Fungal Genetics and Biology **25,** 143–156 (1998)
Article No. FG981102

REVIEW

Sex and Crime: Heterotrimeric G Proteins in Fungal Mating and Pathogenesis

Michael Bölker[1]

Department of Biology, University of Marburg, Karl-von-Frisch-Strasse, 35032 Marburg, Germany

Accepted for publication October 22, 1998

Bölker, M. 1998. Sex and crime: Heterotrimeric G proteins in fungal mating and pathogenesis. *Fungal Genetics and Biology* 25, 143–156. Heterotrimeric G proteins act as signal transducers that couple cell-surface receptors to cytoplasmic effector proteins. In fungi, G proteins play essential roles during sexual and pathogenic development. They are part of the pheromone signaling cascade in both ascomycetes and basidiomycetes, which is crucial for the recognition and fusion of cells of opposite mating type. In addition, G proteins affect a number of developmental and morphogenetic processes which determine the virulence of plant and human fungal pathogens. Cloning and targeted disruption of genes encoding α subunits of G proteins allowed the attribution of specific functions to these signal transducing molecules. Several lines of evidence indicate that many of the known fungal G proteins influence the intracellular level of cAMP by either stimulating or inhibiting adenylyl cyclase.

Index Descriptors: G proteins; signal transduction; pheromone response; pathogenicity; mating; receptors; cAMP; adenylyl cyclase; fungal development; hypovirulence.

Recognition of environmental stimuli plays a fundamental role for all eukaryotic cells. Many different signals are perceived at the surface of cells and transmitted by transmembrane signaling pathways to elicit specific responses such as the induction of gene transcription, protein phosphorylation, or cytoskeleton reorganization. Fungal cells can sense many different chemical and physical cues such as nutrients, osmolarity, pH, light, or surface hydrophobicity. In particular, fungi sense the presence of potential mating partners by recognizing sex-specific pheromones secreted by cells of opposite mating type. Pathogenic fungi depend on signals that allow them to recognize their plant or animal hosts. Fungi have evolved intricate systems that allow them to respond appropriately to these different signals and survive in various environments.

To understand the complex mechanisms of signal perception and signal processing, the study of "simple" systems that allow the efficient genetic and biochemical dissection of signal transduction pathways has been of great value. Regulatory networks identified in microorganisms often serve as paradigms for that of vertebrates. One of the most impressive examples is the pheromone response pathway in the yeast *Saccharomyces cerevisiae.* The discovery of regulatory components that are highly conserved between yeast and human (Neiman *et al.,* 1993) sheds light on the importance of studying fungal genetics and biology. In this review, only a short description of the yeast pheromone response system will be provided to serve as a framework for other fungal systems (for comprehensive reviews, see Kurjan, 1993; Herskowitz, 1995; Leberer *et al.,* 1997). Fungal G proteins have been recently reviewed (Borkovich, 1996), but since then a number of additional G

[1] To whom correspondence should be addressed. Fax: +49-6421-288971. E-mail: boelker@mailer.uni-marburg.de.

1087-1845/98 $25.00

proteins have been identified in different fungal species. For many of them targeted gene disruptions have revealed interesting clues about their functions. Fungal G proteins not only play pivotal roles in mating interactions and during pathogenic development, but are also involved in fungal development and morphogenesis. In this review, I summarize recent findings in diverse fungal systems and speculate on common themes of G protein function in fungi.

GENERAL PROPERTIES OF HETEROTRIMERIC G PROTEINS

Heterotrimeric G proteins were named by their ability to bind guanine nucleotides and consist of three different subunits α, β, and γ, with Gα being the nucleotide binding component (for review, see Gilman, 1987; Hamm and Gilchrist, 1996). The Gα subunit adopts different conformations depending on whether GTP or GDP is bound. Heterotrimeric G proteins were first implicated in transmembrane signaling by the requirement of GTP for hormonal activation of adenylyl cyclase (Rodbell *et al.*, 1971). G proteins are critical for the transduction of signals that are perceived by a distinct family of cell surface receptors. Although only slightly related on amino acid sequence level, these receptors share a common domain structure. They all contain seven stretches of hydrophobic amino acids that span the cytoplasmic membrane. The N-terminus of these seven transmembrane (7TM) receptors is located outside of the cell and the C-terminus in the cytoplasm (Dohlman *et al.*, 1991). Members of this class of receptors include the light sensing rhodopsin in retinal cells, β-adrenergic receptors in neurons, and olfactory receptors in nose neuroepithelial cells. Binding of the specific ligand induces a structural change of the receptor. The signal is then transduced to the heterotrimeric G protein which contacts the receptor at the inner membrane. This leads to decreased affinity of the Gα subunit for GDP which is then replaced by GTP. The α subunit assumes a different conformation and dissociates from the βγ heterodimer. In this activated state, the Gα subunit is supposed to be diffusible in the cytoplasm and can interact with intracellular effectors. The α subunit contains an intrinsic GTPase activity that catalyzes the rapid hydrolysis of GTP to GDP and P_i. Therefore, the activated state of the Gα subunit persists only a short time. This mechanism prevents the G protein from continuous signaling once it is activated (for review see Gilman, 1987).

In mammalian systems, heterotrimeric G proteins with different functions have been identified. The specificity of these G proteins is mainly determined by their α subunits which have been placed in different subfamilies based on their function and amino acid sequence similarity. Members of the $G\alpha_s$ subfamily stimulate adenylyl cyclase. $G\alpha_i$ and $G\alpha_o$ proteins contain a characteristic consensus sequence (CXXX) at the carboxy terminus that is susceptible to modification by pertussis toxin. Activated $G\alpha_i$ subunits lower intracellular cAMP levels by inhibiting adenylyl cyclase and $G\alpha_o$ is implicated in phosphoinositide metabolism. Other Gα subunits have been shown to be involved in the regulation of potassium channels, phospholipase C, and cyclic GMP phosphodiesterase (for review see Simon *et al.*, 1991).

Despite of the extensive biochemical evidence for the function of G proteins in diverse signaling pathways in higher organisms only very few mutants are known that are affected in G protein function. Many fungi, however, are genetically tractable and loss-of-function mutants can be constructed by targeted gene disruption. In addition, constitutive active and dominant negative variants of Gα subunits can be generated by single amino acid exchanges within the primary structure (Kurjan *et al.*, 1991). The phenotype of such mutants could thus help to elucidate the biological function of the signaling pathway that contains this G protein. In conjunction with methods that allow the identification of interacting partners, e.g., the yeast two-hybrid system, G proteins thus can serve as a starting point to isolate signaling components that act either upstream or downstream of heterotrimeric G proteins.

DIVERSITY OF FUNGAL G PROTEINS

Up to 24 different genes encoding Gα subunits have been isolated from fungal organisms (see Table 1). Most of the genes have been identified by DNA hybridization with heterologous probes or by PCR with degenerate primers deduced from highly conserved regions of the Gα sequences. Only the genes encoding the yeast Gpa1, the *Ustilago hordei* Fil1 and the *Aspergillus nidulans* FadA proteins have been isolated by the phenotype of respective mutants. The *Ustilago maydis gpa4* gene was identified by its distinct expression pattern. A phylogenetic tree generated by multiple alignment of the fungal G protein sequences reveals three major subgroups (I–III) within the family of Gα proteins (Fig. 1). Members of subgroups I and III can be related to the mammalian $G\alpha_i$ and $G\alpha_s$

