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1998

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Kronstad, James; De Maria, Adriana; Funnell, Deanna L.; Laidlaw, R. David; Lee, Nancy; Moniz de Sá, Mário; and Ramesh, Marilee, "Signaling via cAMP in Fungi: Interconnections with Mitogen-Activated Protein Kinase Pathways" (1998). *Papers in Plant Pathology*. 211.
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Submitted June 5, 1998; accepted September 11, 1998.

MINI-REVIEW

Signaling via cAMP in Fungi: Interconnections with Mitogen-Activated Protein Kinase Pathways

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Abstract

The cAMP signal transduction pathway controls a wide variety of processes in fungi. For example, considerable progress has been made in describing the involvement of cAMP pathway components in the control of morphogenesis in *Saccharomyces cerevisiae*, *Ustilago maydis*, and *Magnaporthe grisea*. These morphological processes include the establishment of filamentous growth in *S. cerevisiae* and *U. maydis*, and the differentiation of an appressorial infection structure in *M. grisea*. The discovery that appressorium formation requires cAMP signaling provides an immediate connection to fungal virulence. This connection may have broader implications among fungal pathogens because recent work indicates that cAMP signaling controls the expression of virulence traits in the human pathogen *Cryptococcus neoformans*. In this fungus, cAMP also influences mating, as has been found for *Schizosaccharomyces pombe* and as may occur in *U. maydis*. Finally, cAMP and mitogen-activated protein kinase pathways appear to function coordinately to control the response of certain fungi, e.g., *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, to environmental stress. There are clues that interconnections between these pathways may be common in the control of many fungal processes.

Keywords: Signal transduction, Fungi, Stress, Virulence, Sexual development, Mating, Appressorium and Dimorphism

Abbreviations: *PKA* Protein kinase A (cAMP-dependent protein kinase), *MAPK* Mitogen-activated protein kinase, *STRE* Stress response element and *HMG* High-mobility-group protein

Fungi employ cAMP signaling in a variety of processes including the control of differentiation, sexual development, and virulence in addition to the monitoring of nutritional status and stress. Furthermore, the cAMP pathway influences transcription and cell cycle progression. New insights into the role of cAMP in morphogenesis and stress are emerging in the well-characterized yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and recent forays into other fungi including pathogens of plants and animals have provided revelations about cAMP signaling in development and virulence. It is clear that we are approaching an understanding of the environmental signals that influence the activity of cAMP-dependent protein kinase (PKA) via upstream components such as receptors, G-proteins and adenylyl cyclase. In addition, we are beginning to collect factors that function downstream of PKA. A theme that has emerged from the analysis of signaling in fungi is that interactions frequently exist between cAMP signaling and mitogen-activated protein kinase (MAPK) pathways involved in mating, morphogenesis, virulence, and stress response. This review will briefly highlight recent experiments in selected fungi to provide insights into the function of cAMP and, where applicable, to illustrate connections with MAPK signaling. Figure 1 presents an overview of signals and processes that are known to be controlled by cAMP signaling in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Ustilago maydis*. Known connections with MAPK cascades are also indicated. For reference, Table 1 provides a list of the fungal genes that encode the signal transduction components that are discussed in this review.

Saccharomyces cerevisiae

In *S. cerevisiae*, an increase in the activity of PKA correlates with sensitivity to environmental stress, growth defects on carbon sources other than glucose, the loss of carbohydrate reserves, a transient arrest in G1, a block in sporulation, and enhanced pseudohyphal growth (also referred to here as filamentous growth). Activation

