# Glucose Monitoring in Fission Yeast via the gpa2 Gα, the git5 Gβ and the git3 Putative Glucose Receptor

**Robert M. Welton and Charles S. Hoffman**

*Department of Biology, Boston College, Chestnut Hill, Massachusetts 02467*

Manuscript received January 31, 2000
Accepted for publication June 9, 2000

ABSTRACT

The fission yeast *Schizosaccharomyces pombe* responds to environmental glucose by activating adenylate cyclase. The resulting cAMP signal activates protein kinase A (PKA). PKA inhibits glucose starvation-induced processes, such as conjugation and meiosis, and the transcription of the *fbp1* gene that encodes the gluconeogenic enzyme fructose-1,6-bisphosphatase. We previously identified a collection of *git* genes required for glucose repression of *fbp1* transcription, including *pka1/git6*, encoding the PKA catalytic subunit, *git2/cyr1*, encoding adenylate cyclase, and six "upstream" genes required for adenylate cyclase activation. The *git8* gene, identical to *gpa2*, encodes the alpha subunit of a heterotrimeric guanine-nucleotide binding protein (Gα) while *git5* encodes a Gβ subunit. Multicopy suppression studies with $gpa2^{+}$ previously indicated that *S. pombe* adenylate cyclase activation may resemble that of the mammalian type II enzyme with sequential activation by Gα followed by Gβγ. We show here that an activated allele of *gpa2* ($gpa2^{R176H}$, carrying a mutation in the coding region for the GTPase domain) fully suppresses mutations in *git3* and *git5*, leading to a refinement in our model. We describe the cloning of *git3* and show that it encodes a putative seven-transmembrane G protein-coupled receptor. A *git3* deletion confers the same phenotypes as deletions of other components of the PKA pathway, including a germination delay, constitutive *fbp1* transcription, and starvation-independent conjugation. Since the *git3* deletion is fully suppressed by the $gpa2^{R176H}$ allele with respect to *fbp1* transcription, *git3* appears to encode a G protein-coupled glucose receptor responsible for adenylate cyclase activation in *S. pombe*.

ENVIRONMENTAL glucose is an important regulator of gene expression and other biological processes in both unicellular organisms and mammalian cells. As such, considerable research has been devoted to the study of glucose detection and the associated signal transduction pathways in a variety of model organisms. These studies have revealed surprising differences with respect to how two key model systems, the bacterium *Escherichia coli* and the budding yeast *Saccharomyces cerevisiae*, detect and respond to glucose.

*E. coli* employs a phosphoenolpyruvate-dependent phosphotransferase system (PTS) that is responsible for both the sensing of glucose and its translocation and phosphorylation to glucose-6-phosphate (reviewed by Postma *et al.* 1993). Therefore, glucose detection in *E. coli* is intrinsically linked to its uptake. The glucose PTS system regulates alternative carbon source utilization by inhibiting the transport of other carbon sources (inducer exclusion) and by reducing adenylate cyclase activity, thus lowering intracellular cAMP levels. The reduced cAMP level causes a reduction in DNA binding by the cAMP receptor protein, a positive regulator of transcription of operons subject to glucose repression.

*Corresponding author:* Charles S. Hoffman, Department of Biology, Boston College, Higgins Hall 401B, Chestnut Hill, MA 02467. E-mail: hoffmacs@bc.edu

Glucose detection in *S. cerevisiae* occurs through multiple mechanisms that are still actively under examination. One type of glucose sensor, encoded by *RGT2* and *SNF3*, resembles a 12-transmembrane hexose transporter (reviewed by Ozcan and Johnston 1999). A second glucose detection system is responsible for adenylate cyclase activation. The *GPR1* and *GPA2* genes, encoding a putative seven-transmembrane protein and a heterotrimeric G protein alpha subunit (Gα), respectively, are key components in this glucose-detection pathway (Colombo *et al.* 1998; Xue *et al.* 1998; Yun *et al.* 1998; Kraakman *et al.* 1999; Lorenz *et al.* 2000). While the Gpa2 Gα does not appear to interact with a classical Gβγ dimer, it is unclear whether it functions as a monomer or within some other protein complex. Gpr1 and Gpa2 have also been implicated in the control of pseudohyphal growth (Lorenz and Heitman 1997; Ansari *et al.* 1999; Pan and Heitman 1999), with key roles postulated for both the Mep2 permease as an ammonium sensor and Gpr1 as a carbon source sensor (Lorenz and Heitman 1998; Lorenz *et al.* 2000).

The fission yeast *Schizosaccharomyces pombe* monitors glucose to regulate a wide range of biological processes. Our studies focus on the transcriptional regulation of the glucose-repressed *fbp1* gene that encodes the gluconeogenic enzyme fructose-1,6-bisphosphatase (Vassarotti and Friesen 1985). Previously, we identified mutations in genes that confer constitutive *fbp1* transcription

(Hoffman and Winston 1990). These *git* (glucose insensitive transcription) genes act in a PKA pathway (Hoffman and Winston 1991; Byrne and Hoffman 1993). The *git2* gene, identical to *cyr1* (Yamawaki-Kataoka *et al.* 1989; Young *et al.* 1989; Maeda *et al.* 1990), encodes adenylate cyclase (Hoffman and Winston 1991); the *git6* gene, identical to *pka1* (Maeda *et al.* 1994), encodes the catalytic subunit of PKA (Jin *et al.* 1995). The remaining six genes, *git1*, *git3*, *git5*, *git7*, *git8*, and *git10*, are required for glucose-triggered adenylate cyclase activation. Mutations in these "upstream" *git* genes are suppressed by multicopy $git2^+$ or by exogenous cAMP (Hoffman and Winston 1991), and strains carrying mutations in any of these genes fail to elevate intracellular cAMP levels in response to glucose (Byrne and Hoffman 1993). The *git8* gene, identical to *gpa2*, encodes a Gα subunit (Isshiki *et al.* 1992; Nocero *et al.* 1994). Multicopy $gpa2^+$ partially suppresses mutations in *git3* and *git5* but not in the other upstream *git* genes (Nocero *et al.* 1994; Landry *et al.* 2000). The *git5* gene encodes a Gβ subunit that acts as a positive regulator of the gpa2 Gα (Landry *et al.* 2000).

In this article, we further characterize the genetic interactions between *gpa2* and the other upstream *git* genes through the use of an "activated" allele of *gpa2* whose product is defective in its autoinhibitory GTPase activity. Furthermore, we describe the cloning and characterization of *git3* and provide genetic evidence that it encodes the G protein-coupled receptor responsible for the activation of adenylate cyclase through gpa2.

## MATERIALS AND METHODS

**Yeast strains and growth media:** *S. pombe* strains used in this study are listed in Table 1. The $fbp1::ura4^+$ allele is a disruption of the *fbp1* gene by the coding region of the *ura4* gene, creating a translational fusion that is under the transcriptional control of the *fbp1* promoter. The *ura4::fbp1-lacZ* allele is a disruption of the *ura4* gene by an *fbp1-lacZ* translational fusion (Hoffman and Winston 1990).