TABLE 1
The Fungal G Alpha Subunits

Name	Subgroup	Organism	Taxonomic class	Mutants	Biological function	Accession no.	Reference
Gpa1 (=Scg1)	I/II	*Saccharomyces cerevisiae*	Ascomyceteous yeasts	+	Acts as negative regulator of the active G-protein βγ heterodimer in pheromone signalling. Haploid mutants are lethal.	P08539	Dietzel and Kurjan, 1987 Miyajima *et al.*, 1987 Nakafuku *et al.*, 1987
Gpa2	III ($G\alpha_s$)			+	Regulation of filamentation in response to nitrogen starvation. Nutrient sensing.	P10823	Nakafuku *et al.*, 1988
Gpa2	III ($G\alpha_s$)	*Kluyveromyces lactis*		+	cAMP regulation.	P54111	Savinon-Tejeda *et al.*, 1996
Cag1	I/II	*Candida albicans*		+	Can replace the yeast Gpa1 in pheromone signalling.	P28868	Sadhu *et al.*, 1992
Gpa1	II	*Schizosaccharomyces pombe*		+	Pheromone signaling.	P27584	Obara *et al.*, 1991
Gpa2	III ($G\alpha_s$)			+	Sensing of nitrogen starvation.	Q04665	Isshiki *et al.*, 1992
Gna-1	I ($G\alpha_i$)	*Neurospora crassa*	Ascomycetes	+	Female fertility, slower hyphal extension.	Q05425	Turner and Borkovich, 1993
Gna-2	II			+	Unknown. *gna-2/gna-1* mutants exhibit a more pronounced *gna-1* phenotype.	Q05424	Turner and Borkovich, 1993 Baasiri *et al.*, 1997
FadA	I ($G\alpha_i$)	*Aspergillus nidulans*		+	Negative regulator of asexual development.	Q00743	Yu *et al.*, 1996
Cpg1	I ($G\alpha_i$)	*Cryphonectria parasitica*		+	Virulence. Expression suppressed by hypovirus. Required for fertility.	Q00580	Choi *et al.*, 1995
Cpg2	III ($G\alpha_s$)			+	Unknown.	L32177	
MagA	III ($G\alpha_s$)	*Magnaporthe grisea*		+	Required for virulence.	AF011340	Liu and Dean, 1997
MagB	I ($G\alpha_i$)			+	Ascospore development.	AF011341	
MagC	II			+	Ascospore development.	AF011342	
Ctg1	I ($G\alpha_i$)	*Colletotrichum trifolii*		−	Unknown.	AF044894	Truesdall and Dickman, direct submission
Pcg1	II	*Pneumocystis carinii* f. sp. *carinii*		−	Unknown.	U30791	Smulian *et al.*, 1995
Pcg1	II	*Pneumocystis carinii* f. sp. *ratti*		−	Unknown.	U30790	
Gpa1	I ($G\alpha_i$)	*Ustilago maydis*	Basidiomycetes	+	Unknown.	P87032	Regenfelder *et al.*, 1997
Gpa2	II			+	Unknown.	P87033	
Gpa3	III ($G\alpha_s$)			+	Pheromone signaling. Morphogenesis and pathogenicity.	P87034	
Gpa4	IV			+	Unknown. Unusual sequence. Expression induced during pathogenic development.	P87035	
Fil1	III ($G\alpha_s$)	*Ustilago hordei*		+	Mutants show filamentous growth.	U76672	Lichter and Mills, 1997
Gpa1	III ($G\alpha_s$)	*Cryptococcus neoformans*		+	Required for mating and virulence. Involved in cAMP regulation.	P54853	Tolkacheva *et al.*, 1991
Gpa1	I ($G\alpha_i$)	*Coprinus congregatus*		−	Blue light regulation.	P30675	Kozak *et al.*, 1995

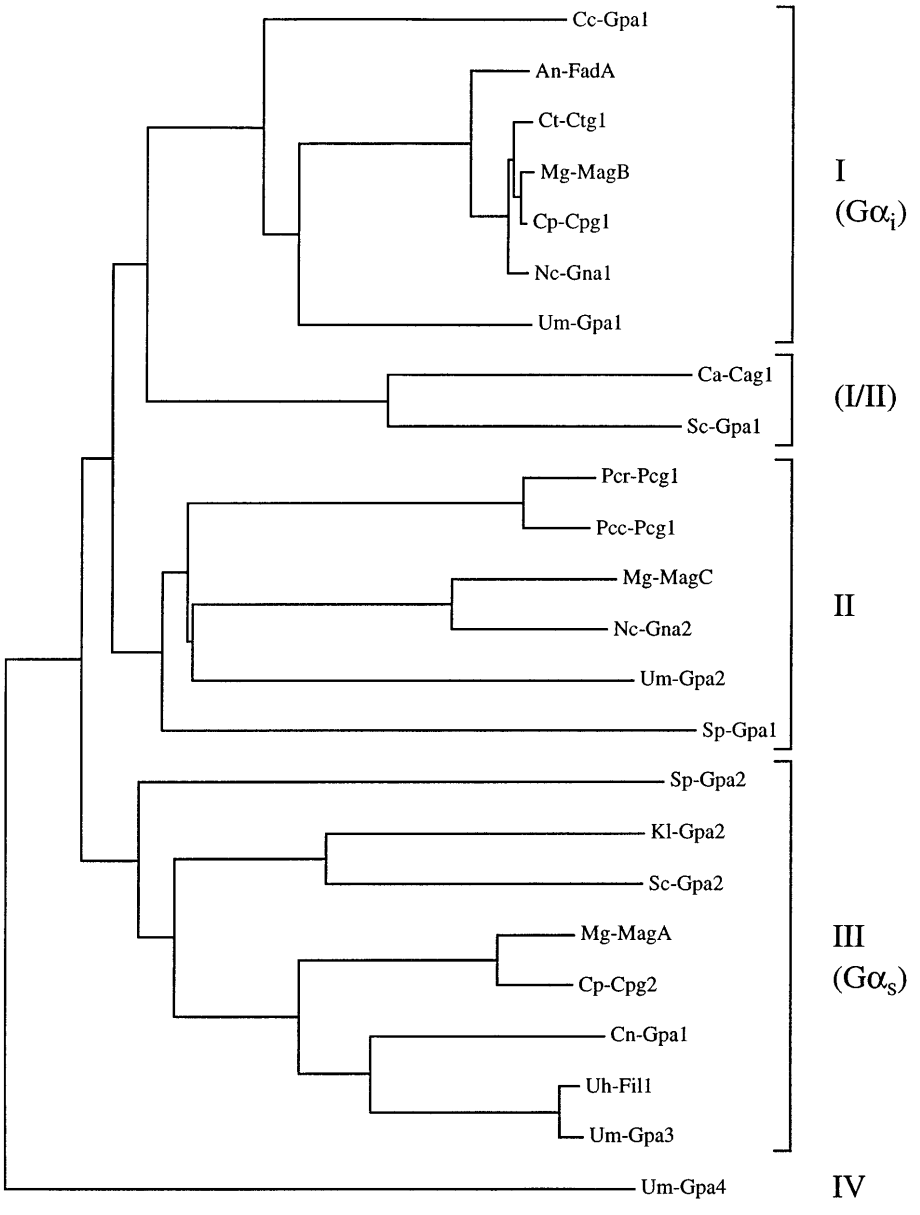

FIG. 1. Relationship of fungal Gα sequences. The amino acid sequences have been compared using the CLUSTAL W program (Thompson *et al.*, 1994). The rather unusual sequence of the *U. maydis* Gpa4 protein has been used as outgroup. Abbreviations of species: An, *Aspergillus nidulans;* Ca, *Candida albicans;* Cc, *Coprinus congregatus;* Cn, *Cryptococcus neoformans;* Cp, *Cryphonectria parasitica;* Ct, *Collectotrichum trifolii;* Kl, *Kluyveromyces lactis;* Mg, *Magnaporthe grisea;* Nc, *Neurospora crassa;* Pcr, *Pneumocystis carinii* f. sp. ratti; Pcc, *Pneumocystis carinii* f. sp. carinii; Sc, *Saccharomyces cerevisiae;* Sp, *Schizosaccharomyces pombe;* Uh, *Ustilago hordei;* Um, *Ustilago maydis.* For gene names and accession numbers see Table 1.

proteins, respectively, that either inhibit or stimulate the activity of adenylyl cyclase. Group II fungal Gα proteins have no corresponding mammalian counterparts that would allow clues to their function. The protein sequences within each subgroup are more closely related to each other than different G proteins of a single organism. This indicates that these proteins must be derived from ancestor molecules that have diverged before the different classes of fungi such as ascomycetes and basidiomycetes were originated. Only for the ascomycete *Magnaporthe grisea* (MagA-C) and the basidiomycete *U. maydis* (Gpa1-3) have members of each subgroup been identified so far. Mem-

bers of the group I Gα subunits are highly related on the amino acid sequence level. The presence of both a potential myristoylation site (MGXXXS) at the N-terminus and the consensus site CXXX for ADP ribosylation by cholera toxin (Simon *et al.*, 1991) at the carboxy-terminus has been taken as evidence that members of this group are homologous to the mammalian $G\alpha_i$ subunits that inhibit adenylyl cyclase (Turner and Borkovich, 1993). Indeed, there is genetic evidence for some fungi that members of this group can lower the intracellular cAMP level (see below) although the mechanism by which this occurs is still unknown.

