Published in final edited form as:

Biochem Soc Trans. 2005 February ; 33(0 1): 257–260. doi:10.1042/BST0330257.

Glucose sensing via the protein kinase A pathway in *Schizosaccharomyces pombe*

C.S. Hoffman

Biology Department, Boston College, Chestnut Hill, MA 02467, U.S.A

C.S. Hoffman: hoffmacs@bc.edu

Abstract

The fission yeast *Schizosaccharomyces pombe* primarily detects glucose via a cAMP-signalling pathway. Components of this pathway include the Git3 GPCR and a heterotrimeric G-protein, from which the Gpa2 G alpha subunit activates adenylate cyclase (Git2/Cyr1). Three additional proteins, Git1, Git7 and Git10 are required to generate a cAMP response even in a strain expressing an activated form of Gpa2, which is capable of bypassing the loss of the GPCR and G β - γ dimer. Therefore, Git1, Git7 and Git10 either act in a G-protein-independent manner or are required to stabilize or assemble a functional signalling complex. Although prior data suggested that the Cgs2 cAMP phosphodiesterase (PDE) do not regulate the cAMP response, we now have evidence that along with adenylate cyclase regulation, PDE activation is important for limiting the response to glucose. Finally, regulation of PKA activation appears to involve both traditional post-translational regulation of the function of the components of the cAMP pathway and glucose-dependent transcriptional regulation of some of these cAMP pathway genes.

Keywords

adenylate cyclase; cAMP; *fbp1* transcription; G-protein; *Schizosaccharomyces pombe*; PKA

Introduction

Nutrient sensing is critical to the survival of both prokaryotic and eukaryotic micro-organisms, which adjust their metabolism to utilize the resources available in their growth environment or enact developmental programmatic changes in response to nutrient stress. In particular, carbon source sensing has been heavily studied with a recurrent theme of catabolite repression [1], in which glucose detection represses transcription of genes required for the utilization of less optimal carbon sources. In *Escherichia coli*, cells respond to a glucose-rich environment by lowering cAMP levels to down-regulate the activity of the CRP (cAMP receptor protein) transcriptional activator [2]. Surprisingly, in both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, glucose detection results in a transient rise in cAMP levels to activate the cAMP-dependent protein kinase A (PKA) [3,4]. Studies of catabolite repression in *S. cerevisiae* have identified

glucose detection pathways in addition to the PKA pathway [5], while work in *S. pombe* suggests that glucose detection acts largely through the PKA pathway [6,7], as strains with reduced PKA activity fully mimic glucose-starved cells.

Genes encoding components of the *S. pombe* PKA pathway have been identified based on their sequence or functional homology to orthologous genes or on their role in glucose repression of sexual development or transcription of *fbp1*, which encodes the gluconeogenic enzyme fructose-1,6-bisphosphatase. Interest in the *S. pombe* cAMP pathway initially grew from the discovery that Ras proteins activate adenylate cyclase in budding yeast [8]. This led three laboratories to independently clone the *S. pombe* *cyr1* adenylate cyclase gene, also known as *git2*, by hybridization using the *S. cerevisiae* *CYR1* gene as a probe [7,9,10]. However, unlike the *S. cerevisiae* enzyme, *S. pombe* adenylate cyclase is not regulated by Ras and is not essential for cell viability [6,7,11]. A hybridization screen was also used to clone *gpa2*, which encodes a $G\alpha$ subunit required for adenylate cyclase activation [12]. In addition, the *cap* gene, which encodes the cyclase-associated protein, was cloned based on its ability to complement a deletion of the *S. cerevisiae* *CAP* gene [13], while the *pka1* gene, which encodes the catalytic subunit of PKA, was cloned by its ability to suppress a dominant-negative allele of the *S. cerevisiae* *RAS2* gene [14]. Loss of these activities allows cells to conjugate and sporulate in the absence of a starvation signal, which is normally required for *S. pombe* sexual development. Conversely, mutations in genes that increase PKA activity inhibit sexual development. This underlies the identification of *cgs1* and *cgs2* [15], encoding the regulatory subunit of PKA and the cAMP phosphodiesterase (PDE), respectively, by mutations that suppress the unregulated meiosis conferred by a conditional allele of the *ran1/pat1* gene. The PDE gene was also identified as *pde1* in a screen for multicopy suppressors of a meiotic defect due to high cAMP levels in an *S. pombe* strain that overexpressed the catalytic domain of *S. cerevisiae* adenylate cyclase [16], while the *pka1* gene was cloned as a multicopy inhibitor of meiotic entry [17]. Finally, my laboratory has employed a genetic selection for mutations that reduce or eliminate glucose repression of *fbp1* transcription, which identified eight *git* (glucose insensitive transcription) genes including the *git2/cyr1* adenylate cyclase gene, the *git6/pka1* PKA gene, the *git8/gpa2* gene and five additional genes required for adenylate cyclase activation. I will review here our current understanding of the *S. pombe* PKA signalling pathway based on our analyses of these genes.