Standard rich media YEA and YEL (Gutz *et al.* 1974) were supplemented with 2% casamino acids. PM media (Watanabe *et al.* 1988) were supplemented with required nutrients at 75 mg/liter, except for leucine which was at 150 mg/liter. Glucose was present at a concentration of 3%, unless otherwise specified. Sensitivity to 5-fluoro-orotic acid (5-FOA) was determined on SC solid medium containing 0.4 g/liter 5-FOA and 8% glucose as previously described (Hoffman and Winston 1990). Strains were grown at 30°. Crosses were performed on SPA (Gutz *et al.* 1974) following pregrowth on PM medium.

**Epistasis testing:** Epistasis tests were conducted by examining progeny from tetrad dissections of crosses of RWP4 ($gpa2^{R176H}$) with strains carrying mutations in *git1*, *git2*, *git3*, *git5*, *pka1/git6*, *git7*, and *git10*. Following germination and colony formation, progeny were transferred to a fresh YEA plate, grown 1 day, and then replica plated to 5-FOA-containing medium. 5-FOA resistance was determined 2–3 days after replica plating.

**TABLE 1**

**Strain list**

| Strain | Genotype |
|---|---|
| FWP72 | $h^-$ *leu1-32 fbp1::ura4*$^+$ *ura4::fbp1-lacZ* |
| FWP94 | $h^-$ *leu1-32 his7-366 ura4::fbp1-lacZ* |
| FWP112 | $h^-$ *leu1-32 his7-366 ade6-M216 fbp1::ura4*$^+$ *ura4::fbp1-lacZ* |
| FWP113 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git1-1* |
| FWP190 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git2*Δ*::LEU2*$^+$ |
| CHP7 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git2-7* |
| CHP14 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3-14* |
| CHP27 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git7-27* |
| CHP61 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git2-61* |
| CHP75 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git5-75* |
| CHP107 | $h^+$ *leu1-32 his7-366 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ pka1-107* |
| CHP459 | $h^+$ *leu1-32 ade6-M216 ura4::fbp1-lacZ gpa2*Δ*::ura4*$^+$ |
| CHP478 | $h^+$ *leu1-32 his7-366 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git5*Δ*::his7*$^+$ |
| CHP482 | $h^{90}$ *leu1-32 ade6-M216 gpa2*Δ*::ura4*$^+$ |
| CHP483 | $h^{90}$ *leu1-32 ade6-M216 ura4::fbp1-lacZ* |
| CHP495 | $h^-$ *leu1-32 his7-366 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git2*Δ*::his7*$^+$ |
| CHP567 | $h^+$ *leu1-32 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git10-201* |
| CHP616 | $h^+$ *leu1-32 his3-D1 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3*$^-$*::his3*$^+$ |
| RWP4 | $h^-$ *leu1-32 ade6-M216 fbp1::ura4*$^+$ *ura4::fbp1-lacZ* $gpa2^{R176H}$ |
| RWP9 | $h^-$ *leu1-32 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3*Δ::kanMX6 |
| RWP22 | $h^+$ *leu1-32 ura4::fbp1-lacZ gpa2*Δ*::ura4*$^+$ *git3*Δ::kanMX6 |
| RWP28 | $h^{90}$ *leu1-32 ura4::fbp1-lacZ git3*Δ::kanMX6 |
| RWP30 | $h^-$ *leu1-32 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git5*Δ*::his7*$^+$ $gpa2^{R176H}$ |
| RWP32 | $h^+$ *leu1-32 his7-366 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3*Δ::kanMX6 $gpa2^{R176H}$ |
| RWP35 | $h^+$ *leu1-32 his7-366 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3*Δ::kanMX6 *pka1::ura4*$^+$ |
| RWP39 | $h^+$ *leu1-32 ade6-M210 fbp1::ura4*$^+$ *ura4::fbp1-lacZ git3-14* $gpa2^{R176H}$ |

**β-Galactosidase assays:** β-Galactosidase activity, expressed from the *fbp1-lacZ* reporter, was determined as previously described (Nocero *et al.* 1994). Strains were pregrown in PM medium under repressing conditions (8% glucose) to exponential phase, washed twice with sterile water, and then subcultured in PM medium containing 8% glucose (repressing conditions) or 0.1% glucose plus 3% glycerol (derepressing conditions) for 24 hr. Soluble protein extracts were prepared by glass bead lysis. Two volumes of each extract were assayed to determine β-galactosidase activity. Total soluble protein was measured by BCA assay (Pierce Chemical Co., Rockford, IL) to calculate β-galactosidase-specific activity in extracts.

**Recombinant DNA methodology:** Standard recombinant DNA techniques, including DNA restriction digests, ligations, and *E. coli* transformations, were performed according to Ausubel *et al.* (1998). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA). Plasmid transformations of yeast were carried out as previously described (Dal Santo *et al.* 1996). DNA sequencing was performed using ABI's (Foster City, CA) big dye terminators on an ABI 377XL automated sequencer by Bioserve Biotechnologies, Ltd. (Laurel, MD) with custom oligonucleotides to sequence both strands of the *git3* gene at least once. The GenBank accession no. of this sequence is AF085162.

**Cosmid and P1 filter hybridization:** Plasmid pRW1 carries a 4.2-kb fragment of *S. pombe* chromosome 3 genomic DNA from the 5′ end of the *git3* gene through to the 3′ end of the *rec7* (Figure 1A). Cosmid and P1 library clones carrying the intact *git3* gene were identified by probing high-density filters from the Resource Center/Primary Database of the German Human Genome Project (RZPD, Berlin, Germany; Zehetner and Lehrach 1994) containing cosmid and P1-cloned *S. pombe* genomic libraries. Filters (cosmid library no. 60 and P1 library no. 705) were probed with a 2.9-kb *Sac*I fragment from plasmid pRW1 (Figure 1A, "filter probe") that had been gel purified and labeled by random priming. Prehybridization and hybridization of filters were done according to the protocol provided by the RZPD. Grid coordinates of positive clones were determined by probing filters with nick-translated pBluescript II SK+. Positive cosmid and P1 clones were obtained from the RZPD.

**Cloning of the *git3* gene:** DNA from the candidate cosmid (ICRFc60A231Q) and from six P1 clones (ICRFP705: L127Q, F024Q, E051Q, E161Q, I221Q, and O132Q) was digested with *Sac*I and examined by Southern hybridization analysis using nick-translated plasmid pRW1. A 0.4-kb fragment identified clones carrying the *git3* gene. On the basis of the restriction map surrounding the *git3* locus, a 4.5-kb *Sac*I fragment from cosmid ICRFc60A231Q was gel purified and inserted into *Sac*I-digested pSP1 (Cottarel *et al.* 1993) to create plasmid pRW2. Complementation of the *git3-14* mutation in strain CHP14 by plasmid pRW2 indicated that this plasmid carried a functional copy of the *git3* gene. Plasmids pRW3 and pRW4 were constructed by digesting plasmid pRW2 with *Hin*dIII or *Spe*I, respectively, and religating with T4 DNA ligase to drop out portions of the pRW2 insert DNA as diagrammed in Figure 1B.