Only for a few members of subfamily II fungal G proteins, a biological function or a distinct phenotype has been observed so far. Deletion of the *magC* gene in *M. grisea* reduces conidia formation and interferes with ascospore development (Liu and Dean, 1997). In *Schizosaccharomyces pombe*, the Gpa1 protein has been implicated in the transduction of the pheromone signal. It has been shown that a MAP kinase cascade is activated by concerted action of Gpa1 and the *S. pombe* Ras1 protein (Xu *et al.*, 1994). However, the direct effectors for fungal Gα subunits of this group still remain to be identified.

The Gpa1 proteins of *S. cerevisiae* and *Candida albicans* show some sequence relationship to subgroup I. However, they lack the consensus site for pertussis toxin dependent ribosylation which is characteristic for $G\alpha_i$ subunits. Thus they cannot be placed unambiguously into one of the subfamilies and constitute here the intermediate subgroup I/II. This might reflect the unique function of these G-protein α subunits acting as negative regulators of the βγ heterodimer which is the active signal transducing component during the yeast pheromone response (see below).

The group III of fungal G proteins contains some members that are implicated to positively influence the internal cAMP level. Overexpression of *S. cerevisiae* Gpa2 leads to an increase in internal cAMP, and disruption of the *S. pombe gpa2* gene results in a decrease of the cAMP level. Therefore, members of this subfamily have been here tentatively assigned as adenylyl cyclase stimulating fungal $G\alpha_s$ subunits in analogy to the mammalian $G\alpha_s$ family.

During a screen for *U. maydis* genes that are induced during the pathogenic phase of its life cycle, a fourth Gα subunit (Gpa4) has been identified. Interestingly, this protein has some quite unusual features. Gpa4 is larger than other fungal Gα proteins and exhibits a number of amino acid exchanges in otherwise highly conserved regions (Regenfelder *et al.*, 1997). This casts some doubt on whether this gene has the same properties as other Gα subunit in terms of GDP/GTP binding and GTPase activity. Although the disruption of the *gpa4* gene induces no discernible phenotype, the observed expression pattern of Gpa4 makes it unlikely to be a pseudogene. Homologs of *U. maydis* Gpa4 might exist in other fungal species as well, but have escaped detection so far. Most fungal Gα subunit genes have been cloned by PCR amplification using only a limited set of primer pairs. If these primers fail to amplify potential homologs of Gpa4 these would remain undetected.

For many of the fungal Gα subunits, targeted gene disruptions have been generated to study the biological function of these G-protein signaling pathways (see Table 1). In many cases, Gα mutants exhibited phenotypes which resemble that of mutants affected in the regulation of internal cAMP levels. cAMP is generated by the membrane bound adenylyl cyclase and acts as a secondary messenger mainly through activation of cAMP-dependent protein kinase. This serine/threonine kinase consists of two catalytic and two regulatory subunits with the latter repressing the kinase activity in the absence of cAMP. Binding of cAMP to the regulatory subunit leads to its dissociation from the catalytic subunit which then can phosphorylate specific target proteins. The role of cAMP signaling in virulence of phytopathogenic fungi has been reviewed by Kronstad (1997).

In the following sections, Gα proteins will be discussed according to their function in mating recognition and pathogenic development. However, gene disruption mutants of Gα subunits are often pleiotropic and sometimes defective in both mating and pathogenic processes. Therefore, these G proteins are discussed in the context of their original function, even though they may have additional roles.

G PROTEINS IN MATING AND DEVELOPMENT

Saccharomyces cerevisiae

The role of G proteins in fungal mating was first established during the study of the pheromone response in *S. cerevisiae.* In 1987, three different groups independently isolated the *GPA1* (=*SCG1*) gene (Dietzel and Kurjan, 1987; Miyajima *et al.*, 1987; Nakafuku *et al.*, 1987), which encodes a homolog of mammalian G protein α

subunits. Yeast cells exist in two different mating types (**a** and α) and secrete mating-type specific peptide pheromones. Recognition of these mating factors occurs by membrane bound receptors. Addition of pheromone to cells of opposite mating type results in a morphological response (shmoo formation) and cell cycle arrest (for review see Kurjan, 1993). Interestingly, the disruption of the *GPA1* gene leads to constitutive activation of the pheromone response pathway and is lethal in haploid strains due to the induced cell cycle arrest. In diploid **a**/α cells, however, *gpa1* mutants have no apparent phenotype (Dietzel and Kurjan, 1987). A number of sterility (*ste*) mutants that are defective in the mating pathway downstream of the Gα protein have been identified by their ability to rescue haploid Gα mutants. It has been shown that the signal received by the pheromone receptors is transmitted to a MAP kinase cascade by the βγ heterodimer that is activated by its dissociation from the α subunit (for a recent review see Leberer *et al.,* 1997). Thus the Gα subunit plays only a negative role in pheromone signaling by repressing the βγ dimer. This is the first indication for an active function of the βγ dimer in signaling processes which has been later demonstrated also for mammalian heterotrimeric G proteins (for review see Clapham and Neer, 1993).

The second Gα subunit from yeast, Gpa2, is implicated in sensing nitrogen starvation (Nakafuku *et al.,* 1988). *gpa2* mutant strains are viable and show no obvious mating defect. The presence of multiple copies of *GPA2* leads to a marked increase in intracellular cAMP concentration and can rescue a temperature sensitive *ras2* mutant (Nakafuku *et al.,* 1988). *GPA2* is also required for the induction of pseudohyphal growth in diploid yeast cells under nitrogen starvation (Kübler *et al.,* 1997; Lorenz and Heitman, 1997). This role of Gpa2 is independent of the MAP kinase pathway that was previously shown to be involved in pseudohyphal growth (Liu *et al.,* 1993). Since the defect in filament formation can be rescued by the addition of exogenous cAMP, Gpa2 most likely stimulates the activity of adenylyl cyclase. In yeast, adenylyl cyclase is also controlled by the small GTP binding proteins Ras1 and Ras2 (Toda *et al.,* 1985). It was shown that *ras2 gpa2* double mutants exhibit near synthetic lethality, indicating that Ras2 and Gpa2 proteins can replace each other in their stimulatory effect on adenylyl cyclase (Kübler *et al.,* 1997; Lorenz and Heitman, 1997; Xue *et al.,* 1998). When both proteins are missing, the cAMP level might drop to such a low level that cell division is affected.

The mechanism by which the yeast cells sense nitrogen starvation remains unclear. Two candidate genes have been proposed to encode potential sensor molecules. The *GPR1* gene was identified by the yeast two-hybrid system. It encodes a protein of 961 amino acids that can interact with Gpa2 (Yun *et al.,* 1997; Xue *et al.,* 1998). The Gpr1 protein contains seven hydrophobic potential membrane spanning domains and may function as a a G protein coupled receptor that monitors extracellular conditions such as nutrition (Yun *et al.,* 1997; Xue *et al.,* 1998). Another potential sensor is the *MEP2* ammonium permease which is required for pseudohyphal differentiation in response to ammonium limitation (Lorenz and Heitman, 1998). The membrane bound Mep2 protein might act as an ammonium sensor and generate a signal to regulate filamentous growth in response to ammonium starvation (Lorenz and Heitman, 1998).

A recent report demonstrated that in *S. cerevisiae* Gpa2 is also required for the glucose-stimulated transient increase in intracellular cAMP levels (Colombo *et al.,* 1998). A similar function had been previously observed for Gα proteins in *Schizosaccharomyces pombe* (Isshiki *et al.,* 1992) and *Kluyveromyces lactis* (Saviñon-Tejeda *et al.,* 1996).

Since the entire *S. cerevisiae* genome has been sequenced, it is now clear that yeast has only two different Gα subunits. The fact that at least three different Gα subfamilies have been identified both in other ascomycetes and basidiomycetes indicates that *S. cerevisiae* could have lost some of these genes during evolution.

