MINIREVIEW

Except in Every Detail: Comparing and Contrasting G-Protein Signaling in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*

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When asked to explain the difference between the mechanisms controlling mating type switching in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, Brandeis University’s Jim Haber once replied, “It’s exactly the same, except in every detail.” He may as well have been referring to how these two yeasts utilize guanine nucleotide-binding proteins (G proteins) to detect the two most essential objects in the extracellular milieu, sexual partners and food. Both pheromone signaling and glucose signaling in these yeasts occur through the action of G-protein-coupled receptors (GPCRs) and associated G proteins, though the composition of the G proteins and the relative roles of individual subunits vary among the four pathways. In this review, I will compare and contrast these *S. cerevisiae* and *S. pombe* G-protein-mediated signaling pathways. I will also discuss the genetic implications of G-protein-mediated signaling as well as the need to revisit some generally accepted ideas regarding receptor-mediated G-protein signaling. For more comprehensive reviews on fungal signaling pathways, see recent reviews by Lengeler et al. and Kays and Borkovich (48, 61). Note that recent studies of mitotic spindle positioning in *Drosophila melanogaster* and *Caenorhabditis elegans* (36) have uncovered both receptor-independent regulation of and novel biological roles for Gα subunits, providing further evidence that our understanding of G-protein signaling is far from complete.

G-protein-mediated signaling is one of the most important mechanisms by which eukaryotic cells sense extracellular stimuli and convert them into intracellular signals. Such signaling is often used to regulate transcriptional activators and repressors to control cell function and development. Interest in developing a detailed understanding of the *S. cerevisiae* and *S. pombe* G-protein pathways comes from two distinct areas of biology. Firstly, G-protein signaling is critical to human development, with many human inherited diseases as well as tumors being associated with mutations in genes encoding GPCRs, their cognate G proteins, and their downstream effectors (27, 77, 81). In addition, more than half of all pharmaceuticals act on GPCRs, which continue to be viewed as the premier class of drug targets (4, 20). Secondly, both fungal human pathogens such as *Candida albicans* and *Cryptococcus neoformans* (1, 26, 70) and fungal plant pathogens such as *Cryptonectria paradoxa*, *Magnaporthe grisea*, and *Ustilago maydis* (30, 54, 55, 63, 79) utilize G-protein signaling pathways to control processes required for virulence (61). The identification of G-protein signaling pathway genes in the genetically pliable model yeasts *S. cerevisiae* and *S. pombe* motivates the study of homologs in the aforementioned less genetically tractable fungal systems.

**TEXTBOOK G-PROTEIN SIGNALING**

The G-protein activation-inactivation cycle has been a common feature of cell and molecular biology textbooks for the past 20 years, suggesting a completeness of understanding that belies the true state of the field. During this time, the only significant modifications to this model that have gained wide acceptance include the recognition that some effectors are activated by Gβγ dimers and that GTPase-activating proteins function in some pathways to restore the G protein to its inactive state. As shown in Fig. 1, the G protein converts the event of extracellular ligand binding by a GPCR into the activation of a downstream effector. In the absence of the ligand, the G protein is found as a heterotrimer of Gα, Gβ, and Gγ subunits, with the Ga bound to GDP (Fig. 1A). Upon agonist binding, the GPCR causes the Gα subunit to release the GDP and subsequently bind the more abundant GTP (Fig. 1B). GTP binding promotes a conformational change in the Gα and its dissociation from the Gβγ dimer, which itself does not undergo a conformational change (84). Depending on the signaling pathway, the Gα, the Gβγ dimer, or both can go on to regulate downstream effectors (Fig. 1C, shown as “A” and “B”). The cycle is completed when the Gα subunit, which has an intrinsic GTPase activity, hydrolyzes the bound GTP to GDP and returns to the GDP-bound “inactive” conformation that reassociates with a Gβγ dimer (Fig. 1C). For some Gα subunits, the GTPase activity is stimulated by GTPase-activating proteins, also known as RGS proteins (regulators of G-protein signaling). While this general view of the G-protein cycle is widely accepted, at least two aspects of it have been questioned. First, though most studies support a model in which G-protein activation results in Gα dissociation from the Gβγ dimer, some studies challenge this notion. The ability to chemically cross-link Gα subunits to Gβ subunits is not altered by activation of the Gα, and Gα subunits within chemically cross-linked G proteins can be charged with GTP and convert...
to the activated conformation (102). In yeast, a translational fusion of the $G_{\alpha}/H9251$ and $G_{\alpha}/H9252$ of the pheromone pathway continues to function normally, although it is obviously unable to fully dissociate (52). Second, contrary to the model in Fig. 1, in which activation of the G protein causes it to shuttle from the receptor to the effector, a receptor-G-protein-effector preactivation complex may exist prior to signaling, and G-protein activation could simply alter the nature of the interactions among the various components of the signaling pathway (15).