**Construction of a *git3* deletion:** Deletion of the *git3* gene was accomplished by a PCR-based strategy according to the protocol of Bahler *et al.* (1998). Oligonucleotides *git3*-fordelta, 5′-CTCACCTCCTCTCCTCTCTTTTTTTTTCCTGTCTTCTTGAAGAGAAGTTTAAACTTTGTAATAAGGAACAAGGTTGCTCCGGATCCCCCGGGTTAATTAA-3′ and *git3*-reverse, 5′-CCATCTTCCAATCCGAGCCTTTTGCCAAGTTAAGTAGTGCCAAAATTTAATACTTCGAAATAAATTCTGGGAAGCGAGAATTCGAGCTCGTTTAAA C-3′ (Integrated DNA Technologies, Coralville, IA) were used to PCR amplify the *kan*-selectable marker from plasmid pFA6a-GFP(S65T)-kanMX6 (Wach *et al.* 1997) such that the amplified marker was flanked with sequences from either side of the *git3* open reading frame. The 2.6-kb PCR product was used to transform strain FWP72 to G418-resistance using the DMSO transformation protocol to ensure a high efficiency of homologous recombination (Bahler *et al.* 1998). Stable G418-resistant transformants were analyzed by Southern hybridization for the presence of a 5.1-kb *Sac*I fragment when probed with the 4.5-kb *Sac*I fragment from plasmid pRW2, indicating homologous insertion of the PCR product and the deletion of the *git3* gene.

## RESULTS

**Epistatic relationship between *gpa2*$^{R176H}$ and "upstream" *git* mutant alleles:** The *gpa2*, *git1*, *git3*, *git5*, *git7*, and *git10* genes are all required for both glucose repression of *fbp1* transcription and for the production of a glucose-triggered cAMP response in *S. pombe* (Hoffman and Winston 1990, 1991; Isshiki *et al.* 1992; Byrne and Hoffman 1993; Nocero *et al.* 1994). It was previously shown that multicopy *gpa2*$^{+}$ suppresses mutations in *git3* and *git5* but has no effect on mutations in *git1*, *git7*, and *git10* (Nocero *et al.* 1994). However, a quantitative analysis of the multicopy *gpa2*$^{+}$ suppression of a *git5*Δ deletion allele has shown that suppression is only partial (Landry *et al.* 2000). Since Gα subunits are post-translationally modified and their activity depends upon the exchange of the bound guanine nucleotide from GDP to GTP (Casey 1994), overexpression of a Gα subunit would not necessarily bypass the need for its proper modification and regulation. Therefore, the failure of multicopy *gpa2*$^{+}$ to suppress mutations in *git1*, *git7*, or *git10* does not eliminate the possibility that these genes function to regulate gpa2 activity. Similarly, the fact that multicopy *gpa2*$^{+}$ only partially suppresses mutations in *git3* or *git5* does not prove additional roles for these genes beyond the activation of gpa2. We reexamined these genetic interactions using an activated allele of *gpa2* that carries a mutation predicted to inactivate the GTPase domain responsible for converting the gpa2 Gα from its GTP-bound active state to its GDP-bound inactive state (Isshiki *et al.* 1992). Strains carrying this mutant allele display a partial mating defect and increased cAMP levels (when combined with a mutation in the *pde1/cgs2* cAMP phosphodiesterase gene; Isshiki *et al.* 1992) along with a partial defect in derepressing *fbp1* transcription (see below). These phenotypes are consistent with an increase in adenylate cyclase activation.

To test the ability of the *gpa2*$^{R176H}$ mutant allele to suppress mutations in *git1*, *git3*, *git5*, *git7*, or *git10*, we performed pairwise crosses between strain RWP4 (*gpa2*$^{R176H}$) and strains carrying mutations in these genes. The *git* mutant strains are 5-FOA-sensitive (5-FOA$^{S}$) due to a defect in glucose repression of an integrated *fbp1-ura4* reporter (Hoffman and Winston 1990), while strains that glucose repress *fbp1-ura4* transcription are 5-FOA resistant (5-FOA$^{R}$). If *gpa2*$^{R176H}$ fails to suppress the *git* mutation in the mating partner, all tetrads should

display a 2:2 5-FOA$^R$:5-FOA$^S$ pattern based on segregation of the *git* mutant allele. If suppression occurs, only *git*$^-$ *gpa2*$^+$ progeny will be 5-FOA$^S$. Thus tetratype and nonparental ditype tetrads will contain more than two 5-FOA$^R$ progeny. While a 2:2 pattern would also result if only parental ditypes were examined, our sample size of 9–15 tetrads makes this highly unlikely given the frequency of tetratype tetrads present in similar crosses involving *gpa2* loss-of-function alleles (Hoffman and Winston 1990). By this analysis, the *gpa2*$^{R176H}$ allele suppresses mutations in *git3* and *git5*, but not in *git1*, *git7*, or *git10*. As expected, *gpa2*$^{R176H}$ fails to suppress either a *git2*Δ deletion allele or a *pka1-107* mutation. Similarly, *gpa2*$^{R176H}$ fails to suppress *git2-7* or *git2-61*. These *git2* alleles display intragenic complementation, suggesting that they affect two distinct functions of adenylate cyclase (Hoffman and Winston 1991).

**Effect of *gpa2*$^{R176H}$ on *fbp1-lacZ* expression:** While the *gpa2*$^{R176H}$ suppression of a *git3* or *git5* mutation is qualitatively similar to the multicopy *gpa2*$^+$ suppression data (Nocero *et al.* 1994), we observe distinct quantitative differences in the suppression. Multicopy *gpa2*$^+$ suppression of a *git5*Δ allele is only fivefold (Landry *et al.* 2000). This partial suppression supports a model in which *S. pombe* adenylate cyclase is activated by a mechanism similar to that of the type II mammalian enzyme (Tang and Gilman 1991) with the gpa2 Gα carrying out an initial activation step and the git5 Gβ carrying out a subsequent activation step. Alternatively, partial suppression may simply indicate that much of the overexpressed wild-type gpa2 is in the inactive, GDP-bound form. Unlike multicopy *gpa2*$^+$, the *gpa2*$^{R176H}$ allele completely suppresses a *git3* mutation or a *git5* deletion in cells grown under repressing conditions (Table 2). In addition, the *gpa2*$^{R176H}$ allele on its own inhibits *fbp1-lacZ* expression in derepressed cells by approximately fourfold. Since suppression by *gpa2*$^{R176H}$ is complete, git3 and git5 may function solely to activate gpa2. Thus, the activation of *S. pombe* adenylate cyclase is not like that of the type II mammalian enzyme.

**TABLE 2**

**Suppression of *git3* and *git5* mutations by *gpa2*$^{R176H}$**

| Strain | Relevant genotype | β-Galactosidase activity | |
|---|---|---|---|
| | | Repressed | Derepressed |
| FWP112 | Wild type | 20 ± 4 | 1666 ± 21 |
| CHP14 | *git3-14* | 760 ± 68 | 2815 ± 256 |
| CHP478 | *git5*Δ | 1050 ± 78 | 2291 ± 179 |
| RWP4 | *gpa2*$^{R176H}$ | 5 ± 1 | 442 ± 167 |
| RWP39 | *git3-14 gpa2*$^{R176H}$ | 5 ± 0 | 354 ± 145 |
| RWP30 | *git5*Δ *gpa2*$^{R176H}$ | 8 ± 2 | 510 ± 249 |

β-Galactosidase activity was determined from two to six independent cultures as described in materials and methods. The average ± SE represents specific activity per milligram soluble protein.