Candida albicans

The *CAG1* gene from the human fungal pathogen *C. albicans* has been isolated using the *gpa1* gene from *S. cerevisiae* as a probe (Sadhu *et al.,* 1992). *CAG1* encodes a G protein α subunit that is most similar to the *S. cerevisiae* Gpa1 protein (See Fig. 1). Introduction of *CAG1* into *S. cerevisiae* complemented both the growth and the mating defects of *gpa1* null mutants (Sadhu *et al.,* 1992). Interestingly, transcription of the *CAG1* gene was observed only in haploid *S. cerevisiae* cells. Thus, the *C. albicans* gene is subject to control by the yeast a1-α2 mating-type regulation pathway. Therefore it has been inferred that *C. albicans* may have an analogous mating-type controlling system, although haploid cells of *C. albicans* have so far remained undetected (Sadhu *et al.,* 1992). *cag1* heterozygous and homozygous null mutants had no discernible morphological phenotypes when incubated in human serum (Sadhu *et al.,* 1992; Ward *et al.,* 1997). Upon incubation in nutrient-rich medium, however, a major

increase in the level of germ tube formation was observed in the mutant strains (Ward *et al.,* 1997). This may indicate that, analogous to the situation in *S. cerevisiae,* the free βγ dimer might constitutively transmit a morphogenetic signal which is normally induced by human serum (Ward *et al.,* 1997).

Schizosaccharomyces pombe

In sharp contrast to the situation in *S. cerevisiae,* the Gα subunit plays an active role during pheromone signaling in *S. pombe.* Disruption of the *gpa1* gene results in sterility but does not lead to constitutive activation of the pheromone response pathway (Obara *et al.,* 1991). Interestingly, diploid *S. pombe gpa1* mutants are defective in meiosis and sporulation, which indicates that the pheromone signaling is required for sexual development even after cell fusion (Obara *et al.,* 1991). Binding of pheromone to the receptor leads to the activation of a MAP kinase cascade and the transcription factor Ste11 that belongs to the family of HMG box proteins (for review, see Yamamoto, 1996). Genetic analysis revealed that either *ras1* or *gpa1* mutants fail to transduce the pheromone signal, indicating that in *S. pombe* the heterotrimeric G protein acts in concert with the small GTP binding protein Ras1 (Xu *et al.,* 1994).

A second G protein α subunit (Gpa2) in *S. pombe* may be involved in monitoring of the nutritional status (Isshiki *et al.,* 1992). Disruption of the *gpa2* gene leads to a slow growth phenotype, but is not lethal. *gpa2* mutants can mate and sporulate in rich medium by bypassing the requirement for nitrogen starvation. As suggested by its sequence (see Fig. 1), Gpa2 acts most likely as a positive regulator of adenylyl cyclase because in *gpa2* mutant cells the intracellular level of cAMP level is only one third of the wild-type level (Isshiki *et al.,* 1992).

In *S. pombe,* the *gpb1* gene encoding a G-protein β subunit has been isolated and used to generate a disruption mutant (Kim *et al.,* 1996). *gpb1* mutants show precocious mating that is no longer dependent on nitrogen starvation. The G protein β subunit apparently acts as a negative regulator of sexual development. The authors proposed that Gpb1 inhibits pheromone signaling by binding to Gpa1, which acts as a positive activator of mating factor signaling (Kim *et al.,* 1996). However, in this model it is assumed that the Gpa1 protein can signal without being part of a heterotrimeric complex. An alternative explanation for the observed phenotype of *gpb1* mutants is the possibility that Gpb1 is coupled to the nutrient sensing Gα protein Gpa2. *gpa2* mutants can mate on nitrogen-rich media (Isshiki *et al.,* 1992) and thus exhibit the same phenotype as *gpb1* mutants.

Neurospora crassa

The *gna-1* and *gna-2* genes of *N. crassa* were the first Gα encoding genes that were identified in a filamentous fungus (Turner and Borkovich, 1993). Recently, a third gene encoding a Gα subunit (*gna-3*) has been isolated from *N. crassa* (K. Borkovich, pers. commun.). Sequence comparison and the presence of a consensus site for pertussis toxin dependent ADP-ribosylation indicate that Gna-1 belongs to the subfamily of $G\alpha_i$ proteins that act as inhibitors of adenylyl cyclase. Targeted disruption of *gna-1* resulted in multiple phenotypes during vegetative and sexual development (Ivey *et al.,* 1996). *gna-1* mutants exhibit altered colony morphology with slower apical extension and hyperbranching of the mycelium. On solid media, *gna-1* mutants exhibit a significant reduction in the accumulation of dry mass. In liquid media, however, no effect of *gna-1* on mass accumulation was observed (Ivey *et al.,* 1996). This difference could be explained by the colonial phenotype of *gna-1* mutants that might affect nutrient assimilation on solid medium but not in liquid medium.

gna-1 mutants are female sterile, suggesting that Gna-1 might also be involved in pheromone signaling during the directed growth of the female trichogyne toward male micro- or macroconidia. It has been known that diffusible pheromones are involved in the *N. crassa* mating process (Bistis, 1983) but so far no genes encoding these pheromones have been identified. Disruption of the *N. crassa gna-2* gene did not lead to any abnormalities during vegetative and sexual development. Constitutive active mutants of *gna-2* caused only subtle defects in aerial hypha formation and conidial germination (Baasiri *et al.,* 1997). However, *gna-2 gna-1* double mutants have more pronounced defects in female fertility and slower rates of hyphal extension than *gna-1* mutants (Baasiri *et al.,* 1997).

Aspergillus nidulans

A role for G-protein signaling has been implicated during development in the ascomycete *A. nidulans.* A mutant, *flbA,* has been isolated that shows a fluffy phenotype and is blocked in the asexual reproductive pathway prior to the formation of conidiophores. The corresponding gene encodes a protein with high homology to the yeast Sst2 protein that belongs to the RGS family (Lee and

Adams, 1994). RGS proteins enhance the intrinsic GTPase activity of G protein α subunits and thus act as negative regulators of heterotrimeric G proteins (Koelle, 1997). A dominant mutation in a second gene, *fadA*, resulted in a very similar phenotype as observed for the *flbA* mutant (Yu *et al.*, 1996). *FadA* codes for a G protein α subunit, the putative target of negative regulation by FlbA. The mutant FadA protein carries a single amino acid change located in the GTPase domain predicted to result in constitutive signaling of this α subunit. Deletion of *fadA* does not block sporulation but in fact suppresses the phenotype of the *flbA* mutant, indicating that negative regulation of this G protein α subunit by FlbA is required for sporulation (Yu *et al.*, 1996). The authors proposed that some unknown growth factors present during early colony growth interacts with a receptor to activate the FadA signaling pathway. The high degree of sequence similarity between FadA and other fungal $G\alpha_i$ subunits of the subgroup I (Fig. 1) makes it likely that FlbA blocks G protein signaling to increase the internal cAMP level, which is apparently required for conidiophore development. In this respect, it would be interesting to test whether the phenotype of the dominant *fadA* mutation can be suppressed by addition of exogenous cAMP.

In a recent report, the FadA protein was shown to be also involved in the regulation of mycotoxin synthesis (Hicks *et al.*, 1997). *A. nidulans* synthesizes a toxic and carcinogenic secondary metabolite called sterigmatocystin, a precursor of the fungal toxin aflatoxin. Dominant active mutations in *fadA* block sterigmatocystin production, indicating that sterigmatocystin biosynthesis requires inactivation of the FadA-dependent signaling pathway by FlbA (Hicks *et al.*, 1997). Thus in *A. nidulans*, the FadA Gα subunit participates in different signaling pathways.