Signalling occurs through the Git3 GPCR and its cognate G-protein composed of Gpa2, Git5 and Git11

Similar to mammalian cAMP pathways, four *S. pombe* genes required for glucose-triggered adenylate cyclase activation encode the Git3 seven-transmembrane protein and a heterotrimeric G-protein composed of the Gpa2 $G\alpha$, the Git5 $G\beta$ and the Git11 $G\gamma$. The first of these genes to be identified was the *gpa2* $G\alpha$ gene [12], which was later shown by genetic linkage and complementation tests to be identical to *git8* [18]. At the same time, we showed that overexpression of Gpa2 suppresses the defect in *fbp1* transcriptional repression caused by *git3* or *git5* mutations, suggesting that Gpa2 functions downstream from Git3 and Git5. Subsequent cloning of these genes showed that *git5* encodes the $G\beta$ of a heterotrimeric G-

protein [19] and Git3 encodes the putative glucose receptor [20]. Recent unpublished work from my laboratory supports the presumption that Git3 acts as a GPCR. We have shown that Git3 interacts with Gpa2 in a two-hybrid assay. This interaction is facilitated by the Git5 $G\beta$ and abolished by a mutation in *gpa2* that would constitutively activate Gpa2, thus mimicking the model for G-protein interactions with their cognate receptors (D.A. Kelly and C.S. Hoffman, unpublished results). In addition, preliminary results suggest that cells expressing a translational fusion of Git3 and the Gpa1 $G\alpha$ of the pheromone pathway respond to glucose with a transient activation of the pheromone pathway. Thus, Git3 appears to be an authentic GPCR whose ligand is glucose.

The *git5* $G\beta$ gene was used in a two-hybrid screen to identify Git5-binding partners, leading to the cloning of the *git11* $G\gamma$ gene [21]. Git5 and Git11 have also been shown to interact in *S. pombe* by co-immunoprecipitation, and *git11* deletion strains display cAMP pathway defects including elevated *fbp1* transcription and starvation-independent sexual development. As with *git3* and *git5*, the effect of a *git11* deletion on *fbp1* transcription is suppressed by overexpression of wild-type *gpa2*⁺ (S. Landry and C.S. Hoffman, unpublished results) or by a chromosomal copy of the activated *gpa2*^{R176H} allele (R. Welton and C.S. Hoffman, unpublished results). Thus, Gpa2 is responsible for the activation of adenylate cyclase. Indeed, recent studies have identified a binding site for Gpa2 in the amino-terminus of adenylate cyclase, and a direct role for Gpa2 in the activation of adenylate cyclase (F.D. Ivey and C.S. Hoffman, unpublished results).