**Cloning of the *git3* gene:** In the course of a study to create novel *git* mutant strains by nonhomologous plasmid insertion, we identified strain CHP616 carrying an insertion of plasmid pAF1 (Ohi *et al.* 1996) in the previously uncloned *git3* gene as judged by linkage and complementation tests (Hoffman and Welton 2000). Plasmid p616E was obtained by *Eco*RI restriction digestion and ligation of CHP616 genomic DNA, followed by transformation of *E. coli* to ampicillin resistance (Hoffman and Welton 2000). This plasmid carries 0.9 kb of genomic DNA adjacent to the site of pAF1 insertion in strain CHP616. DNA sequence analysis of this insert indicated that this region of the genome had not been previously cloned or sequenced.

To clone an intact copy of the *git3* gene, we first rescued a larger region of the flanking genomic DNA using *Sac*II to digest the genomic DNA prior to ligation. This provided us with both additional sequence information and a larger hybridization probe with which to detect cosmid and P1 library clones carrying the *git3* gene. Plasmid pRW1 carries 4.2 kb of genomic DNA (Figure 1A). Restriction mapping of pRW1 identified the positions of *Sac*I sites within this region, while DNA

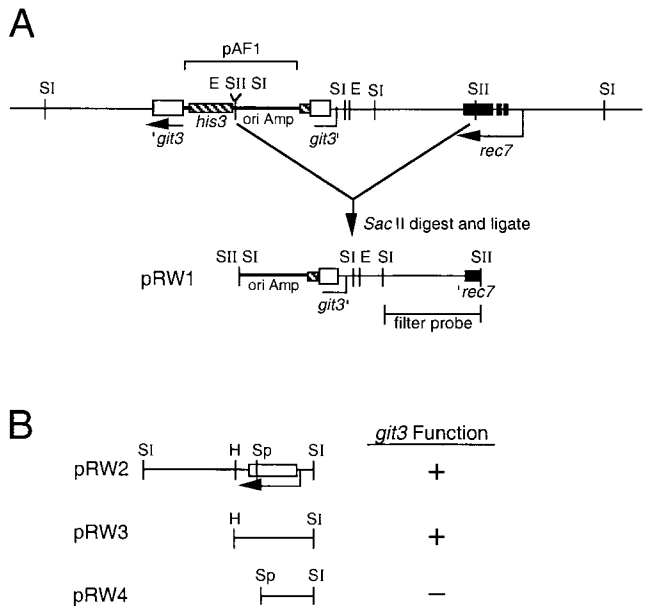


Figure 1.—Schematic of *git3* disruption by plasmid pAF1 (Ohi *et al.* 1996) and subcloning analyses. (A) Structure of the pAF1 insertion into the *git3* gene in strain CHP616. Sites for restriction enzymes *Sac*I (SI), *Sac*II (SII), and *Eco*RI (E) are indicated. The *git3* (open box), *rec7* (solid box), and *his3* (hatched box, the selectable marker on pAF1) are also shown. Plasmid pRW1, rescued by *Sac*II digestion and ligation of CHP616 genomic DNA (Hoffman and Welton 2000), carries 4.2 kb of flanking genomic DNA from the 5′ end of *git3* (*git3*′) to the 3′ end of *rec7* (′*rec7*). The "filter probe" is a 2.9-kb *Sac*I restriction fragment from pRW1. (B) Restriction maps of inserts in plasmids pRW2, pRW3, and pRW4. Plasmids pRW2 and pRW3 carry functional *git3* clones, while pRW4 is nonfunctional. Restriction sites for *Sac*I (SI), *Hin*dIII (H), and *Spe*I (Sp) are shown.

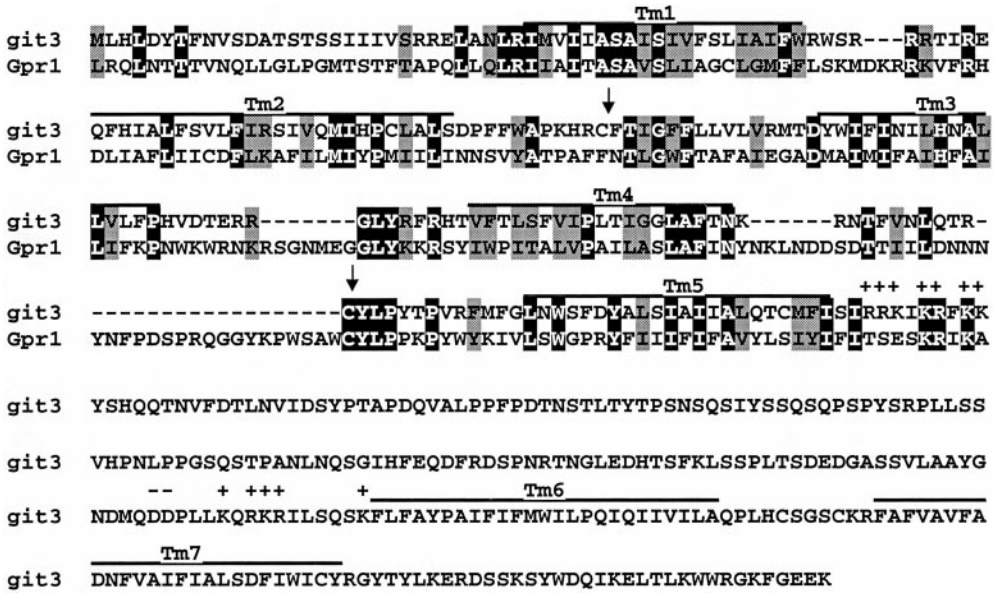


FIGURE 2.—The amino-acid sequence alignment between the predicted git3 protein and a portion of the *S. cerevisiae* Gpr1 putative G protein-coupled receptor. The putative git3 protein (accession no. AAC-69337) was aligned with residues 25–280 of the 961-amino-acid Gpr1 sequence (accession no. NP010249) using the Clustal W (version 1.8) sequence alignment program (THOMPSON *et al.* 1994) and displayed using BOXSHADE. This region includes the first five of the seven predicted transmembrane domains (Tm1–Tm7) in git3. Additional features of G protein-coupled receptors present in git3, a cysteine residue in both the first and second extracellular loops (indicated by arrows between Tm2 and Tm3, and between Tm4 and Tm5), and two clusters of charged residues (+ and −) at the N-terminal and C-terminal ends of the third intracellular loop (between Tm5 and Tm6), are shown.

sequencing across the *Sac*II cloning junction indicated that *git3* is adjacent to *rec7* (LIN *et al.* 1992). Using a 2.9-kb *Sac*I fragment from pRW1 as a hybridization probe (one *Sac*I site comes from the cloning polylinker of plasmid pAF1 while the other lies between *rec7* and *git3*; Figure 1A, filter probe), we identified candidate P1 and cosmid clones of *git3* from high density library filters (data not shown). Six P1 and one cosmid candidate indicated by filter hybridization were obtained from the Resource Center/Primary Database of the RZPD and analyzed by Southern hybridization. Three of six P1 clones and the one cosmid clone contained three *Sac*I insert fragments that hybridized with a pRW1 probe. The *Sac*I fragments included the 0.4-kb fragment predicted from the restriction map of pRW1 (Figure 1A), along with a 4.5- and a 6.1-kb fragment. On the basis of restriction mapping of the original *rec7* clone, we inferred that the 6.1-kb fragment represents the region extending beyond *rec7*, while the 4.5-kb fragment is likely to encompass *git3* (diagrammed in Figure 1A). The 4.5-kb *Sac*I fragment from cosmid ICRFc60A231Q was cloned into pSP1 (COTTAREL *et al.* 1993) to create pRW2. Plasmid pRW2 carries *git3* as it confers 5-FOA resistance upon strain CHP14 (*git3-14*). The *git3* gene was further localized within the 4.5-kb *Sac*I fragment in pRW2 to a 2.15-kb *Hin*dIII-*Sac*I fragment by subcloning and testing for *git3* function (Figure 1B) as determined by suppression of the 5-FOA$^S$ phenotype of strain CHP14 (*git3-14*).