EXACTLY THE SAME

Both $S. cerevisiae$ and $S. pombe$ possess two known G-protein-mediated signaling pathways. These pathways are used to detect extracellular pheromones in order to activate a mitogen-activated protein kinase (MAPK) cascade and to detect glucose in order to activate adenylate cyclase to produce a cyclic AMP (cAMP) signal. At the level of the receptors and effectors, these two signaling pathways appear to be conserved between the two yeasts. The $S. cerevisiae$ Ste2 $\alpha$-factor receptor and the Ste3 $\alpha$-factor receptor of the pheromone pathway show sequence homology to the $S. pombe$ Mam2 $P$-factor receptor and the Map3 $M$-factor receptor, respectively. Similarly, the $S. cerevisiae$ Gpr1 receptor of the glucose/cAMP pathway is related to the $S. pombe$ Git3 receptor of the same pathway. The same relationship holds true for the downstream effectors. The pheromone signals activate MAPK and p21-activated kinase (PAK) pathways comprised of related kinases, while the glucose signals activate adenylate cyclase to produce transient cAMP signals that activate the cAMP-dependent protein kinase PKA.

At first glance, $S. cerevisiae$ and $S. pombe$ also utilize a similar catalog of G-protein subunits with distinct $G_{\alpha}$ subunits for the pheromone and glucose pathways but a single $G_{\beta}$ subunit and $G_{\gamma}$ subunit which function as a dimer in only one of the two pathways. Thus, each yeast employs a traditional heterotrimeric G protein coupled to the receptor of one pathway and a monomeric $G_{\alpha}$ subunit coupled to the receptor of the other pathway. (In this review, the term monomeric $G_{\alpha}$ subunit indicates the absence of a $G_{\beta}/G_{\gamma}$ dimer but allows for the possibility that the $G_{\alpha}$ interacts with other proteins.) In addition, in each yeast, the monomeric small GTP-binding protein Ras acts in one of these two pathways in a mechanism parallel to effector activation by the monomeric $G_{\alpha}$ subunit. Thus, there appears to be considerable congruity between G-protein-mediated signaling in $S. cerevisiae$ and in $S. pombe$.

EXCEPT IN EVERY DETAIL

While both $S. cerevisiae$ and $S. pombe$ possess two $G_{\alpha}$ subunits, one $G_{\beta}$ subunit, and one $G_{\gamma}$ subunit to mediate pheromone and glucose signaling, the use of these four subunits in FIG. 1. The G-protein activation-inactivation cycle. (A) In the absence of an extracellular ligand, the G protein is present as an inactive heterotrimer ($G_{\alpha}$ is in green, $G_{\beta}$ is in blue, and $G_{\gamma}$ is in red), with the $G_{\alpha}$ subunit bound to GDP. The G protein may also be bound to its cognate GPCR in a preactivation complex. Downstream effectors “A” and “B” are shown as physically separate from the G protein, although the receptor-G-protein-effector model (15) suggests that these proteins could interact with the inactive G protein, although not in a manner that leads to effector activation. (B) Ligand binding by the receptor causes the $G_{\alpha}$ subunit to release GDP and bind the more abundant GTP nucleotide. This binding results in a conformational change in the $G_{\alpha}$ and either dissociation from the $G_{\beta}/G_{\gamma}$ dimer or remodeling of the interaction between these subunits. The $G_{\beta}/G_{\gamma}$ dimer does not undergo a conformational change. (C) Once activated, the $G_{\alpha}$, the $G_{\beta}/G_{\gamma}$ dimer, or both components of the G protein can activate downstream effectors. Eventually, the $G_{\alpha}$ subunit hydrolyzes the bound GTP nucleotide to GDP. This causes the $G_{\alpha}$ subunit, and thus the G protein, to return to the inactive conformation to complete the cycle.
the two pathways is completely different. In *S. cerevisiae*, the pheromone signal is sent through a heterotrimeric G protein, resulting in the activation of a MAPK signaling pathway by the Gβγ dimer. This same Gβγ dimer activates a PAK family member, which is required both for activating the MAPK pathway and for a second role in mediating polarized cell growth in order to facilitate contact and cell fusion between mating partners. Meanwhile, *S. cerevisiae* glucose/cAMP signaling is mediated by a monomeric Gα subunit as well as by Ras proteins. Conversely, in *S. pombe*, it is the glucose/cAMP signaling pathway that utilizes the heterotrimeric G protein, in which the Go subunit activates the downstream effector adenylate cyclase. Similar to the *S. cerevisiae* glucose/cAMP pathway, the *S. pombe* pheromone pathway involves both a monomeric Go subunit and a Ras homolog which activate both a MAPK and a PAK signaling pathway. In the following section, these four pathways will be described in greater detail.