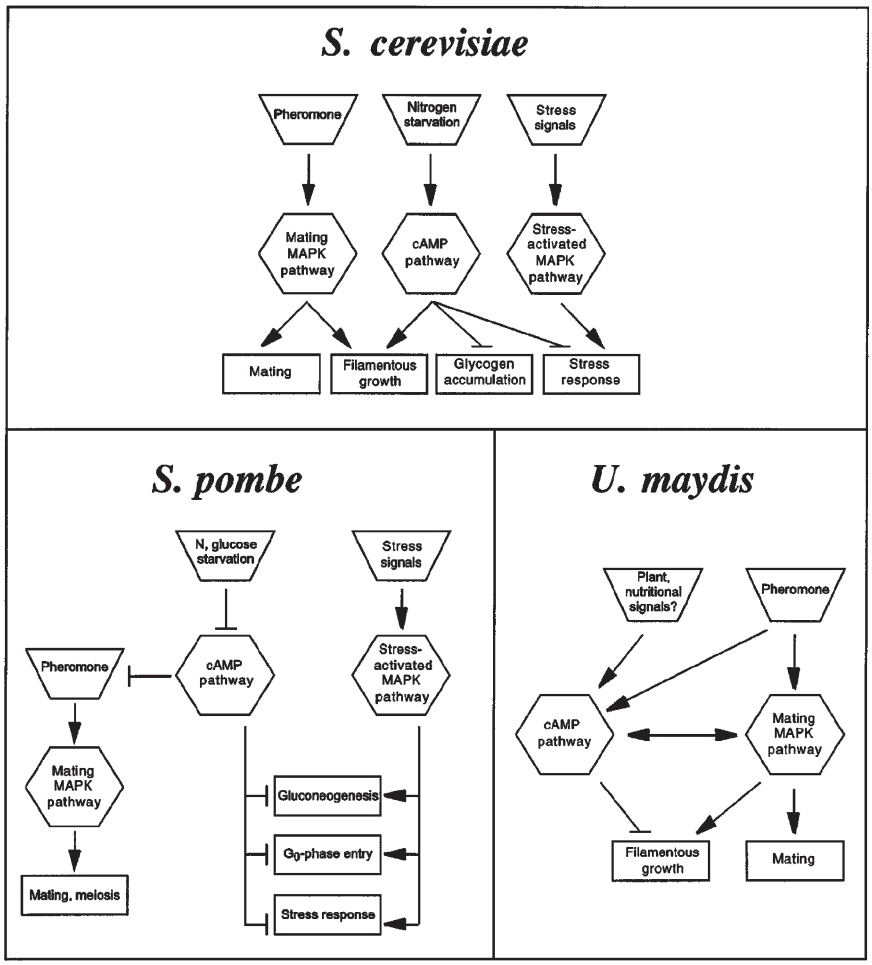

Figure 1. Examples of processes controlled by cAMP and mitogen-activated protein kinase (MAPK) signaling pathways in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Ustilago maydis*. The signals that impinge on cAMP and MAPK signaling pathways are shown at the top of each diagram. These signals represent examples of the best characterized environmental influences that feed into the signaling pathways in the three fungal species. Note that some signals, such as the proposed plant signals that may influence *U. maydis* morphogenesis, have yet to be demonstrated. The diagrams are intended to provide an overview of the processes influenced by cAMP and MAPK signaling pathways and to reinforce the idea that these pathways coordinately control many of the same processes. The involvement of a MAPK pathway in the control of mating and filamentous growth in *U. maydis* has yet to be described in detail

of PKA can be achieved by introduction of the dominant $RAS2^{Val19}$ mutation or a null mutation in the *BCY1* gene, which encodes the regulatory subunit of the enzyme. The analysis of filamentous growth provides a useful example that illustrates recent discoveries about cAMP signaling and connections with MAPK cascades. Phenotypically, filamentous growth in diploid strains is characterized by synchronous unipolar budding, incomplete cell separation, cell elongation, and invasive growth [reviewed by Kron (1997)]. A filamentous growth morphology also occurs in haploid strains in response to nutrient limitation. In both diploid and haploid cells, members of the pheromone-response MAPK cascade, namely Ste20p, Ste11p, Ste7p, Kss1p and Ste12p, regulate filamentous development [reviewed by Madhani and Fink (1998)].

Two G-proteins, Ras2p and Gpa2p, are thought to influence cAMP levels via regulation of adenylyl cyclase in *S. cerevisiae*. Mutants defective in either gene alone do not show growth defects, but double mutants exhibit a very slow growth phenotype on rich medium (Kübler et al. 1997; Xue et al. 1998). This defect can be suppressed by exogenous cAMP or mutations in the *PDE2* gene encoding cAMP phosphodiesterase (Kübler et al. 1997; Lorenz and Heitman 1997; Xue et al. 1998).