**Subcloning and sequence analysis of *git3*:** DNA sequencing of the insert in plasmid pRW3 (GenBank accession no. AF085162) reveals a single open reading frame encoding a 466-amino-acid protein. BLASTP analysis reveals that git3 is distantly related to the *S. cerevisiae* Gpr1 putative G protein-coupled receptor (Figure 2A). The git3 protein is predicted to possess seven transmembrane domains and displays additional features of a seven-transmembrane G protein-coupled receptor. These include a series of charged residues at either end of the third cytoplasmic loop (BALDWIN 1994) and cysteine residues in both the first and second extracellular domains that are likely to form a disulfide bond (NODA *et al.* 1994).

**A *git3* deletion confers phenotypes associated with defects in glucose detection:** Deletion of *git3* (*git3*Δ; see MATERIALS AND METHODS) confers phenotypes associated with other mutations in the PKA glucose monitoring pathway. Spores lacking git3 (Figure 3; progeny 2C and 3D) display a germination delay similar to those lacking pka1 (Figure 3; progeny 2A). Loss of both git3 and pka1 (Figure 3; progeny 1B, 1C, 2D, 3A, 4B, and 4D) does not create any additional delay in germination, indicating that the two proteins act in the same pathway.

Phenotypic characterization of *git3*Δ strains shows that they resemble adenylate cyclase deletion (*git2*Δ) strains. These strains are derepressed for *fbp1-ura4* expression, thereby allowing significant growth on glucose-rich medium lacking uracil and conferring sensitivity to 5-FOA (Figure 4). In addition, these strains fail to glucose repress gluconate uptake that is negatively regulated by PKA (CASPARI 1997), thus abolishing the growth lag observed in wild-type cells upon transfer from a glucose-rich medium to a gluconate-based medium (Figure 4).

When crossed into a homothallic ($h^{90}$) strain background, the *git3* deletion confers the same starvation-independent mating and sporulation phenotype seen in a *gpa2*Δ strain (Figure 5; ISSHIKI *et al.* 1992) and in

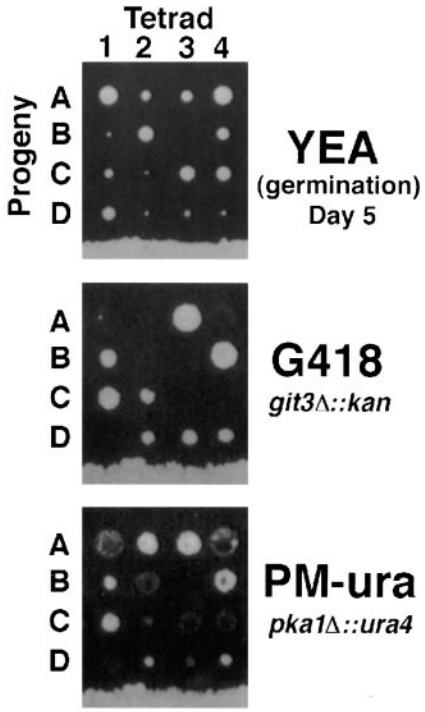


FIGURE 3.—Both *git3*Δ and *pka1*Δ spores display a germination delay. Tetrads from a cross of FWP94 (*git3*$^{+}$ *pka1*$^{+}$) by RWP35 (*git3*Δ*::kan pka1*Δ*::ura4*$^{+}$) were dissected on YEA at 30° and photographed after 5 days. The colonies were grown 1 additional day before replica plating to YEA + G418 (to identify *git3*Δ strains) and to PM-ura (to identify *pka1*Δ strains). These plates were incubated 2 days at 30° and photographed.

*git5*, *git2*, or *pka1* mutant strains (LANDRY *et al.* 2000; MAEDA *et al.* 1990, 1994). The addition of cAMP to the growth medium suppresses this defect, suggesting that the unregulated mating of the *git3*Δ cells is due to a defect in glucose-triggered adenylate cyclase activation (Figure 5).

***git3*Δ strains display a more pronounced defect in *fbp1-lacZ* regulation than do *git3* spontaneous mutants:** Our initial studies indicated that the derepression of *fbp1* transcription caused by mutations in *git3* is half that conferred by a mutation or deletion of the *gpa2* (*git8*) gene (HOFFMAN and WINSTON 1990, 1991; NOCERO *et al.* 1994). Since the *git3*Δ mutation confers the same high-level *fbp1-lacZ* expression as does a *gpa2*Δ allele (Table 3) and twice that conferred by the *git3-14* mutation (Table 2), we presume that the spontaneous *git3* mutant alleles retain some function.

We previously observed that a *git3* mutation causes a further increase in *fbp1* expression in strains carrying a *gpa2* point mutation or deletion (HOFFMAN and WINSTON 1990; NOCERO *et al.* 1994). Consistent with these results, a *git3*Δ *gpa2*Δ double deletion strain (RWP22) expresses the *fbp1-lacZ* reporter to higher levels than either *git3*Δ or *gpa2*Δ strains (Table 3). This may suggest that git3 has a gpa2-independent function. However,

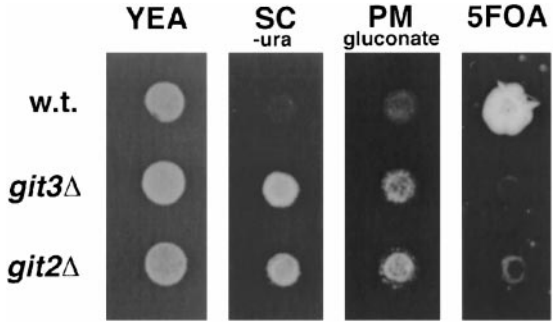


FIGURE 4.—Growth characteristics of a *git3*Δ strain reflect a defect in glucose monitoring. Strains FWP72 (wild type), RWP9 (*git3*Δ), and CHP495 (*git2*Δ) were grown to exponential phase in PM liquid medium (8% glucose), washed and adjusted to $10^7$ cells/ml in water, and spotted to YEA, SC-ura, PM (3% gluconate), and 5-FOA media. The growth was recorded after 2 days for the YEA and SC-ura plates and after 5 days for the PM (3% gluconate) and 5-FOA plates.

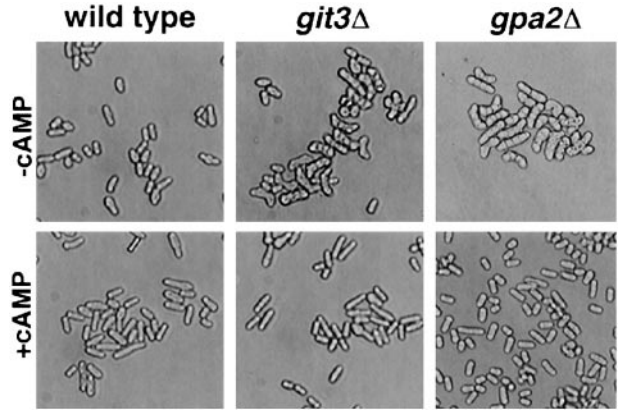


FIGURE 5.—Starvation-independent sexual development in a *git3*Δ homothallic strain. Cells of homothallic strains CHP483 (wild type), RWP28 (*git3*Δ), and CHP482 (*gpa2*Δ) were grown to log phase in PM liquid medium (at 37° to inhibit conjugation) and then diluted to $10^6$ cells/ml in PM liquid medium with or without 5 mM cAMP. These cells were incubated 24 hr at 30° without shaking and photographed.