**S. CEREVISIAE AND S. POMBE G-PROTEIN PATHWAYS**

*S. cerevisiae* pheromone MAPK pathway. The *S. cerevisiae* pheromone pathway is arguably the most thoroughly studied G-protein signaling pathway, certainly in microorganisms (6, 22, 25). Even so, there have been recent discoveries with regard to the function of the G-protein subunits in regulating the response to pheromone detection. Haploid yeast cells express either the Ste2 or Ste3 GPCR, which detects the α-factor or α-factor pheromones, respectively, which are secreted by potential mating partners (11, 35). These receptors are coupled to a heterotrimeric G protein composed of the Gpa1/Seg1 Gα, the Ste4 Gβ, and the Ste18 Gγ subunits (21, 95). Upon activation, the Gβγ dimer activates a MAPK pathway composed of the Ste11 MAPK kinase kinase (MAPKKK), the Ste7 MAPK kinase (MAPKK), and the Fus3 MAPK by binding the Ste5 scaffold protein, which holds the three kinases of this cascade together, and recruiting this complex to the plasma membrane (78, 96). The Ste4-Ste18 Gβγ dimer also binds Ste20, a PAK family member, also known as a MAP4K (MAPKKK kinase), which appears to phosphorylate and activate the Ste11 MAPKKK (59). Thus, by targeting both Ste5 and Ste20, the Ste4-Ste18 Gβγ dimer activates the Fus3 MAPK pathway at two levels. Additional targets of the Gβγ dimer include Cdc24, Far1, and Rho1 (of these, only Cdc24, the guanine-nucleotide exchange factor [GEF] for Cdc42, is shown in Fig. 2A), which are involved in the regulation of polarized cell growth (5, 12, 73, 105). Meanwhile, the Gpa1 Gα subunit plays at least three roles in the signaling pathway. In the absence of pheromone signaling, Gpa1 binds to the Ste4-Ste18 Gβγ dimer to prevent activation of the MAPK pathway. Upon activation, Gpa1 binds the Fus3 MAPK, in a role that may reduce the abundance of nuclear Fus3, which is involved in the transcriptional response to pheromone, while redirecting activated Fus3 to the sites of polarized cell growth to positively regulate such growth and cellular fusion (9, 69). Finally, Gpa1 plays a positive role in signaling through an interaction with the polyribosome-associated mRNA-binding protein Srp160 (34), although the biological effect of this interaction is not understood at a mechanistic level. The prototypical RGS protein Sst2 accelerates Gpa1 GTPase activity to return Gpa1 to its GDP-bound form and desensitize the cells to the pheromone present in the growth environment (8, 23, 24).

