whether or not members of the Git7p/Sgt1p family act to regulate adenylate cyclase in mammalian cells, even though human Sgt1p can provide that function in *S. pombe*. For example, although mammalian Ras proteins are able to replace *S. cerevisiae* Ras proteins in the budding yeast cAMP signaling pathway (10, 28), they do not appear to regulate cAMP signaling in mammalian cells (8). Thus, additional studies are needed to determine whether members of the Git7p/Sgt1p family are universally involved in cAMP signaling in yeasts, plants, and animals.

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