As the $G\alpha$ in this pathway signals to the downstream effector, there is a cooperative genetic relationship between the $G\alpha$ and the $G\beta\gamma$ dimer. Our two-hybrid data described above suggest that the $G\beta\gamma$ dimer facilitates the interaction between Gpa2 and the Git3 GPCR and that this interaction is disrupted upon activation of Gpa2. As such, the GPCR and $G\beta\gamma$ are required for the activation of Gpa2, which presumably involves GDP release and GTP binding, leading to a conformational change in Gpa2. This contrasts with the *S. cerevisiae* pheromone-signalling pathway in which the Ste4-Ste18 $G\beta\gamma$ signals to the downstream effector [22]. In this pathway, loss of the Gpa1 $G\alpha$ activates signalling by releasing the $G\beta\gamma$ dimer, which has no additional requirements for it to bind its downstream effector [23]. Thus, the genetic relationship between $G\alpha$ -encoding genes and $G\beta$ - or $G\gamma$ -encoding genes is fundamentally different depending on whether the $G\alpha$ or the $G\beta\gamma$ dimer signals to the downstream effector.

The Git1, Git7 and Git10 proteins are also required for cAMP signalling

Unlike *git3*, *git5* and *git11*, mutations in *git1*, *git7* and *git10* are not suppressed by overexpression of wild-type *gpa2*⁺ or a genomic copy of *gpa2*^{R176H} [18,20]. Therefore, Git1, Git7 and Git10 may be involved in a G-protein-independent adenylate cyclase activation mechanism, acting in concert with Gpa2. Alternatively, these proteins may be required for Gpa2-mediated activation to occur by controlling the localization or stability of adenylate cyclase or Gpa2, or by facilitating the assembly of a functional signalling complex. The *git7* gene [24] encodes a member of the Sgt1 protein family, named after the *S. cerevisiae* Sgt1 protein originally identified as having a role in budding yeast kinetochore assembly [25]. Sgt1 is also involved in *S. cerevisiae* cAMP signalling [26], although, as with

Git7, the mechanism of action remains unclear. Proteins of the Sgt1 family contain two domains that suggest a co-chaperone function, a tetratricopeptide repeat (TPR) domain and a CS (found in CHORD and Sgt1 proteins) domain [27]. In *Arabidopsis*, two orthologues of Sgt1 are involved in pathogen resistance and interact with the HSP90 chaperone protein [28]. Remarkably, we have recently shown that the *git10-201* mutation is an unusual allele of the *S. pombe* *swol* HSP90 gene in that while conferring a defect in cAMP signalling, it does not cause the temperature-sensitive growth associated with other *swol* alleles (M. Alaamery and C.S. Hoffman, unpublished results; [29]). Finally, the *git1* gene has been recently cloned and encodes an *S. pombe*-specific protein with very little sequence homology to other proteins (R. Kao, E. Morreale and C.S. Hoffman, unpublished results). Git1 possesses one recognizable motif, a C2 domain that has been shown in proteins such as protein kinase C to promote association with phospholipids in a calcium-dependent manner, although C2 domains can act in a calcium-independent manner and bind proteins as well as phospholipids [30].

Both adenylate cyclase and cAMP phosphodiesterase regulate cAMP signalling

Intracellular cAMP levels are a function of the relative rates of cAMP production from ATP by adenylate cyclase and cAMP conversion to AMP by PDE. Initial studies of *S. pombe* cAMP signalling suggested that glucose detection triggers adenylate cyclase activation while PDE activity remains constant. Some strains carrying point mutations in *git2/cyr1* that alter adenylate cyclase activity display normal basal cAMP levels, but are defective in their cAMP response upon exposure to glucose, while a strain carrying the *cgs2-2* PDE mutant allele possesses elevated basal cAMP levels and responds to glucose with a similar fold increase in cAMP as seen in wild-type cells [4]. In addition, loss of PKA activity results in an increase in basal cAMP levels and a defect in the restoration of basal levels after glucose addition to cells, suggesting that PKA is responsible for feedback regulation of the pathway, presumably by attenuating the activation of adenylate cyclase.