*S. pombe* pheromone MAPK pathway. The *S. pombe* pheromone signaling pathway also employs two pheromone-specific GPCRs, the Mam2 F-factor receptor and the Map3 M-factor receptor (19, 51, 85). However, these receptors are coupled to Gpa1, which appears to be a monomeric Gα subunit (56, 75). While one report suggested that the Gpb1/Git5 Gβ is a negative regulator of Gpa1 (50), it was subsequently shown that the enhanced conjugation observed in cells lacking this Gβ is due to a loss of the starvation requirement for conjugation in *S. pombe* due to a defect in glucose/cAMP signaling and not to an enhancement of pheromone signaling (58). Unlike *S. cerevisiae* cells, which are able to conjugate under nutrient-rich conditions, wild-type *S. pombe* cells conjugate only under starvation conditions. As in *S. cerevisiae*, pheromone signaling leads to the activation of a MAPK pathway composed of the Byr2 MAPKKK, the Byr1 MAPKK, and the Spk1 MAPK. Gpa1 and Ras1, the only Ras homolog in *S. pombe*, functionally converge on the Byr2 MAPKKK of the pathway (99). Ras1 directly binds Byr2 (66), while direct targets of Gpa1 are not known. Ras1 is also part of a protein complex involving the Shk1 PAK/MAP4K family member (14), which is similar to the *S. cerevisiae* Ste20 MAP4K. Activation of Ras1 in response to pheromone signaling appears to be due to pheromone-induced expression of the Ste6 GEF (43), suggesting that Ras1 is not directly activated by the pheromone receptors. While pheromone signaling in *S. pombe* and *S. cerevisiae* results in the activation of homologous MAPK pathways, and this activation involves targeting of both the core MAPK cassette and the MAP4K, the *S. pombe* MAPK pathway is clearly not activated by a Gβγ dimer. This may not be surprising in that the *S. pombe* MAPK pathway has not been shown to possess an Ste5-like scaffold protein and therefore may involve very different protein-protein interactions from those observed in *S. cerevisiae*. Clearly, many basic questions remain to be answered with regard to the regulation and role of Gpa1 in *S. pombe* pheromone signaling, although like *S. cerevisiae* Gpa1, *S. pombe* Gpa1 is negatively regulated by an RGS protein (93).

*S. cerevisiae* glucose/cAMP pathway. The *S. cerevisiae* glucose/cAMP pathway (39, 86, 92) was originally shown to be a Ras-dependent pathway in which Ras is coupled to adenylyl cyclase (Cyr1) (89). This pathway was subsequently linked to glucose detection in a study of glucose-induced trehalase activation and trehalose mobilization (68). Ras is activated by the Cdc25 GEF, although the glucose detection mechanism that leads to Ras activation has yet to be determined (86). More recently, the glucose/cAMP pathway was shown to be G-protein mediated with core components including the Gpr1 GPCR and the Gpa2 Gα subunit (17, 53, 64, 65, 100, 103, 104) (Fig. 2C). Gpr1 is a seven-transmembrane domain protein that, based on cAMP response assays with various monosaccharide concentrations, behaves like a low-affinity glucose receptor and a higher-affinity sucrose receptor (60). One study suggested that Gpr1 and Gpa2 are solely responsible for glucose-triggered activation of Cyr1, while Ras-mediated activation of Cyr1 occurs in response to internal acidification and not glucose detection (17). However, recent work with a more sensitive assay of Ras activation has shown that GTP charging of Ras occurs in response to glucose signaling but is indepen-
dent of Gpr1 and Gpa2 function (18, 82). Therefore, two distinct signaling pathways result in Gpa2 and Ras activation in order to activate adenylate cyclase. In addition, Ras- and Gpa2-mediated activation of adenylate cyclase is not redundant as the Ras proteins and adenylate cyclase are essential in S. cerevisiae, while Gpa2 is not (47, 67, 72). To some degree, the relationship of the S. cerevisiae Gpa2 and Ras proteins resembles that of the S. pombe Gpa1 and Ras1 proteins as they converge on the activation of the Byr2 MAPKKK in the pheromone pathway (Fig. 2B). As in the two pheromone pathways described above, an RGS protein, Rgs2, has been identified as a negative regulator of Gpa2-mediated signaling (91).

The S. cerevisiae genomic sequence suggests the presence of a single G6 gene, STE4', which appears to function only in the pheromone pathway, as its overexpression or deletion has no effect on cAMP signaling (J. Thevelein, personal communication) and loss of the Ste4 protein has no effect on filamentous growth (62), which is a cAMP-regulated process involving Gpr1 and Gpa2 (see below). Therefore, considerable efforts have been made to identify either an atypical Gß6 dimer or proteins that functionally substitute for a Gß6 in this pathway. Coimmunoprecipitation analyses suggest that the interaction between Gpr1 and Gpa2 is dependent on the presence of the Plc1 phospholipase C protein, while Plc1 itself interacts with either Gpa2 or Gpr1 in the absence of the other component of the G-protein pathway (3). However, there has been no independent verification or follow-up studies supporting such a role for Plc1 in this pathway. It is also not clear whether Plc1 would play a structural role in the coupling of Gpr1 with Gpa2 or whether its role might be an indirect function of its requirement for glucose-triggered calcium signaling (87, 88). Other studies have found that the Gpb1/Krh2 and Gpb2/Krh1 proteins bind to Gpa2 and that these Kelch repeat proteins should fold into a structure resembling the beta propeller seen for Gß6 subunits (7, 38). Gpb1/Krh2 and Gpb2/Krh1 appear to act together with Gpg1, whose only resemblance to Gß6 subunits is...
its relatively small size of only 126 residues (38). However, as Gpb1/Krh2 and Gpb2/Krh1 do not resemble Gβ subunits at the sequence level, there is no compelling reason for Gpg1 to resemble a Gγ subunit. While these proteins have been proposed to serve as Gβγ mimics, they do not function in the traditional role of a Gβγ to facilitate coupling of the GPCR and the Go subunit in that the physical interaction between Gpr1 and Gpa2 is not reduced by the loss of Gpb1/Krh2 and Gpb2/Krh1 (38). In addition, loss of Gpb1/Krh2 and Gpb2/Krh1 actually increases diploid filamentous growth and haploid invasive growth, indicating an activation of this pathway, while gpa2Δ and gpr1Δ mutants are reduced for such growth (7, 38).