**TABLE 3**

**Effect of mutations in *git3* and *gpa2* on *fbp1-lacZ* expression**

| Strain | Relevant genotype | β-Galactosidase activity | |
|---|---|---|---|
| | | Repressed | Derepressed |
| RWP9 | *git3*Δ | 1501 ± 241 | 3363 ± 390 |
| CHP459 | *gpa2*Δ | 1403 ± 214 | 2800 ± 100 |
| RWP22 | *git3*Δ *gpa2*Δ | 2350 ± 298 | 3165 ± 97 |
| RWP32[a] | *git3*Δ *gpa2*$^{R176H}$ | 7 ± 0 | 491 ± 61 |

β-Galactosidase activity was determined from two to four independent cultures as described in MATERIALS AND METHODS. The average ± SE represents specific activity per milligram soluble protein.

[a] Strain RWP32 is 5-FOA$^{R}$ due to suppression of the *git3* deletion by *gpa2*$^{R176H}$.

since *gpa2*$^{R176H}$ fully suppresses *git3*Δ (RWP32, Table 3), it appears that the primary function of git3 is to activate the gpa2 Gα.

**Multicopy *git3*$^{+}$ fails to suppress mutations in other genes required for adenylate cyclase activation:** Multicopy suppression analyses have been used to identify functional relationships among genes of the glucose/cAMP pathway. The *git2* (adenylate cyclase) gene was originally cloned as a multicopy suppressor of a *git1* mutation and was also shown to suppress mutations in *git3*, *git5*, *git7*, *gpa2* (*git8*), and *git10* (Hoffman and Winston 1991). This suppression led us to identify these genes as encoding components of an adenylate cyclase pathway in that they are required for the production of a glucose-triggered cAMP signal (Byrne and Hoffman 1993). Multicopy *gpa2*, encoding the Gα subunit, suppresses mutations in *git5* (Gβ) and *git3*, indicating that these two genes act to regulate the activation of the gpa2 Gα (Nocero *et al.* 1994). We tested whether multicopy *git3* could suppress mutations in *git1*, *git3*, *git5*, *git7*, *gpa2*, or *git10*. While transformation by plasmid pRW2 (see Figure 1B) restores *git3* mutant strains to 5-FOA resistance due to glucose repression of the *fbp1-ura4* reporter, it has no effect on *git1*, *git5*, *git7*, *gpa2*, or *git10* mutant strains (data not shown).

## DISCUSSION

Both the gpa2 Gα and git5 Gβ are required for *S. pombe* adenylate cyclase activation; however, the precise role of the Gβ subunit has been unclear (Isshiki *et al.* 1992; Nocero *et al.* 1994; Landry *et al.* 2000). Mammalian adenylate cyclases are also regulated by G proteins, with the various adenylate cyclase isoforms displaying different sensitivities to the individual G protein subunits (reviewed by Choi *et al.* 1993; Taussig and Gilman 1995). While all are stimulated by $G_s\alpha$ subunits, the type I enzyme is inhibited by Gβγ dimers, the type II enzyme is further activated by Gβγ, and the type III enzyme is insensitive to Gβγ (Tang and Gilman 1991; Tang *et al.* 1992). Thus, there is no single model for the mode of action of G protein subunits in adenylate cyclase activation. The git5 Gβ may simply be important for the activation of the gpa2 Gα by facilitating efficient coupling of the Gα to a receptor that promotes GDP release to allow Gα to bind GTP and attain the active conformation. Alternatively, or in addition, the Gβ may directly activate adenylate cyclase as in the case of the type II mammalian enzyme. This possibility was supported by the fact that multicopy *gpa2*$^{+}$ only partially suppressed the loss of the *git5* Gβ gene (Nocero *et al.* 1994; Landry *et al.* 2000). However, we have shown here that the *gpa2*$^{R176H}$ activated allele restores full repression of *fbp1* transcription in the absence of the git5 Gβ subunit. This complete suppression suggests that the Gβ subunit may have no role beyond delivery of the Gα to the receptor.

The genetic interactions between *gpa2* and *git5* are very different from those observed in the G protein-mediated *S. cerevisiae* pheromone response pathway, in which the Gβγ dimer activates a mitogen-activated protein kinase (MAPK) pathway (Hirsch and Cross 1992). In this system, the Gα subunit negatively regulates signaling by sequestering the Gβγ dimer in the inactive heterotrimer. The pathway is activated when the pheromone receptor binds the appropriate pheromone and causes the activation of the G protein. The Gα subunit presumably undergoes a conformational change as it releases GDP and binds GTP, causing it to dissociate from the Gβγ dimer that is then free to activate the MAPK pathway. As such, a mutation in the *GPA1/SCG1* Gα gene activates the pathway by alleviating the regulation of Gβγ, while mutations in either the *STE4* Gβ gene or the *STE18* Gγ gene prevent pathway activation (Whiteway *et al.* 1989). The opposing phenotypes associated with a defect in Gα *vs.* Gβγ are due to the fact that the activation of Gβγ simply requires its release from Gα with no additional conformational change occurring in Gβγ as indicated by crystal structure studies (Sondek *et al.* 1996). On the other hand, the conformation of Gα is not dependent upon the presence or absence of Gβγ, but on the binding of GDP (inactive conformation) *vs.* GTP (active conformation). Therefore, the loss of Gβγ in a system in which Gα activates the downstream effector would not only fail to activate the pathway, but would likely reduce signaling by reducing the interaction between the Gα subunit and the receptor. This model is consistent with our data regarding *gpa2*, *git5*, and adenylate cyclase activation in *S. pombe*.

Of the six upstream *git* genes required for glucose-triggered adenylate cyclase activation, *gpa2*$^{R176H}$ only suppressed mutations in *git5* (Gβ) and *git3* (Table 2). This genetic interaction, along with the sequence analysis of the *git3* gene (Figure 2), suggests that *git3* encodes a G protein-coupled glucose receptor responsible for adenylate cyclase activation. Cells lacking git3 display a profound defect in glucose detection (Table 3; Figures 3–5), consistent with our previous observation that a *git3* mutant fails to produce a cAMP response to glucose (Byrne and Hoffman 1993). The *git3*Δ phenotypes include a germination delay, derepression of *fbp1* transcription, a defect in glucose repression of gluconate uptake, and starvation-independent conjugation and sporulation. These are very distinct processes, but are all subject to inhibition by PKA. In addition, multicopy *git3* fails to suppress mutations in the other upstream *git* genes, as expected for a gene acting at the top of a signaling pathway. Also consistent with a role as a glucose receptor, the germination delay due to the loss of git3 is not additive with the delay due to the loss of PKA (Figure 3). This result also argues against the possibility that git3 activates other nutrient sensing pathways. We previously showed that loss of both PKA and the sck1

kinase results in a dramatic enhancement of both the germination delay and the exit from stationary phase seen in cells lacking PKA alone (Jin *et al.* 1995). We would have expected a similar enhancement if git3 stimulated other signaling pathways.