Ustilago maydis and Ustilago hordei

In the phytopathogenic basidiomycete *U. maydis*, the biallelic *a* mating type locus contains structural genes for farnesylated peptide pheromones and for pheromone receptors that belong to the family of G protein coupled transmembrane receptors (Bölker *et al.*, 1992). Pheromone signaling is required not only for the process of cell recognition during mating but also for the maintenance of filamentous growth of the dikaryon after cell fusion. Upon pheromone stimulation, the expression of specific genes is induced by the transcription factor Prf1 that belongs to the family of HMG box proteins. Prf1 binds to short stretches of DNA, the pheromone response elements, located upstream of pheromone inducible promoters (Hartmann *et al.*, 1996; Urban *et al.*, 1996). The pheromone signal was proposed to be transduced via a MAP kinase cascade because Prf1 contain potential MAP kinase phosphorylation sites whose deletion resulted in loss of function (Hartmann, 1997). To identify the G-protein α subunit that is involved in pheromone signaling, four different genes encoding Gα proteins have been cloned and characterized (Regenfelder *et al.*, 1997). For all four *gpa* genes, targeted gene disruption mutants have been generated and tested for their ability to respond to pheromones (Regenfelder *et al.*, 1997). Disruption of either *gpa1*, *gpa2*, or *gpa4* did not result in any discernible phenotype; mutants defective for *gpa3*, however, were unable to mate. Transcription of pheromone inducible genes is significantly reduced in *gpa3* mutants and cannot be stimulated by the addition of exogenous pheromone (Regenfelder *et al.*, 1997). Introduction of a constitutive active variant Gpa3 into haploid cells leads to an significant increase in the expression of pheromone responsive genes. This was taken as evidence that the Gα subunit encoded by the *gpa3* gene plays an active role during pheromone signaling in *U. maydis* (Regenfelder *et al.*, 1997).

In addition to their mating defect, *gpa3* mutant cells are elongated and form clumps in liquid media. This morphological phenotype is reminiscent to the filamentous phenotype of adenylyl cyclase mutants (Gold *et al.*, 1994) and suggests that Gpa3 can increase the intracellular cAMP level by stimulating adenylyl cyclase. This notion was supported by the fact that the morphological phenotypes associated with disruption of *gpa3* can be suppressed by the addition of exogenous cAMP (Krüger et al., 1998). Thus Gpa3 appears to be also involved in the transmission of nutrient signals that are known to affect the expression levels of pheromone regulated genes (Regenfelder *et al.*, 1997). Two scenarios are possible: Gpa3 may be coupled to two different receptors and activates adenylyl cyclase in response to both nutrient and pheromone stimuli. Alternatively, Gpa3 transmits only the nutrient signal that is required for the transcription of mating type genes and the pheromone receptors are coupled to yet another G alpha subunit that remains to be discovered.

In the related fungus *U. hordei*, a mutant with a similar filamentous phenotype has recently been identified. By complementation, the *fil1* gene encoding a Gα subunit, which is highly related to *U. maydis* Gpa3, was isolated (Lichter and Mills, 1997). In *U. hordei* the phenotype of *fil1* mutants can also be suppressed by the addition of

exogenous cAMP, implicating conserved functions of the Gα subunits in these dimorphic fungi (Lichter and Mills, 1997).

Interestingly, disruption of *gpa3* also affects the virulence of *U. maydis* by an as yet unknown mechanism. Since pheromone signaling is not required for pathogenic development, Gpa3 has been proposed to be involved in other signaling processes that are critical during this phase of its life cycle (Regenfelder *et al.*, 1997). This could include the sensing of specific plant signals that trigger fungal responses required for either tumor induction or fungal growth inside the plant tissue. Quite surprisingly, the constitutive active variant Gpa3 induces aberrant tumor development. Early tumor induction appears to be normal; in later stages of infection, however, galls do not show extensive chlorosis and retain their ability to form shoot-like structures. Within the galls, fungal development is markedly retarded and only very few spores can be detected even after several weeks of tumor development (Regenfelder and Kahmann, unpublished). This phenotype of the constitutive active Gpa3 implicates a role in the recognition of signals that normally in early stages of tumor development prevent spore formation; lack of this signal in later stages induces a developmental switch from proliferative growth to karyogamy and spore formation. As potential signals one could think of nutrient or nitrogen availability that may become restrictive during late stages of pathogenic development.

ROLE OF G PROTEINS FOR FUNGAL PATHOGENESIS

During the recent years, it became apparent that signaling processes are crucial for the ability of fungal pathogens to attack their plant or human hosts. It has long been known that the interaction between parasites and hosts involves complex recognition pattern that regulates virulence and resistance (Baker *et al.*, 1997). Surface proteins or cell wall components can trigger the race specific defense mechanisms that prevent the penetration or the proliferation of the pathogen. On the fungal side, elaborate mechanisms and structures have been developed to overcome physical and chemical barriers and to establish themselves within the host tissue. The cost of energy required for these processes implies that regulatory mechanisms exert a tight control of these developmental decisions to prevent the untimely or constitutive activation of these specific virulence traits. For many fungal pathogens heterotrimeric G proteins have been found to play pivotal roles in these recognition processes.

Cryphonectria parasitica

The devastating chestnut blight that has destroyed most of the North American chestnut trees in the first half of this century is caused by the filamentous ascomycete *C. parasitica.* Hypovirulent strains of *C. parasitica* unable to induce severe symptoms were initially observed in Europe. These strains can transmit their hypovirulent phenotype to other virulent strains. Hypovirulence is caused by a double-stranded RNA virus (hypovirus) that can spread within a population by exchange of cytoplasmic material during hyphal anastomosis. This transmissible hypovirulence caused considerable interest because it offered the chance to use this virus as an effective biological control agent of chestnut blight (for review, see Nuss, 1992). Infection by the hypovirus affects not only virulence but also developmental processes such as asexual sporulation, female fertility, colony morphology, and pigmentation. The expression of many genes is affected in hypovirulent strains, indicating that global regulatory networks that normally control the transcription of these genes might be perturbed (Kazmierczak *et al.*, 1996; Chen *et al.*, 1996). In an attempt to study the function of signaling components for the mechanism of fungal hypovirulence, two genes encoding Gα subunits (*cpg-1* and *cpg-2*) have been cloned and characterized in *C. parasitica* (Choi *et al.*, 1995). Using polyclonal antibodies directed against a peptide derived from the C-terminus of the CPG-1 protein, it was observed that accumulation of CPG-1 was significantly reduced in hypovirulent strains of *C. parasitica* (Choi *et al.*, 1995). To test whether G protein signaling is required for virulence, null mutants have been generated by targeted gene disruption for both *cpg-1* and *cpg-2* genes. Whereas loss of *cpg-2* results in only a slight reduction in growth rate and asexual sporulation, *cpg-1* mutants were severely attenuated in virulence and showed multiple morphological phenotypes that were even more severe than that of virus infected strains (Gao and Nuss, 1996). In *cpg-1* deficient strains, elevated levels of cAMP were detected, indicating a role for CPG-1 in inhibiting adenylyl cyclase activity. This is supported by the fact that CPG-1 belongs to the fungal subfamily of $G\alpha_i$ proteins (Fig. 1).

Interestingly, G protein signaling might also be involved in mating processes since virus infected strains are female sterile. Furthermore, in a screen for genes that are specifically suppressed in hypovirulent strains, genes encoding precursors of secreted lipopeptide pheromones have

been identified (Kazmierczak *et al.,* 1996; Zhang *et al.,* 1998). Expression of these mating factor genes occurs only in Mat-2 strains and thus these genes have been termed *Mf2/1* and *Mf2/2,* respectively. The Mat-1 specific pheromone precursor gene *Mf1* was cloned and its expression was also shown to be suppressed in hypovirulent strains (Zhang *et al.,* 1998). To study the possible role of G proteins in this regulation, Zhang *et al.* (1998) used the susceptibility of $G\alpha_i$ subunits to ADP-ribosylation (Turner and Borkovich, 1993) to determine the relative levels of G protein accumulation in virus free and virus infected strains. They detected a single membrane protein that could be labeled by this method and surprisingly the intensity of this band was not altered in virus infected strains (Zhang *et al.,* 1998). If this protein correlates to the $G\alpha_i$ protein CPG-1 this result would be in disagreement with the observation that the level of CPG-1 expression is severely reduced in hypovirulent strains (Choi *et al.,* 1995). To clarify this, mutant strains deleted for *cpg-1* should be tested as to whether they contain the 41-kDa band susceptible to ADP-ribosylation by pertussis toxin.