Thus, it appears that a Gβγ structural mimic has occupied the void created by the absence of an authentic Gβγ dimer in the S. cerevisiae glucose/cAMP pathway. As this dimer negatively regulates a pathway that would have been positively regulated by a true Gβγ, we have referred to this dimer as a pseudostructural inhibitor of the S. cerevisiae glucose/cAMP pathway (45).

The precise role of the S. cerevisiae Gpa2 protein in adenylate cyclase activation is not known. While loss of Gpa2 confers a defect in cAMP signaling, it has yet to be demonstrated that Gpa2 directly binds and activates Cyr1 (Fig. 2C, indicated by the question mark between Gpa2-GTP and Cyr1). Conversely, the Ras proteins, in particular Ras2, have been shown to be direct activators by the ability to reconstitute Ras-mediated activation by coexpressing adenylate cyclase and Ras2 in Escherichia coli (90). Further studies have identified a region in the leucine-rich repeat domain of adenylate cyclase required for Ras activation and have shown that a peptide of this region would be bound by Ras-GTP but not Ras-GDP (49). Thus, Ras is a direct activator of S. cerevisiae adenylate cyclase.

S. pombe glucose/cAMP pathway. The S. pombe glucose/cAMP signaling pathway involves the Git3 GPCR and a heterotrimeric G protein composed of the Gpa2 Ga, the Git5 Gβ, and the Git11 Gγ (44, 57, 58, 74, 94), with no role for the Ras1 protein (29, 41). Unlike the S. cerevisiae pheromone pathway, which also possesses a heterotrimeric G protein (Fig. 2A), the S. pombe Gpa2 Ga subunit is responsible for activation of the downstream effector, adenylate cyclase (Cyr1/Git2). Loss-of-function mutations affecting the Git3 GPCR or the Git5-Git11 Gβγ dimer confer a defect in cAMP signaling as measured directly and as inferred by the loss of glucose repression of transcription of the fbp1+ gene, which encodes the gluconeogenic enzyme fructose-1,6-bisphosphatase (13, 41, 42). Mutations that activate S. pombe Gpa2 fully suppress deletions of git3Δ, git5Δ, or git11Δ, with respect to fbp1+ transcriptional regulation, but have no effect on mutations in three other genes (git1+ , git7+ , and git10+) required for cAMP signaling (58, 94). Therefore, Git1, Git7, and Git10 are not required for coupling Gpa2 to Git3 and are not included in Fig. 2D. Recent work from my laboratory has shown that adenylate cyclase activation involves the direct binding of Gpa2 to the Git2/Cyr1 adenylate cyclase (F. D. Ivey and C. S. Hoffman, unpublished data). Of the four G-protein-mediated signaling pathways in budding and fission yeast, this is the only pathway for which an RGS protein has not been identified. Analysis of the completely sequenced S. pombe genome (97) suggests that there may be only a single RGS protein, Rgs1 (93), which acts in the pheromone pathway and displays sequence homology to the S. cerevisiae Sst2 RGS protein outside of the RGS domain. However, the sequence conservation among the three yeast RGS proteins is relatively weak, such that a functional RGS for the S. pombe Gpa2 Ga may be found only through a functional screen for genes that when overexpressed confer phenotypes similar to those of strains lacking Gpa2.