It remains possible that git3 carries out some additional function besides the activation of gpa2 in response to glucose detection. Expression from the *fbp1-lacZ* reporter is higher in a *git3*Δ *gpa2*Δ double deletion strain (RWP22) than in a *gpa2*Δ single deletion strain (CHP459; Table 3). If git3 only acted to regulate gpa2, the deletion of *git3* in a *gpa2*Δ background would have had no effect. However, the complete suppression of a *git3* deletion by the *gpa2*$^{R176H}$ allele indicates that any G protein-independent activity of git3 only plays a minor role in glucose monitoring.

Since *gpa2*$^{R176H}$ *git3*Δ cells are still able to respond to glucose starvation by derepressing *fbp1-lacZ* expression 70-fold (Table 3), the glucose starvation response is git3 independent. Glucose starvation is one of several environmental stresses that activate the spc1/sty1 MAPK pathway in *S. pombe* (Degols *et al.* 1996; Shiozaki and Russell 1996; Stettler *et al.* 1996; Samejima *et al.* 1997). This MAPK activates the atf1-pcr1 transcriptional activator that is partially responsible for derepression of *fbp1* transcription (Takeda *et al.* 1995; Kanoh *et al.* 1996; Shiozaki and Russell 1996; Wilkinson *et al.* 1996). Glucose repression of *fbp1* transcription in wild-type cells is due to a combination of an inhibitory signal from the PKA pathway and the lack of an activating signal from the MAPK pathway. Glucose starvation triggers derepression of *fbp1* transcription by reducing PKA repression and stimulating MAPK-dependent transcriptional activation. Therefore, it is likely that the starvation response observed in *gpa2*$^{R176H}$ *git3*Δ cells is due to activation of the MAPK pathway.

While the glucose detection system described here most resembles the *S. cerevisiae* Gpr1-Gpa2 pathway that is also responsible for adenylate cyclase activation, there are some interesting differences. The git3 and Gpr1 proteins are only somewhat related, with the homology limited to transmembrane domains 1–5. Gpr1, a 961-residue protein, is predicted to have a 350-residue third cytoplasmic loop and a 280-residue cytoplasmic tail, while git3, a 466-residue protein, is predicted to have a 159-residue third cytoplasmic loop and a 35-residue cytoplasmic tail (Figure 2A). In addition, Gpr1 possesses a poly-asparagine track, not present in git3. The *S. cerevisiae* Gpa2 Gα is also significantly larger than the *S. pombe* gpa2 Gα, with an additional 90 amino acids at the N terminus (Nakafuku *et al.* 1988; Isshiki *et al.* 1992). These regions of the *S. cerevisiae* Gpr1 and Gpa2 proteins not found in the *S. pombe* git3 and gpa2 proteins may allow for efficient coupling in the absence of a Gβγ dimer in *S. cerevisiae*, whereas the git5 Gβ is required for normal glucose detection in *S. pombe* (Landry *et al.* 2000). Further characterization of the structure, function, and regulation of the git3 protein, as well as the gpa2-containing G protein, will provide us with a valuable model system for the study of G protein signaling that is fundamentally different from the Gβγ-driven *S. cerevisiae* pheromone signaling pathway.

We thank Masayuki Yamamoto for the original *gpa2*$^{R176H}$ strain and Jürg Bähler for materials and advice regarding PCR-based gene disruptions. We thank Junona Moroianu for the use of her microscope. This work was funded by National Institutes of Health grant GM-46226 to C.S.H.

## LITERATURE CITED

Ansari, K., S. Martin, M. Farkasovsky, I. M. Ehbrecht and H. Kuntzel, 1999 Phospholipase C binds to the receptor-like GPR1 protein and controls pseudohyphal differentiation in *Saccharomyces cerevisiae*. J. Biol. Chem. **274:** 30052–30058.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1998 *Current Protocols in Molecular Biology*. Wiley Interscience, New York.

Bahler, J., J. Q. Wu, M. S. Longtine, N. G. Shah, A. McKenzie, 3rd *et al.*, 1998 Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast **14:** 943–951.

Baldwin, J. M., 1994 Structure and function of receptors coupled to G proteins. Curr. Opin. Cell Biol. **6:** 180–190.

Byrne, S. M., and C. S. Hoffman, 1993 Six *git* genes encode a glucose-induced adenylate cyclase activation pathway in the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. **105:** 1095–1100.

Casey, P. J., 1994 Lipid modifications of G proteins. Curr. Opin. Cell Biol. **6:** 219–225.

Caspari, T., 1997 Onset of gluconate-$H^+$ symport in *Schizosaccharomyces pombe* is regulated by the kinases Wis1 and Pka1, and requires the *gti1*$^+$ gene product. J. Cell Sci. **110:** 2599–2608.

Choi, E. J., Z. Xia, E. C. Villacres and D. R. Storm, 1993 The regulatory diversity of the mammalian adenylyl cyclases. Curr. Opin. Cell Biol. **5:** 269–273.

Colombo, S., P. Ma, L. Cauwenberg, J. Winderickx, M. Crauwels *et al.*, 1998 Involvement of distinct G-proteins, Gpa2 and Ras, in glucose- and intracellular acidification-induced cAMP signalling in the yeast *Saccharomyces cerevisiae*. EMBO J. **17:** 3326–3341.

Cottarel, G., D. Beach and U. Deuschle, 1993 Two new multipurpose multicopy *Schizosaccharomyces pombe* shuttle vectors, pSP1 and pSP2. Curr. Genet. **23:** 547–548.

Dal Santo, P., B. Blanchard and C. S. Hoffman, 1996 The *Schizosaccharomyces pombe* pyp1 protein tyrosine phosphatase negatively regulates nutrient monitoring pathways. J. Cell Sci. **109:** 1919–1925.

Degols, G., K. Shiozaki and P. Russell, 1996 Activation and regulation of the Spc1 stress-activated protein kinase in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **16:** 2870–2877.

Gutz, H., H. Heslot, U. Leupold and N. Loprieno, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics*, edited by R. C. King. Plenum Press, New York.

Hirsch, J. P., and F. R. Cross, 1992 Pheromone response in yeast. Bioessays **14:** 367–373.

Hoffman, C. S., and R. Welton, 2000 Mutagenesis and gene cloning in *Schizosaccharomyces pombe* via nonhomologous plasmid integration and rescue. Biotechniques **28:** 532–536, 538, 540.

Hoffman, C. S., and F. Winston, 1990 Isolation and characterization of mutants constitutive for expression of the *fbp1* gene of *Schizosaccharomyces pombe*. Genetics **124:** 807–816.

Hoffman, C. S., and F. Winston, 1991 Glucose repression of transcription of the *Schizosaccharomyces pombe fbp1* gene occurs by a cAMP signaling pathway. Genes Dev. **5:** 561–571.

Isshiki, T., N. Mochizuki, T. Maeda and M. Yamamoto, 1992 Characterization of a fission yeast gene, *gpa2*, that encodes a G alpha subunit involved in the monitoring of nutrition. Genes Dev. **6:** 2455–2462.

Jin, M., M. Fujita, B. M. Culley, E. Apolinario, M. Yamamoto *et al.*, 1995 *sck1*, a high copy number suppressor of defects in the

cAMP-dependent protein kinase pathway in fission yeast, encodes a protein homologous to the *Saccharomyces cerevisiae* SCH9 kinase. Genetics **140:** 457–467.