C. parasitica is the first filamentous fungus in which also the gene for a β subunit of heterotrimeric G proteins has been cloned and characterized (Kasahara and Nuss, 1997). The deduced amino acid sequence of the *cpgb-1* gene shows a high degree of sequence similarity to other eukaryotic Gβ subunits. Remarkably, the CPGB-1 protein is significantly more similar to mammalian Gβ subunits than to the yeast Gβ protein level Ste4 (Kasahara and Nuss, 1997). Disruption of the *cpgb-1* gene resulted in an increased vegetative growth phenotype rather than a reduced growth as observed for *cpg-1* (Gα) mutants (Kasahara and Nuss, 1997). This is reminiscent of the enhanced mating and sporulation phenotype of *S. pombe* Gβ mutant strains (Kim *et al.,* 1996), implying that the *C. parasitica* Gβγ may also act as a negative regulator of Gα function. In this respect, it would be interesting to determine whether introduction of a constitutive active Gα subunit can result in a similar phenotype.

Magnaporthe grisea

Magnaporthe grisea causes rice blast disease, which is a major limiting factor in rice production. The infection process of *M. grisea* starts with germination of asexual spores on the surface of grass leaves and the formation of a highly specialized structure, the appressorium. This dome-shaped highly melanized fungal cell generates an enormous turgor pressure that allows the direct penetration of the plant cell cuticle. The induction of this morphogenetic process is dependent on the sensing of both chemical and physical cues (Bourett and Howard, 1990; Gilbert *et al.,* 1996; Lee and Dean, 1994). *M. grisea* efficiently forms appressoria on artificial hydrophobic surfaces such as polymer sheets. cAMP dependent processes have been implicated in the transduction of these signals since addition of cAMP induces appressorium formation also on hydrophilic surfaces (Lee and Dean, 1993). Appressorium formation can also be induced by 3-isobutyryl-1-methylxanthine (IBMX) which blocks phosphodiesterase and thus increases the internal cAMP level. The catalytic subunit of cAMP dependent protein kinase was cloned and targeted disruption of this gene (*CPKA*) was reported to result in failing to form appressorium (Mitchell and Dean, 1995). Recent experiments, however, demonstrated that the CPKA gene is dispensable for appressorium formation but may be essential for appressorial penetration (Xu *et al.,* 1997). Appressorium formation is delayed for a few hours, which most likely accounts for the difference between these findings, because Mitchell and Dean (1995) examined appressorium formation only up to 8 or 9 h. *cpka* mutants are still responsive to addition of cAMP, suggesting the presence of additional cAMP-dependent protein kinases in *M. grisea* (Xu *et al.,* 1997). Recently, a mitogen activated protein (MAP) kinase signaling cascade required for appressorium formation was identified by Xu and Hamer (1996). It was found that disruption of the MAP-kinase gene (PMK1) inhibits appressorium formation and results in loss of pathogenicity.

To study the role of G proteins in these signal transduction pathways in *M. grisea,* three genes (*magA, magB,* and *magC*) encoding α subunits of heterotrimeric G proteins have been cloned and characterized (Liu and Dean, 1997). The phenotypes of targeted disruption mutants were examined to determine the biological functions of these genes. Whereas the deletion of *magA* and *magC* did not affect either vegetative growth or appressorium formation, disruption of *magB* resulted in significant reduction of vegetative growth, conidiation, and appressorium formation (Liu and Dean, 1997). Interestingly, appressorium formation can be still induced in *magB* mutants by hexadecanediol. This indicates that different signaling pathways, some of which are independent of G-protein signaling, interact in the regulation of appressorium formation in *M. grisea.* Although MagB appears to be a member of the $G\alpha_i$ class, the defect in appressorium formation could be restored by the addition of exogenous cAMP (Liu and Dean, 1997). This was quite surprising since deletion of an inhibitory Gα gene would normally be expected to

result in an increase of the internal cAMP level. However, there is evidence that both activated $G\alpha_i$ and $\beta\gamma$ dimer can act as inhibitors of adenylyl cyclase (Taussig *et al.*, 1993). This would suggest that constitutive inhibition of adenylyl cyclase by the free $\beta\gamma$ dimer occurs in *M. grisea magB* mutants due to the absence of the corresponding $G\alpha_i$ subunit.

Interestingly, it was recently reported that yeast α factor can inhibit appressorium formation in *M. grisea* MAT1-2 strains (Beckerman *et al.*, 1997). The same inhibition activity was also detected in yeast extract and *M. grisea* culture supernatants. The activity of *M. grisea* culture supernatants was mating type specific, making it likely that inhibition of appressorium formation is induced by the binding of secreted pheromones to their cognate receptors (Beckerman *et al.*, 1997). This apparent cross-talk between pheromone signaling and regulation of pathogenicity orchestrates the intricate interplay between different signaling pathways in filamentous fungi.

Cryptococcus neoformans

C. neoformans is a major opportunistic fungal pathogen of immunocompromised patients. It has been found that the virulence of this species depends on its mating type with MATα strains being more virulent than MAT**a** strains (Kwon-Chung *et al.*, 1992). Within a large DNA region that is specific for the α mating type, the gene encoding the potential precursor for a farnesylated lipopeptid pheromone of the yeast **a**-factor class has been identified (Moore and Edman, 1993). Clinical and environmental isolates are predominantly of α mating type. This biased mating type ratio has been attributed to the higher virulence of MATα strains and the ability of MATα cells to produce basidiospores by haploid fruiting (Wickes *et al.*, 1996). The high resistance of basidiosphores to desiccation and the easier penetration of the lung parenchyma by the small spores ($<$2 μm) may explain the preponderance of the α mating type in the environment (Wickes *et al.*, 1996). To study the involvement of signaling molecules for the virulence of *C. neoformans*, a gene encoding a G-protein α subunit has been cloned (Tolkacheva *et al.*, 1994). Its amino acid sequence is most closely related to the Fil1 and Gpa3 proteins of *U. hordei* and *U. maydis*, respectively, and belongs to the subfamily of fungal $G\alpha_s$ subunits (Fig. 1). The *gpa1* gene has been disrupted by homologous recombination and mutants exhibited a mating defect in response to nitrogen starvation (Alspaugh *et al.*, 1997). Interestingly, the mutants were also unable to induce melanin synthesis and capsule production in response to glucose starvation and iron deprivation, respectively. Accordingly, *gpa1* mutants are significantly attenuated in virulence in an animal model (Alspaugh *et al.*, 1997). The authors propose that Gpa1 has a role in sensing environmental signals that are required for mating and virulence. The observed defects in mating and production of virulence factors can be restored by the addition of exogenous cAMP, indicating that Gpa1 in its active conformation indeed stimulates adenylyl cyclase (Alspaugh *et al.*, 1997). Interestingly, restoration of these responses by cAMP did not alleviate the need for the specific signals (nitrogen starvation or iron deprivation). This indicates that additional signaling pathways independent of cAMP signaling are involved in this regulation. As a possible mechanism one could imagine that in addition to the $G\alpha$ subunit, the $\beta\gamma$ dimer also participates in signaling, e.g., by activating a MAP kinase cascade that, together with the elevated cAMP level, causes the specificity of the response.

FUTURE PERSPECTIVES

The past years saw much progress in the study of fungal G proteins, but many questions remain. For many of these signal transducers, the specific signaling pathways in which they participate are still unknown. In particular, only very few receptors that are coupled to G proteins have been identified in fungi. On the other hand, only some potential effectors of G proteins such as adenylyl cyclase and MAP kinase cascades have been implicated in G protein signaling. The advantage of fungal systems for studying G protein function lies in the ease with which mutants can be generated in many fungal species. This will help to identify some of the missing components by a thorough genetic and biochemical analysis.

One of the major unsolved problems in G protein signaling is how the specificity of the response is reached. For some fungal $G\alpha$ subunits, it has been demonstrated that they are involved in different signaling pathways. The responses are often, however, specific for the signal that has elicited the signaling pathway. There are some possible explanations of how this specificity can be reached. It could be either the activated receptor or the free $\beta\gamma$ dimer that also participates in signaling. The specific reaction could be the result of combining a more general pathway like cAMP signaling with a more specific response, e.g., activation of a MAP kinase cascade. Another possibility could involve the compartmentalization of signaling pathways in the cytoplasm. The specificity of the response

would then be reached by coupling the receptor not only to a specific G protein but to a complete signaling pathway.