The S. pombe glucose/cAMP pathway provides an interesting challenge to some accepted notions regarding Gβγ dimer structure and function. Crystal structure analysis of a bovine Gβγ dimer shows that the Gγ subunit lies cradled in a groove between the N-terminal coiled coil and the beta propeller of the Gβ subunit (83). The Git5 Gβ is unique in that is completely lacks the N-terminal coiled-coil domain present in all other Gβ subunits (58). While studies in mammalian systems have indicated that this domain is required for Gγ binding and thus proper folding of the Gβ subunit (31, 32), this is clearly not true in S. pombe, in which the Git5 Gβ functions together with a traditional Gγ subunit encoded by the git11+ gene (57). In this system, the Gγ appears to be required for Gβ localization but not folding, as a deletion of the git11+ gene confers a relatively weak defect in glucose repression of fbp1+ transcription, and this defect can be suppressed by fusing the CAAX box of the Git11 Gγ to the C terminus of the Git5 Gβ (57). The link between Gβγ dimerization and Gβ folding may exist only in organisms that express a variety of these subunits as a mechanism to reduce inappropriate signaling by subunits that have not assembled into signaling complexes.

In S. pombe, a second putative seven-transmembrane protein, Stm1, has been implicated in the cAMP signaling pathway, although it may not be involved in glucose signaling (16). Stm1 appears to be able to bind Gpa2, however, deletion or overexpression of stm1+ has no effect on fbp1+ transcriptional regulation (R. Kao and C. S. Hoffman, unpublished data). Other experiments suggest that under certain conditions, overexpression of Stm1 lowers cAMP levels (16). Since stm1+ transcription is induced by nitrogen starvation, Stm1 may function not as a receptor but as a pseudostructural inhibitor by titrating Gpa2 out of the glucose/cAMP pathway in cells undergoing nitrogen starvation. However, more work needs to be done with Stm1 to clearly demonstrate a role in the S. pombe cAMP pathway.

GENETICS OF Ga VERSUS Gβγ SIGNALING

As described above, loss-of-function mutations affecting the Gpa1 Ga of the S. cerevisiae pheromone pathway confer the opposite phenotype from those affecting the Ste4 Gβ or Ste18 Gγ, while mutations affecting the Gpa2 Ga, the Git5 Gβ, or the Git11 Gγ of the S. pombe glucose pathway confer similar mutant phenotypes. This difference in the genetic relationships among these genes encoding G-protein subunits is due to the fact that S. cerevisiae pheromone signaling is mediated by the Gβγ dimer, while S. pombe glucose signaling is mediated by the Ga subunit. The activation of a heterotrimeric G protein involves the exchange of GDP for GTP on the Ga subunit and a resulting conformational change but no change in the conformation of the Gβγ dimer (Fig. 1B). Therefore, activation of a Gβγ simply requires its dissociation from the Ga, such that most loss-of-function mutations in the S. cerevisiae GPA1+ gene result in the activation of the pheromone pathway. On the other hand, in a Ga-mediated signaling pathway, in which Ga...
activation is receptor mediated, removal of the Gβγ dimer from the Gα subunit is not sufficient to activate the Gα, whose active conformation is dependent upon GTP binding. (The same cannot be said for receptor-independent Gα subunits that either display high intrinsic nucleotide exchange rates or are regulated by nonreceptor exchange factors such as the C. elegans RIC-8 protein [36].) The fact that the loss of any of the G-protein subunits in the S. pombe glucose/cAMP pathway results in a cAMP signaling defect supports this idea that the Gβγ dimer acts to couple the Gα with the GPCR. This coupling could be achieved through the targeting of the Gα to the plasma membrane or through enhancing the interaction between the GPCR and the Gα subunit. In mammals, it has been shown that a Gβγ dimer, which is associated with the plasma membrane due to prenylation of the Gγ subunit, plays a role in associating a Gα subunit with the membrane, where the Gα then becomes palmitoylated to further enhance its association with the membrane (28, 71). This role for the S. pombe Git5-Git11 Gβγ dimer is attractive, as the Gpa2 Gα lacks a myristoylation site (a glycine residue at position 2, present in the other three yeast Gα subunits) which normally helps to localize Gα subunits to the plasma membrane prior to palmitoylation. Alternatively, or in addition, the Gβγ dimer could strengthen the physical interaction between the GPCR and the Gα subunit, as has been shown in the S. cerevisiae pheromone pathway, where the interaction between the Gpa1 Gα and the Ste2 GPCR is positively regulated by the Ste4-Ste18 Gβγ (10, 76, 98). This may be due in part to the fact that the Ste4 Gβ subunit also interacts with Ste2 as judged by two-hybrid assay (76). Finally, it has been shown in the fungi Neurospora crassa and Cryptococcus parasitica that the deletion of a Gβ gene results in a reduction in the level of Gα subunits in the cells (46, 101), causing the Gβ deletion strains to share some mutant phenotypes with Gα deletion strains. Thus, the genetic relationship between the Gα subunit and the Gβγ dimer is dependent upon the mechanism of effector activation, while the biochemical relationship, in which the Gβγ positively regulates the interaction between the GPCR and the Gα subunit, remains constant.