The Ras2p and Gpa2p proteins are both required for filamentous growth in *S. cerevisiae*. Signaling through Ras2p is thought to activate both the cAMP and the MAPK pathways to influence filamentous growth (Ward et al. 1995; Mösch et al. 1996). The connection with the cAMP pathway was initially suggested by the finding of Gimeno et al. (1992) that transformation with the dominant activated $RAS2^{Val19}$ allele results in strains with enhanced filamentous growth. Further evidence for a connection came from the finding that overproduction of the cAMP phosphodiesterase Pde2p suppresses both the filamentous phenotype of wild-type strains and the enhanced pseudohyphal growth of strains expressing the dominant activated $RAS2^{Val19}$ allele (Ward et al. 1995). The activation of the MAPK pathway by Ras2p occurs via the GT-Pase Cdc42p and a complex consisting of the Ste20p protein kinase and the 14–3–3 proteins Bmh1p and Bmh2p (Roberts et al. 1997). Bmh1p and Bmh2p are essential for pseudohyphal development and may also be involved in signal transduction by the *RAS2*/cAMP pathway. Mutants with defects in both *BMH1* and *BMH2* are nonviable at 34°C, are defective in sporulation, and accumulate abnormally high levels of glycogen. Introduction of the $RAS2^{Val19}$ allele or overproduction of a catalytic subunit of PKA (encoded by *TPK1*) suppresses glycogen hyperaccumulation by the *bmh1bmh2* strains (Roberts et al. 1997). *RAS2* is known to regulate glycogen accumulation through cAMP and PKA.

Table 1. Genes involved in cAMP and MAPK signaling pathways in fungi

Fungal species	Gene	Gene product
Saccharomyces cerevisiae	*CYR1*	Adenylyl cyclase
	BCY1	Protein kinase A – regulatory subunit
	TPK1	Protein kinase A – catalytic subunit
	BMH1, BMH2	14-3-3 proteins
	RAS2	GTP binding protein
	GPA2	G-protein α-subunit
	GPR1	Gpa2p-coupled receptor
	MEP2	High-affinity ammonium permease
	PDE2	cAMP phosphodiesterase
	ELM1	Protein kinase
	STE20	Protein kinase
	STE11	MAPKKK
	STE7	MAPKK
	KSS1	MAPK
Schizosaccharomyces pombe	*cyr1*	Adenylyl cyclase
	pka1	Protein kinase A – catalytic subunit
	gpa1, gpa2	G-protein α-subunits
	ras1	GTP binding protein
	win1, wis4	MAPKKKs
	wis1	MAPKK
	sty1/spc1/phh1	MAPK
	pyp1, pyp2	Protein tyrosine phosphatases
Ustilago maydis	*uac1*	Adenylyl cyclase
	ubc1	Protein kinase A – regulatory subunit
	adr1	Protein kinase A – catalytic subunit
	uka1	Protein kinase A – catalytic subunit
	gpa3	G-protein α-subunit
	fuz7	MAPKK
Magnaporthe grisea	*MAC1*	Adenylyl cyclase
	CPKA	Protein kinase A – catalytic subunit
	MAGB	G-protein α-subunit
	PMK1	MAPK
Cryptococcus neoformans	*CAC1*	Adenylyl cyclase
	GPA1	G-protein α-subunit

The G-protein α-subunit Gpa2p appears to act coordinately with Ras2p to stimulate filamentous development via the cAMP pathway. Diploid *gpa2/gpa2ras2/ras2* mutants show additive defects in pseudohyphal growth as compared to mutants defective in either gene alone (Kübler et al. 1997). In addition, a dominant active *GPA2* allele enhances pseudohyphal differentiation (Lorenz and Heitman 1997). Mutants that are defective in Gpa2p are more severely compromised for filamentous growth than are mutants defective in Ras2p, although the addition of exogenous cAMP suppresses the defects in filament formation for both types of mutants (Kübler et al. 1997; Lorenz and Heitman 1997). In contrast to the Ras2p protein, Gpa2p does not appear to activate the MAPK cascade because the dominant active Gpa2 protein can partially suppress the pseudohyphal defect of *ste7*, *ste11*, or *ste12* mutants and because mutations in GPA2 do not influence activation of the MAPK-responsive FG(TyA)-*lacZ* reporter (Lorenz and Heitman 1997).