A common theme from the collected data for many fungal species is the tight connection between G protein signaling and the regulation of intracellular cAMP levels. Although biochemical evidence is still lacking it appears likely that in fungi a similar positive and negative regulation of adenylyl cyclase by heterotrimeric G proteins exist as in vertebrates. Based on sequence similarity fungal Gα subunits that are presumed to either stimulate or inhibit adenylyl cyclase show more similarity to each other than to other Gα subunits of the same species. This indicates that this functional dichotomy must have evolved before the different fungal families had been separated.

Remarkably, analogous functions such as the pheromone response have been realized among quite different types of G protein signaling. The pheromone signal during mating is transduced in *S. cerevisiae* by the free βγ dimer, in *S. pombe* by a Gα subunit in concert with a Ras protein, and in *U. maydis* by a stimulatory Gα subunit that activates cAMP production.

A major challenge of future research will be the elucidation of specificity of G protein signaling and the cross-talk with other signaling pathways.

ACKNOWLEDGMENTS

I thank Regine Kahmann, Katherine A. Borkovich, Erika Regenfelder, and Julia Krüger for helpful discussions and for communication of results prior to publication. I am grateful to Reinhard Fischer, Christiane Lehmler, and three anonymous reviewers for their valuable suggestions. The work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

Alspaugh, J. A., Perfect, J. R., and Heitman, J. 1997. *Cryptococcus neoformans* mating and virulence are regulated by the G- protein alpha subunit GPA1 and cAMP. *Genes Dev.* **11:** 3206–3217.

Baasiri, R. A., Lu, X., Rowley, P. S., Turner, G. E., and Borkovich, K. A. 1997. Overlapping functions for two G protein alpha subunits in *Neurospora crassa. Genetics* **147:** 137–145.

Baker, B., Zambryski, P., Staskawicz, B., and Dinesh-Kumar, S. P. 1997. Signaling in plant-microbe interactions. *Science* **276:** 726–733.

Beckerman, J. L., Naider, F., and Ebbole, D. J. 1997. Inhibition of pathogenicity of the rice blast fungus by *Saccharomyces cerevisiae* α-factor. *Science* **276:** 1116–1119.

Bistis, G. N. 1983. Evidence for diffusible, mating-type-specific trichogyne attractants in Neurospora crassa. *Exp. Mycol.* **7:** 292–295.

Bölker, M., Urban, M., and Kahmann, R. 1992. The α mating type locus of *U. maydis* specifies cell signaling components. *Cell* **68:** 441–450.

Borkovich, K. A. 1996. Signal Transduction Pathways and Heterotrimeric G Proteins. In *The Mycota* (R. Brambl and G. A. Marzluf, Eds.), Vol. 3, pp. 211–233. Springer-Verlag, Berlin Heidelberg.

Bourett, T. M., and Howard, R. J. 1990. In vivo development of penetration structures in the rice blast fungus, *Magnaporthe grisea. Can. J. Bot.* **68:** 329–342.

Chen, B., Gao, S., Choi, G. H., and Nuss, D. L. 1996. Extensive alteration of fungal gene transcript accumulation and elevation of G-protein-regulated cAMP levels by a virulence-attenuating hypovirus. *Proc. Natl. Acad. Sci. USA* **93:** 7996–8000.

Choi, G. H., Chen, B., and Nuss, D. L. 1995. Virus-mediated or transgenic suppression of a G-protein alpha subunit and attenuation of fungal virulence. *Proc. Natl. Acad. Sci. USA* **92:** 305–309.

Clapham, D. E., and Neer, E. J. 1993. New roles for G-protein beta gamma-dimers in transmembrane signalling. *Nature* **365:** 403–406.

Colombo, S., Ma, P., Cauwenberg, L., Winderickx, J., Crauwels, M., Teunissen, A., Nauwelaers, D., de Winde, J. H., Gorwa, M.-F., Colavizza, D., and Thevelein, J. M. 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.

Dietzel, C., and Kurjan, J. 1987. The yeast *SCG1* gene: A G alpha-like protein implicated in the **a**- and α-factor response pathway. *Cell* **50:** 1001–1010.

Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. 1991. Model systems for the study of seven-transmembrane-segment receptors. *Annu. Rev. Biochem.* **60:** 653–688.

Gao, S., and Nuss, D. L. 1996. Distinct roles for two G protein alpha subunits in fungal virulence, morphology and reproduction revealed by targeted gene disruption. *Proc. Natl. Acad. Sci. USA* **93:** 14122–14127.

Gilbert, R. D., Johnson, A. M., and Dean, R. A. 1996. Chemical signals responsible for appressorium formation in the rice blast fungus *Magnaporthe grisea. Physiol. Mol. Plant Pathol.* **48:** 335–346.

Gilman, A. G. 1987. G proteins: Transducers of receptor-generated signals. *Annu. Rev. Biochem.* **56:** 615–649.

Gold, S., Duncan, G., Barrett, K., and Kronstad, J. 1994. cAMP regulates morphogenesis in the fungal pathogen *Ustilago maydis. Genes Dev.* **8:** 2805–2816.

Hamm, H. E., and Gilchrist, A. 1996. Heterotrimeric G proteins. *Curr. Opin. Cell Biol.* **8:** 189–196.

Hartmann, H. A. 1997. *Die Pheromonantwort in Ustilago maydis: Kontrolle von Zellfusion und pathogenität.* Thesis, Univ. of Munich.

Hartmann, H. A., Kahmann, R., and Bölker, M. 1996. The pheromone response factor coordinates filamentous growth and pathogenicity in *Ustilago maydis. EMBO J.* **15:** 1632–1641.

Herskowitz, I. 1995. MAP kinase pathways in yeast: For mating and more. *Cell* **80:** 187–197.

Hicks, J. K., Yu, J. H., Keller, N. P., and Adams, T. H. 1997. *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA G alpha protein-dependent signaling pathway. *EMBO J.* **16:** 4916–4923.

Isshiki, T., Mochizuki, N., Maeda, T., and Yamamoto, M. 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.

Ivey, F. D., Hodge, P. N., Turner, G. E., and Borkovich, K. A. 1996. The G alpha i homologue gna-1 controls multiple differentiation pathways in *Neurospora crassa. Mol. Biol. Cell* **7:** 1283–1297.

Kasahara, S., and Nuss, D. L. 1997. Targeted disruption of a fungal G-protein beta subunit gene results in increased vegetative growth but reduced virulence. *Mol. Plant Microbe Interact.* **10:** 984–993.

Kazmierczak, P., Pfeiffer, P., Zhang, L., and Van Alfen, N. K. 1996. Transcriptional repression of specific host genes by the mycovirus *Cryphonectria* hypovirus 1. *J. Virol.* **70:** 1137–1142.

Kim, D. U., Park, S. K., Chung, K. S., Choi, M. U., and Yoo, H. S. 1996. The G protein beta subunit Gpb1 of *Schizosaccharomyces pombe* is a negative regulator of sexual development. *Mol. Gen. Genet.* **252:** 20–32.

Koelle, M. R. 1997. A new family of G-protein regulators—The RGS proteins. *Curr. Opin. Cell Biol.* **9:** 143–147.

Kozak, K. R., Foster, L. M., and Ross, I. K. 1995. Cloning and characterization of a G protein alpha-subunit-encoding gene from the basidiomycete, *Coprinus congregatus. Gene* **163:** 133–137.

Kronstad, J. W. 1997. Virulence and cAMP in smuts, blasts and blights. *Trends Plant Sci.* **2:** 193–199.

Krüger, J., Loubradou, G., Regenfelder, E., Hartmann, H. A., and Kahmann, R. 1998. Crosstalk between cAMP and pheromone signalling in *Ustilago maydis. Mol. Gen. Genet.* **260:** 193–198.

Kübler, E., Mosch, H. U., Rupp, S., and Lisanti, M. P. 1997. Gpa2p, a G-protein alpha-subunit, regulates growth and pseudohyphal development in *Saccharomyces cerevisiae* via a cAMP-dependent mechanism. *J. Biol. Chem.* **272:** 20321–20323.

Kurjan, J. 1993. The pheromone response pathway in *Saccharomyces cerevisiae. Annu. Rev. Genet.* **27:** 147–179.