REWITING THE BOOK ON G-PROTEIN SIGNALING

The most obvious way in which these yeast signaling pathways disregard the “rules” of G-protein signaling is in the apparent ability of the S. cerevisiae Gpa2 Gα of the glucose/cAMP pathway (Fig. 2C) and the S. pombe Gpa1 Gα of the pheromone pathway (Fig. 2B) to couple with a GPCR in the absence of a Gβγ partner. While it remains possible that atypical Gβγ dimers exist in these pathways to couple these Gα subunits to their respective receptors, it seems unlikely. In the S. pombe pheromone pathway, such genes should have turned up in genetic screens for sterile mutants, due to a reduction in Gpa1 coupling with the pheromone receptors. In S. cerevisiae, several studies to identify proteins that interact with Gpa2 have failed to define a functional Gβγ dimer in that pathway. However, the idea of a signaling pathway involving a GPCR-regulated monomeric Gα need not be so controversial. As described above, the major role of the Gβγ dimer with respect to receiving signals from the GPCR is to facilitate the interaction between the Gα subunit and the receptor, although it should be noted that Gβγ dimers have also been shown to negatively regulate signaling by enhancing the affinity of Gα subunits for GDP (40). Thus, in a Gα-mediated signaling pathway, a Gβγ dimer is required only if the unaided interaction between the Gα subunit and the GPCR is insufficient for signaling. Such a requirement is a function of the preactivation occupancy of the GPCR by the monomeric Gα, which reflects the binding affinity and the abundance of these proteins as well as the level of Gα activation needed to mount an appropriate response to receptor binding of the extracellular ligand. If there is either a sufficient interaction between the Gα and the receptor in the absence of a Gβγ dimer or a sufficiently low requirement for Gα activation, then no Gβγ dimer is required. When comparing the interaction of the Gpa2 Gα subunits of the two glucose/cAMP pathways with their cognate GPCRs, it should be noted that the S. cerevisiae Gpa2 protein demonstrates a strong interaction with the C terminus of the Gpr1 GPCR by two-hybrid analysis (100, 103). Conversely, we have observed only a very weak interaction between the S. pombe Git3 GPCR C terminus and the Gpa2 Gα, which is significantly strengthened upon coexpression of the Git5 Gβ (D. A. Kelly and C. S. Hoffman, unpublished data). Thus, the two-hybrid data support the idea that a Gβγ may not be required to couple the receptor and Gα of the S. cerevisiae glucose/cAMP pathway but is required in the S. pombe pathway.

The absence of a Gβγ dimer in some signaling pathways creates a literal opening for novel regulators to become part of the signaling pathway, as may be the case in the S. cerevisiae glucose/cAMP pathway. Gpb1/Krh2 and Gpb2/Krh1, together with Gpbp1, appear to act as pseudostructural inhibitors of the cAMP signaling pathway to negatively regulate haploid invasive, filamentous growth (7, 38). This Gβγ mimic may inhibit signaling by carrying out some, but not all, functions associated with Gβγ dimers, which have both positive and negative roles in G-protein signaling. While Gβγ dimers can positively regulate signaling by aiding the localization of Gα subunits to the plasma membrane and by enhancing the GPCR-Gα interaction (28, 71, 76), the Gβγ dimer used to be better known for a negative role by reducing GDP release and enhancing GTP release (as opposed to GTP hydrolysis) by the Gα subunit (40). A Gβγ mimic might carry out only this negative role in a situation where the normal dimer is not required for GPCR-Gα coupling, although such mimics could also serve as competitive inhibitors of authentic Gβγ dimers. However, in the case of the S. cerevisiae cAMP pathway, the Gpb1/2-Gpbp1 complex must also negatively regulate a target downstream from Gpa2, as a strain lacking Gpa2, Gpb1 (Krhl2), and Gpb2 (Krhl1) displays enhanced filamentation and haploid invasive growth, indicative of increased activation of the PKA pathway. The most likely downstream target would be adenylate cyclase, as this effector enzyme could be in physical contact with the Gpb1/2-Gpbp1 complex in a preactivation complex (15).