The environmental cues that trigger filamentous development in *S. cerevisiae* are not yet completely understood, and it is not clear whether the activation of Ras2p and Gpa2p occurs via shared or different sensors. Additionally, the number of sensors involved in the regulation of filamentous growth has yet to be elucidated. These issues are beginning to be addressed with the identification of a role for genes such as *GPR1* and *MEP2* in filamentous growth. *GPR1* encodes a Gpa2p-coupled, seven-transmembrane domain receptor and may function to sense extracellular signals and regulate cAMP levels via Gpa2p (Yun et al. 1997; Xue et al. 1998). The *MEP2* gene, encoding a high-affinity ammonium permease, has recently been demonstrated to control filamentous growth, i.e., deletion of *MEP2* is sufficient to block filament formation (Lorenz and Heitman 1998). The defect in filamentous growth exhibited by a diploid *mep2/mep2* mutant can be suppressed by activated alleles of *GPA2* and *RAS2* and by the addition of exogenous cAMP. In contrast, introduction of a dominant activated Ste11p (encoded by the *STE11-4* allele) and overexpression of *STE12* does not restore filamentous growth to strains deficient in Mep2p (Lorenz and Heitman 1998). Furthermore, the *mep2* mutation does not influence the MAPK-pathway-dependent activation of the FG(TyA)-*lacZ* reporter (Lorenz and Heitman 1998). Thus, Mep2p appears to lie upstream of both Gpa2p and Ras2p, and

to act independently of the MAPK pathway.

Downstream effectors of the cAMP pathway in *S. cerevisiae* may include Sok2p, a polypeptide with a basic helix-loop-helix motif that is homologous to a novel class of transcription factors essential for fungal differentiation (Ward et al. 1995). The *SOK2* gene is involved in the regulation of several phenotypes related to defects in PKA, and diploids carrying homozygous *SOK2* deletions exhibit enhanced filamentous growth (Ward et al. 1995). Overexpression of a *SOK2* homologue, *PHD1*, also leads to enhanced filamentous growth (Gimeno and Fink 1994), and double mutants deficient in both Phd1p and the transcription factor Ste12p (*phd1*/*phd1ste12*/*ste12*) are completely defective in filamentous growth (Lo et al. 1997). Although Phd1p does not appear to play a role in PKA-dependent growth, Phd1p may act to regulate filamentous growth in concert with Sok2p since diploids with mutations in both genes (*sok2*/*sok2phd1*/*phd1*) are suppressed for the enhanced filamentous growth phenotype of *sok2*/*sok2* single mutants (Ward et al. 1995). Both Phd1p and Sok2p appear to act independently of the MAPK cascade.

Another factor involved in cAMP-regulated filamentous growth is the serine/threonine kinase Elm1p (Koehler and Myers 1997). Diploids with homozygous deletions in *ELM1* are constitutively filamentous, indicating that the kinase functions to repress pseudohyphal development (Blacketer et al. 1993). Haploid *elm1* mutants containing $RAS2^{Val19}$ or overexpressed *TPK1* are suppressed for the elongated cell phenotype of the parental *elm1* mutant, demonstrating that the *elm1* phenotype is influenced by PKA activity (Garrett 1997). Double mutants defective in *ELM1* and *TRP1* display stress-related phenotypes (e.g., heat-shock sensitivity) that are similar to the phenotypes seen in strains with an activated RAS/cAMP pathway. Garrett (1997) has shown that the phenotypes of *elm1* mutants are dependent on tryptophan auxotrophy and that Elm1p may process intracellular signals (such as tryptophan levels) and regulate amino acid transport (Garrett 1997).