More recently, studies of a new allele of the *cgs2* PDE gene indicate that PDE activation is partially responsible for feedback regulation of the cAMP signal (L. Wang and C.S. Hoffman, unpublished results). The *cgs2-s1* allele was identified as a suppressor of the *git2-7* allele, which produces a catalytically active adenylate cyclase that is unable to generate a cAMP response to glucose [4,6]. A *cgs2-s1* strain, with an otherwise wild-type genetic background, possesses wild-type basal cAMP levels, but displays a significantly enhanced cAMP response to glucose. Thus adenylate cyclase activation appears to lead to a commensurate activation of PDE to limit the cAMP response, with the Cgs2-s1 PDE failing to respond to the cAMP signal. It is not clear whether PDE activation occurs through the direct action of PKA, as seen for *S. cerevisiae* Pde1 [31], or if *S. pombe* Cgs2 is allosterically activated by cAMP, as seen for the *Dictyostelium discoideum* PdeE enzyme [32]. These studies demonstrate that *S. pombe* cAMP signalling is controlled by the regulation of both adenylate cyclase and PDE, and may involve both PKA-regulated and PKA-independent mechanisms.

Some cAMP pathway genes are also transcriptionally regulated

We have shown that *cgs1* and *pka1*, encoding the regulatory and catalytic subunits of PKA, are themselves transcriptionally regulated by glucose and the PKA pathway [33]. Such regulation suggests that cAMP levels may not always directly reflect the level of PKA activity in a cell. For example, if glucose detection triggers a greater reduction in *cgs1* transcription than in *pka1* transcription, cells could maintain elevated PKA activity even after cAMP levels have returned to basal levels, by increasing the ratio of catalytic to regulatory subunits of PKA. Recent transcriptional analyses of other cAMP pathway genes indicate that several of these genes are also glucose-repressed in a PKA-dependent manner (M. Grandy and C.S. Hoffman, unpublished results). It is not clear whether this contributes to feedback regulation of the pathway or whether this is simply a mechanism to increase the abundance of the glucose detection apparatus in glucose-starved cells and in spores that detect glucose as the primary signal for germination.

Conclusion

Glucose detection in *S. pombe* occurs chiefly through a cAMP-signalling pathway that shares many features with those of mammalian systems. This includes a seven-transmembrane GPCR (Git3) and a heterotrimeric G-protein composed of the Gpa2 $G\alpha$, the Git5 $G\beta$ and the Git11 $G\gamma$. Glucose signalling through this module leads to the activation of Gpa2, which then activates adenylate cyclase. A mutation that constitutively activates Gpa2 bypasses the need for Git3, Git5 and Git11, but not Git1, Git7 and Git10, suggesting that these latter three proteins act independently from the G-protein. Alternatively, these proteins may be intrinsically required for G-protein signalling by controlling the stability, localization or assembly of a functional signalling complex. Finally, feedback regulation of the cAMP signal is controlled by both PKA activity and the activation of PDE, although it is not known whether this represents one or more distinct mechanisms. In addition, feedback regulation may be controlled at the level of glucose repression of transcription of some cAMP pathway genes.

With the cloning of these *S. pombe* cAMP pathway genes, we are now in a position to investigate both the general function of these proteins and the specific biochemical mechanisms by which they carry out their functions. As the *S. pombe* adenylate cyclase enzyme displays a shared structural organization with other fungal adenylate cyclases, this system should serve as a model for glucose detection and cAMP signalling in fungi, including many pathogens whose virulence depends on the functioning of this pathway.

Acknowledgments

I thank members of my laboratory whose unpublished results are cited here and who critically read this manuscript. This work was supported by National Institutes of Health grant GM46226 to C.S.H.

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Abbreviations used

git glucose insensitive transcription

PKA protein kinase A
PDE phosphodiesterase

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