Kanoh, J., Y. Watanabe, M. Ohsugi, Y. Iino and M. Yamamoto, 1996 *Schizosaccharomyces pombe gad7*$^+$ encodes a phosphoprotein with a bZIP domain, which is required for proper G1 arrest and gene expression under nitrogen starvation. Genes Cells **1:** 391–408.

Kraakman, L., K. Lemaire, P. Ma, A. W. Teunissen, M. C. Donaton *et al.*, 1999 A *Saccharomyces cerevisiae* G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. Mol. Microbiol. **32:** 1002–1012.

Landry, S., M. T. Pettit, E. Apolinario and C. S. Hoffman, 2000 The fission yeast *git5* gene encodes a Gβ subunit required for glucose-triggered adenylate cyclase activation. Genetics **154:** 1463–1471.

Lin, Y., K. L. Larson, R. Dorer and G. R. Smith, 1992 Meiotically induced *rec7* and *rec8* genes of *Schizosaccharomyces pombe*. Genetics **132:** 75–85.

Lorenz, M. C., and J. Heitman, 1997 Yeast pseudohyphal growth is regulated by GPA2, a G protein alpha homolog. EMBO J. **16:** 7008–7018.

Lorenz, M. C., and J. Heitman, 1998 The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. EMBO J. **17:** 1236–1247.

Lorenz, M. C., X. Pan, T. Harashima, M. E. Cardenas, Y. Xue *et al.*, 2000 The G protein-coupled receptor Gpr1 is a nutrient sensor that regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. Genetics **154:** 609–622.

Maeda, T., N. Mochizuki and M. Yamamoto, 1990 Adenylyl cyclase is dispensable for vegetative cell growth in the fission yeast *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **87:** 7814–7818.

Maeda, T., Y. Watanabe, H. Kunitomo and M. Yamamoto, 1994 Cloning of the *pka1* gene encoding the catalytic subunit of the cAMP-dependent protein kinase in *Schizosaccharomyces pombe*. J. Biol. Chem. **269:** 9632–9637.

Nakafuku, M., T. Obara, K. Kaibuchi, I. Miyajima, A. Miyajima *et al.*, 1988 Isolation of a second yeast *Saccharomyces cerevisiae* gene (*GPA2*) coding for guanine nucleotide-binding regulatory protein: studies on its structure and possible functions. Proc. Natl. Acad. Sci. USA **85:** 1374–1378.

Nocero, M., T. Isshiki, M. Yamamoto and C. S. Hoffman, 1994 Glucose repression of *fbp1* transcription of *Schizosaccharomyces pombe* is partially regulated by adenylate cyclase activation by a G protein alpha subunit encoded by *gpa2* (*git8*). Genetics **138:** 39–45.

Noda, K., Y. Saad, R. M. Graham and S. S. Karnik, 1994 The high affinity state of the beta 2-adrenergic receptor requires unique interaction between conserved and non-conserved extracellular loop cysteines. J. Biol. Chem. **269:** 6743–6752.

Ohi, R., A. Feoktistova and K. L. Gould, 1996 Construction of vectors and a genomic library for use with *his3*-deficient strains of *Schizosaccharomyces pombe*. Gene **174:** 315–318.

Ozcan, S., and M. Johnston, 1999 Function and regulation of yeast hexose transporters. Microbiol. Mol. Biol. Rev. **63:** 554–569.

Pan, X., and J. Heitman, 1999 Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:** 4874–4887.

Postma, P. W., J. W. Lengeler and G. R. Jacobson, 1993 Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. Microbiol. Rev. **57:** 543–594.

Samejima, I., S. Mackie and P. A. Fantes, 1997 Multiple modes of activation of the stress-responsive MAP kinase pathway in fission yeast. EMBO J. **16:** 6162–6170.

Shiozaki, K., and P. Russell, 1996 Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. Genes Dev. **10:** 2276–2288.

Sondek, J., A. Bohm, D. G. Lambright, H. E. Hamm and P. B. Sigler, 1996 Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. Nature **379:** 369–374.

Stettler, S., E. Warbrick, S. Prochnik, S. Mackie and P. Fantes, 1996 The wis1 signal transduction pathway is required for expression of cAMP-repressed genes in fission yeast. J. Cell Sci. **109:** 1927–1935.

Takeda, T., T. Toda, K. Kominami, A. Kohnosu, M. Yanagida *et al.*, 1995 *Schizosaccharomyces pombe atf1*$^+$ encodes a transcription factor required for sexual development and entry into stationary phase. EMBO J. **14:** 6193–6208.

Tang, W. J., and A. G. Gilman, 1991 Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits. Science **254:** 1500–1503.

Tang, W. J., J. A. Iniguez-Lluhi, S. Mumby and A. G. Gilman, 1992 Regulation of mammalian adenylyl cyclases by G-protein alpha and beta gamma subunits. Cold Spring Harbor Symp. Quant. Biol. **57:** 135–144.

Taussig, R., and A. G. Gilman, 1995 Mammalian membrane-bound adenylyl cyclases. J. Biol. Chem. **270:** 1–4.

Thompson, J. D., D. G. Higgins and T. J. Gibson, 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680.

Vassarotti, A., and J. D. Friesen, 1985 Isolation of the fructose-1,6-bisphosphatase gene of the yeast *Schizosaccharomyces pombe*. Evidence for transcriptional regulation. J. Biol. Chem. **260:** 6348–6353.

Wach, A., A. Brachat, C. Alberti-Segui, C. Rebischung and P. Philippsen, 1997 Heterologous *HIS3* marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. Yeast **13:** 1065–1075.

Watanabe, Y., Y. Lino, K. Furuhata, C. Shimoda and M. Yamamoto, 1988 The *S. pombe mei2* gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP. EMBO J. **7:** 761–767.

Whiteway, M., L. Hougan, D. Dignard, D. Y. Thomas, L. Bell *et al.*, 1989 The *STE4* and *STE18* genes of yeast encode potential beta and gamma subunits of the mating factor receptor-coupled G protein. Cell **56:** 467–477.

Wilkinson, M. G., M. Samuels, T. Takeda, W. M. Toone, J. C. Shieh *et al.*, 1996 The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. Genes Dev. **10:** 2289–2301.

Xue, Y., M. Batlle and J. P. Hirsch, 1998 *GPR1* encodes a putative G protein-coupled receptor that associates with the Gpa2p Galpha subunit and functions in a Ras-independent pathway. EMBO J. **17:** 1996–2007.

Yamawaki-Kataoka, Y., T. Tamaoki, H. R. Choe, H. Tanaka and T. Kataoka, 1989 Adenylate cyclases in yeast: a comparison of the genes from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **86:** 5693–5697.

Young, D., M. Riggs, J. Field, A. Vojtek, D. Broek *et al.*, 1989 The adenylyl cyclase gene from *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **86:** 7989–7993.

Yun, C. W., H. Tamaki, R. Nakayama, K. Yamamoto and H. Kumagai, 1998 Gpr1p, a putative G-protein coupled receptor, regulates glucose-dependent cellular cAMP level in yeast *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **252:** 29–33.

Zehetner, G., and H. Lehrach, 1994 The reference library system—sharing biological material and experimental data. Nature **367:** 489–491.

Communicating editor: M. Johnston