Recently, the daughter-cell-specific, zinc finger transcription factor, Ash1p, has been found to act downstream of Ras2p but independent of the MAPK pathway that regulates filamentous growth (Chandarlapaty and Errede 1998). While *ste12*/*ste12ash1*/*ash1* double mutants completely suppress the filamentous phenotype of $RAS2^{Val19}$ strains, the double mutant still exhibits filamentous growth when PHD1 is overexpressed (Chandarlapaty and Errede 1998). These results suggest that ASH1 may either be regulated by the cAMP pathway or play a role in a novel pathway activated by Ras2p but parallel to both the MAPK and cAMP pathways.

In *S. cerevisiae*, the cAMP pathway also influences the response to extracellular stresses such as heat shock, nutrient deprivation, low pH, osmotic shock, and treatment with alcohols or weak organic acids. The expression of genes that mediate the stress response is induced by Msn2p and Msn4p, partly as a result of signaling via a MAPK pathway in response to osmotic stress. Msn2p and Msn4p are translocated to the nucleus and bind stress response elements (STREs) upon exposure to stress (Görner et al. 1998). PKA phosphorylation of Msn2p and Msn4p may then cause the nuclear export of these transcription factors since mutations in the PKA consensus sites of Msn2p lead to constitutive nuclear localization of the protein in nonstress conditions. Furthermore, conditions that cause high PKA activity lead to cytoplasmic localization of Msn2p (Görner et al. 1998). Smith et al. (1998) have suggested that Msn2p and Msn4p are responsible for PKA-dependent effects on the stress response and that these proteins may antagonize PKA-dependent growth.

In summary, recent work on the control of filamentous growth in *S. cerevisiae* provides strong evidence for a dual role for the Ras2p protein in signaling via both the cAMP and the MAPK pathway. In addition, upstream elements of the cAMP signaling pathway such as Mep2p, Gpr1p, and Gpa2p are known to influence filamentous growth. Downstream effectors that have been shown to play important roles in cAMP signaling include the putative transcription factors Sok2p, Ash1p, Msn2p, and Msn4p. Msn2p and Msn4p provide a clear connection between the stress response, including the response via the osmosensing MAPK pathway, and the cAMP pathway.

Schizosaccharomyces pombe

In the fission yeast *Schizosaccharomyces pombe*, cAMP signaling plays a role in gluconeogenesis, cell cycle progression, entry into stationary phase, sexual development, and meiosis. Upon nutrient starvation, cells enter stationary phase or, in the presence of a compatible mating partner, undergo conjugation and meiosis [reviewed in Yamamoto (1996)]. The influence of cAMP signaling on sexual development has been particularly well-characterized. For example, mutants that are defective in adenylyl cyclase (encoded by *cyr1*) or the catalytic subunit of PKA (encoded by *pka1*) undergo conjugation and sporulation even in the absence of starvation. Nitrogen starvation causes a decrease in the intracellular level of cAMP, and the resulting decrease in the activity of PKA induces the expression of the *ste11* gene. This gene encodes a transcription factor of the high-mobility-group (HMG) family that mediates the expression of several genes required for sexual development. The influence of nutrient deprivation on mating appears to be mediated by a Gα-subunit encoded by the *gpa2* gene [reviewed in Yamamoto (1996)]. This Gα-subunit, which shows high sequence similarity to Gpa2p from *Saccharomyces cerevisiae*, acts through the cAMP pathway (Isshiki et al. 1992). In contrast to the situation in *Saccharomyces cerevisiae*, Ras in *Schizosaccharomyces pombe* (encoded by the *ras1* gene) does not influence adenylyl cyclase, but rather acts with a second Gα-subunit protein (encoded by *gpa1*) to control transduction of the mating pheromone signal via a MAPK cascade (Xu et al. 1994).