Kurjan, J., Hirsch, J. P., and Dietzel, C. 1991. Mutations in the guanine nucleotide-binding domains of a yeast G alpha protein confer a constitutive or uninducible state to the pheromone response pathway. *Genes Dev.* **5:** 475–483.

Kwon-Chung, K. J., Edman, J. C., and Wickes, B. L. 1992. Genetic association of mating types and virulence in *Cryptococcus neoformans. Infect. Immunol.* **60:** 602–605.

Leberer, E., Thomas, D. Y., and Whiteway, M. 1997. Pheromone signalling and polarized morphogenesis in yeast. *Curr. Opin. Genet. Dev.* **7:** 59–66.

Lee, B. N., and Adams, T. H. 1994. Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation, leads to activation of *brlA* and premature initiation of development. *Mol. Microbiol.* **14:** 323–334.

Lee, Y.-H., and Dean, R. A. 1993. cAMP regulates infection structure formation in the plant pathogenic fungus *Magnaporthe grisea. Plant Cell* **5:** 693–700.

Lee, Y.-H., and Dean, R. A. 1994. Hydrophobicity of contact surface induces appressorium formation of *Magnaporthe grisea. FEMS Microbiol. Lett.* **115:** 71–76.

Lichter, A., and Mills, D. 1997. Fil1, a G-protein alpha-subunit that acts upstream of cAMP and is essential for dimorphic switching in haploid cells of *Ustilago hordei. Mol. Gen. Genet.* **256:** 426–435.

Liu, H., Styles, C. A., and Fink, G. R. 1993. Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* **262:** 1741–1744.

Liu, S., and Dean, R. A. 1997. G protein alpha subunit genes control growth, development, and pathogenicity of *Magnaporthe grisea. Mol. Plant Microbe Interact.* **10:** 1075–1086.

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

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

Mitchell, T. K., and Dean, R. A. 1995. The cAMP-dependent protein kinase catalytic subunit is required for appressorium formation and pathogenesis by the rice blast pathogen *Magnaporthe grisea. Plant Cell* **7:** 1869–1878.

Miyajima, I., Nakafuku, M., Nakayama, N., Brenner, C., Miyajima, A., Kaibuchi, K., Arai, K., Kaziro, Y., and Matsumoto, K. 1987. *GPA1*, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. *Cell* **50:** 1011–1019.

Moore, T. D., and Edman, J. C. 1993. The alpha-mating type locus of *Cryptococcus neoformans* contains a peptide pheromone gene. *Mol. Cell. Biol.* **13:** 1962–1970.

Nakafuku, M., Itoh, H., Nakamura, S., and Kaziro, Y. 1987. Occurrence in *Saccharomyces cerevisiae* of a gene homologous to the cDNA coding for the alpha subunit of mammalian G proteins. *Proc. Natl. Acad. Sci. USA* **84:** 2140–2144.

Nakafuku, M., Obara, T., Kaibuchi, K., Miyajima, I., Miyajima, A., Itoh, H., Nakamura, S., Arai, K., Matsumoto, K., and Kaziro, Y. 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.

Neiman, A. M., Stevenson, B. J., Xu, H. P., Sprague, G. F., Jr., Herskowitz, I., Wigler, M., and Marcus, S. 1993. Functional homology of protein kinases required for sexual differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* suggests a conserved signal transduction module in eukaryotic organisms. *Mol. Biol. Cell* **4:** 107–120.

Nuss, D. L. 1992. Biological control of chestnut blight: An example of virus-mediated attenuation of fungal pathogenesis. *Microbiol. Rev.* **56:** 561–576.

Obara, T., Nakafuku, M., Yamamoto, M., and Kaziro, Y. 1991. Isolation and characterization of a gene encoding a G-protein alpha subunit from *Schizosaccharomyces pombe:* Involvement in mating and sporulation pathways. *Proc. Natl. Acad. Sci. USA* **88:** 5877–5881.

Regenfelder, E., Spellig, T., Hartmann, A., Lauenstein, S., Bölker, M., and Kahmann, R. 1997. G proteins in *Ustilago maydis:* transmission of multiple signals? *EMBO J.* **16:** 1934–1942.

Rodbell, M., Birnbaumer, L., Pohl, S. L., and Krans, H. M. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. V. An obligatory role of guanylnucleotides in glucagon action. *J. Biol. Chem.* **246:** 1877–1882.

Sadhu, C., Hoekstra, D., McEachern, M. J., Reed, S. I., and Hicks, J. B. 1992. A G-protein alpha subunit from asexual *Candida albicans* functions in the mating signal transduction pathway of *Saccharomyces cerevisiae* and is regulated by the a1-α2 repressor. *Mol. Cell. Biol.* **12:** 1977–1985.

Saviñon-Tejeda, A. L., Ongay-Larios, L., Ramirez, J., and Coria, R. 1996.

Isolation of a gene encoding a G protein alpha subunit involved in the regulation of cAMP levels in the yeast *Kluyveromyces lactis. Yeast* **12:** 1125–33.

Simon, M. I., Strathmann, M. P., and Gautam, N. 1991. Diversity of G proteins in signal transduction. *Science* **252:** 802–808.

Smulian, A. G., Ryan, M., Staben, C., and Cushion, M. 1996. Signal transduction in *Pneumocystis carinii:* Characterization of the genes (*pcg1*) encoding the alpha subunit of the G protein (PCG1) of *Pneumocystis carinii carinii* and *Pneumocystis carinii ratti. Infect. Immun.* **64:** 691–701.

Taussig, R., Iniguez-Lluhi, J. A., and Gilman, A. G. 1993. Inhibition of adenylyl cyclase by Gi alpha. *Science* **261:** 218–221.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. 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.

Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. 1985. In yeast, RAS proteins are controlling elements of adenylate cyclase. *Cell* **40:** 27–36.

Tolkacheva, T., McNamara, P., Piekarz, E., and Courchesne, W. 1994. Cloning of a *Cryptococcus neoformans* gene, *GPA1*, encoding a G-protein alpha-subunit homolog. *Infect. Immunol.* **62:** 2849–2856.

Turner, G. E., and Borkovich, K. A. 1993. Identification of a G protein alpha subunit from *Neurospora crassa* that is a member of the Gi family. *J. Biol. Chem.* **268:** 14805–14811.

Urban, M., Kahmann, R., and Bölker, M. 1996. Identification of the pheromone response element in *Ustilago maydis. Mol. Gen. Genet.* **251:** 31–37.

Ward, S. J., Kinsman, O. S., and Adams, D. J. 1997. Evidence of a role for a guanine nucleotide binding protein during growth and morphogenesis in *Candida albicans. Biochem. Soc. Trans.* **25:** 448S.

Wickes, B. L., Mayorga, M. E., Edman, U., and Edman, J. C. 1996. Dimorphism and haploid fruiting in *Cryptococcus neoformans:* Association with the alpha-mating type. *Proc. Natl. Acad. Sci. USA* **93:** 7327–7331.

Xu, H. P., White, M., Marcus, S., and Wigler, M. 1994. Concerted action of RAS and G proteins in the sexual response pathways of *Schizosaccharomyces pombe. Mol. Cell. Biol.* **14:** 50–58.

Xu, J. R., and Hamer, J. E. 1996. MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea. Genes Dev.* **10:** 2696–2706.

Xu, J. R., Urban, M., Sweigard, J. A., and Hamer, J. E. 1997. The CPKA gene of *Magnaporthe grisea* is essential for appressorial penetration. *Mol. Plant Microbe Interact.* **10:** 187–194.

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

Yamamoto, M. 1996. The molecular control mechanisms of meiosis in fission yeast. *Trends Biochem. Sci.* **21:** 18–22.

Yu, J. H., Wieser, J., and Adams, T. H. 1996. The *Aspergillus* FlbA RGS domain protein antagonizes G protein signaling to block proliferation and allow development. *EMBO J.* **15:** 5184–5190.

Yun, C. W., Tamaki, H., Nakayama, R., Yamamoto, K., and Kumagai, H. 1997. G-protein coupled receptor from yeast *Saccharomyces cerevisiae. Biochem. Biophys. Res. Commun.* **240:** 287–292.

Zhang, L., Baasiri, R. A., and Van Alfen, N. K. 1998. Viral expression of fungal pheromone precursor gene expression. *Mol. Cell. Biol.* **18:** 953–959.