Finally, once one accepts the premise of signaling by monomeric Gα subunits, one can then realize that the assumed 1:1 stoichiometry of heterotrimeric G-protein subunits is not necessary for proper regulation of traditional G-protein signaling pathways. In fact, a study that examined the levels of all predicted S. cerevisiae proteins indicates an apparent ratio of the G-protein subunits in the pheromone pathway of 5:3:3 (Ste18 Gγ):3 (Ste4 Gβ) (33). While these measure-
ments may not accurately reflect the abundance of the untagged proteins, the balance of subunits is important, as a mere twofold increase in expression of the Ste4 Gβ is sufficient to activate the pheromone pathway (37). In this pathway, an excess of Ga subunits may be needed to prevent pheromone-independent signaling by the Gβγ dimer. However, we have observed a similar imbalance at the level of transcription of the G-protein subunit genes in the Go-mediated S. pombe glucose/cAMP pathway. Cells growing in either the presence or the absence of glucose, the presumed agonist for the Git3 GPCR, transcribe three times as much of the gpa2 Gα gene as of the git11 Gγ gene and nine to twenty times as much of the gpa2 as of the git5 Gβ gene (M. Grandy and C. S. Hoffman, unpublished data). Once again, the Gβ subunit may be the limiting component of this pathway. The relatively low level of Gβ expression suggests that a single Gβγ dimer may be responsible for sequential loading of Ga subunits onto an agonist-bound GPCR for their activation. While this could involve shuttling of Gpa2 from the receptor to the effector upon activation, it would also fit into the receptor-G-protein-effector preactivation complex model (15) with just a minor modification. In the absence of glucose signaling, along with fully assembled preactivation complexes, the excess monomeric Gpa2 subunits could be associated with adenylate cyclase without activating the enzyme. Upon glucose detection, activation of the existing receptor-G-protein-effector complexes would result in the release of the activated Gpa2-adenylate cyclase complex from the Git3-Gβγ complex, allowing for activation of the excess pool of inactive Gpa2-adenylate cyclase complexes. Whether or not such Ga-effector complexes exist prior to signaling, it is essential that in Ga-mediated pathways, excess monomeric Ga subunits, presumably in the form of Ga-GDP, do not significantly activate the downstream effector. Furthermore, Ga activation must be a function of GTP charging and the resulting conformational change rather than the loss of an interaction with a Gβγ partner. This is consistent with the fact that in the S. pombe glucose/cAMP pathway, cells lacking the Git5 Gβ display the same phenotypes as those lacking the Gpa2 Gα (41, 42, 57, 58, 74). It remains to be seen whether similar imbalances of G-protein subunits are found in other signaling pathways or whether GPCR-mediated monomeric Ga signaling pathways exist in mammals.

**SUMMARY**

Both the budding yeast S. cerevisiae and the fission yeast S. pombe utilize G proteins to respond to ligand binding by receptors that detect pheromones and glucose in the environment. While each yeast employs one heterotrimeric G protein and one monomeric Ga subunit in these pathways, the specific use of the G-protein subunits in the two pheromone pathways and the two glucose pathways has not been conserved. These similarities and differences point to both the advantages and the potential limitations of studies in relatively simple model organisms. The surprising discoveries of signaling by monomeric Ga subunits in the S. cerevisiae glucose/cAMP pathway and the S. pombe pheromone pathway were greatly facilitated by the fact that each yeast possesses only a single Gβ gene which could be genetically shown to act in only one of the two signaling pathways. It will be much harder to identify monomeric Ga-signaling pathways in humans, whose genome possesses 16 Ga, 5 Gβ, and 12 Gγ genes (80). However, it seems unlikely that the ability of monomeric Ga subunits to mediate G-protein signaling pathways is restricted to fungal systems.

A common tendency in model system work is to extrapolate from specific observations to propose broad rules that are applied to all organisms. As Jacques Monod is reported to have said, “What is true for E. coli is also true for elephants, only more so.” As we see here, depending upon the level of specificity, what is true for S. cerevisiae is not necessarily true for S. pombe, and vice versa. However, both organisms provide valuable examples of alternative strategies for ways in which a eukaryote may utilize a similar G-protein-related tool set to accomplish similar goals. It is as important for us to appreciate such mechanistic diversity as it is to understand any specific interaction that occurs in these four signaling pathways.

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