In addition to the control of sexual development, an interesting aspect of cAMP signaling in *Schizosaccharomyces pombe* is the apparent complex interplay between components of the cAMP pathway and a stress-responsive MAPK pathway. This MAPK cascade is encoded by *win1* or *wis4* (MAPKKKs), *wis1* (MAPKK), and *sty1/spc1/phh1* (MAPK), and is activated in response to osmotic stress, oxidative stress, heat shock, UV light, and nutritional starvation (Degols and Russell 1997; Samejima et al. 1997). Induction of *ste11* expression by nutrient starvation requires signaling via the MAPK pathway in response to stress. Activation of the Sty1 kinase induces the expression of *ste11* via Atf1, a transcription factor containing a bZIP domain (Takeda et al. 1995; Wilkinson et al. 1996). That is, *ste11* expression is positively regulated by Atf1, in contrast to the negative regulation by the cAMP pathway mentioned above (Takeda et al. 1995). Atf1 may be present in cells as a heterodimer with Pcr1, another bZip protein, since disruption of $pcr1^+$ results in the same phenotypes as does disruption of the *atf1* gene (Watanabe and Yamamoto 1996; Wilkinson et al. 1996). Atf1 and Pcr1 bind to the CRE/ATF site, the cAMP-responsive element characterized in higher eukaryotes, but there is no evidence that these transcription factors are in fact regulated by PKA. In accordance with their function, disruption of *atf1*, *pcr1*, *sty1* or *wis1* results in hypersensitivity to stress, sterility, and loss of viability in stationary phase. The stress-responsive MAPK pathway is also negatively regulated by *pyp1* and *pyp2*, both encoding protein tyrosine phosphatases that dephosphorylate Spc1 (Millar et al. 1995; Shiosaki and Russell 1995; Degols et al. 1996). Overexpression of *pyp1* can suppress the derepressed sexual development of *pka1* mutants (Dal Santo et al. 1996).

Entry into stationary phase (G_0) is also positively regulated by *atf1* and negatively regulated by cAMP. Recent work indicates that the gene *rsv1*, which encodes a zinc finger protein essential for cell viability during glucose starvation, is transcriptionally induced just before entry into G_0 (Hao et al. 1997). *rsv1* transcription is also negatively regulated by the cAMP pathway, but is not altered in *wis1* mutants, indicating that control of this gene is independent of the stress-responsive pathway. Both induction of *rsv1* expression and glucose depletion during entry into stationary phase are necessary for cell viability (Hao et al. 1997). The cAMP pathway in *Schizosaccharomyces pombe* is also involved in carbon repression during growth and regulates the expression of *fbp1*, the gene encoding the glucose-repressed fructose-1,6-bis-phosphatase. Expression of *fbp1* upon starvation is mediated by Atf1 and also requires an intact stress-responsive MAPK pathway (Takeda et al. 1995; Stettler et al. 1996). Thus, *fbp1* provides another example of a gene or process that is regulated by both the cAMP pathway and the stress-responsive pathway in an antagonistic way.

In the absence of glucose, fission yeast can use gluconic acid as a carbon source. Gluconic acid uptake increases dramatically in the absence of glucose, but is rapidly down-regulated in the presence of glucose (Caspari and Urlinger 1996). Mutants deficient in *cyr1* or *pka1* show high basal rates of gluconate uptake that can be further increased by glucose starvation (Caspari 1997). On the other hand, *wis1* mutants show a basal level of uptake similar to that of wild-type cells, but very low induction by glucose starvation. Therefore, the cAMP and the *wis1* pathways act parallel in the regulation of gluconate transport by glucose (Caspari 1997).

In *Schizosaccharomyces pombe*, mutants of *pka1* display a higher tolerance than do wild-type cells when exposed to severe heat shock (Fernández et al. 1997). Acquisition of thermotolerance in *Saccharomyces cerevisiae* has been associated with the ability to synthesize trehalose and to induce the expression of heat-shock proteins. Similarly, a *Schizosaccharomyces pombe* mutant defective in *tps1*, the gene encoding the trehalose-6-phosphate synthase, displays a reduced ability to acquire thermotolerance under certain conditions. Mutants defective in *pka1* have higher thermotolerance, and this may be partially due to increased trehalose synthesis. However, a *pka1 tps1* double mutant is more thermotolerant than a wild-type strain or a *tps1* mutant, suggesting that cAMP has another effect on thermotolerance that is independent of trehalose synthesis (Ribeiro et al. 1997). The level of *tps1* mRNA is approximately threefold higher in a *pka1* mutant than in a wild-type strain, suggesting that Pka1 also has a role in the expression of genes involved in thermotolerance (Fernández et al. 1997). The transcription of *tps1* is also positively regulated by the *wis1* MAPK cascade in response to heat shock (Degols et al. 1996), indicating again that both the cAMP and the stress-activated pathways act antagonistically to control transcription of common target genes. Neutral trehalase, the main enzyme in the degradation of trehalose, also appears to be post-transcriptionally regulated by Pka1 and another protein kinase in fission yeast, Sck1 (Soto et al. 1997).

In summary, the cAMP-dependent signal transduction pathway in *Schizosaccharomyces pombe* has a role in the control of several processes in response to extracellular signals and conditions, e.g., nutrient starvation. In many cases, the cAMP pathway functions both parallel and antagonistically to the stress-responsive MAPK pathway to regulate common targets in events such as the exit from mitotic cycle into stationary phase, sexual development, stress response, and changes in carbon metabolism. It is clear that cAMP signaling also plays roles in many of these same processes, including response to stress, in *Saccharomyces cerevisiae*. As described in the following sections, there are hints that similar connections may exist between signaling pathways in fungal pathogens of plants and animals.

Given the emerging appreciation for the connections between nutrient sensing and morphogenesis, it should also be noted that the fission yeast *Schizosaccharomyces japonicus* var. *japonicus* has recently been shown to undergo a dimorphic transition in response to nutritional conditions (Sipiczki et al. 1998). It will be interesting to see whether this morphological transition involves cAMP signaling.

Ustilago maydis

In the basidiomycete *U. maydis* and related smut fungi, cAMP signaling appears to control the switch between budding and filamentous growth (Gold et al. 1994). This switch is interconnected with the ability of these fungi to cause diseases on cereals and to complete sexual development. Haploid cells of *U. maydis* have a yeastlike morphology and grow by budding. Upon mating, two compatible haploids will fuse and form a filamentous dikaryon that is the infectious cell type. A hallmark of *U. maydis* infection of corn is the induction of large plant tumors filled with spores of the fungus. Sexual development in *U. maydis* is controlled by two loci, *a* and *b* [reviewed by Kronstad and Staben (1997)]. The *a* locus contains genes that encode pheromones and pheromone receptors that mediate recognition leading to the fusion of mating partners. The *b* locus encodes two homeodomain proteins, bE and bW, which dimerize to establish a factor that controls filamentous growth. Filamentous growth is also seen in haploid strains in response to certain environmental conditions (Ruiz-Herrera et al. 1995).

The initial evidence for a role for cAMP in the filamentous growth of *U. maydis* came from the discovery that mutations in the gene encoding adenylyl cyclase (*uac1*) caused constitutively filamentous growth (Gold et al. 1994). Furthermore, the filamentous phenotype of *uac1* mutants could be reverted to the wild-type budding phenotype by the addition of cAMP. However, *uac1* mutants are not pathogenic as haploid strains, indicating that filamentous growth alone is not sufficient to allow plant infection. Subsequently, a screen for suppressor mutations that restored budding growth to a mutant defective in *uac1* identified the *ubc1* gene (encoding the regulatory subunit of PKA; Gold et al. 1994). Mutants defective in *ubc1* are impaired in cytokinesis and bud site selection, resulting in cells with a multiple budding phenotype (Gold et al. 1994). The *ubc1* gene also appears to be required for pathogenicity. Microscopic observation of infected maize tissue showed that a dikaryon that is defective in both alleles of *ubc1* is capable of growth in the plant, but is incapable of tumor formation (Gold et al. 1997). Similarly, diploid strains lacking both copies of the *ubc1* gene are also incapable of forming tumors on plants (Dürrenberger et al. 1998).

Two genes encoding catalytic subunits of PKA, *adr1* and *uka1*, have been identified in *U. maydis*; *adr1* appears to contribute the majority of PKA activity, while *uka1* appears to make a minor contribution (Dürrenberger et al. 1998). Disruption of *adr1* results in mutants that display a filamentous phenotype quite similar to that of the *uac1* mutant; these mutants are incapable of causing infection in plants (Dürrenberger et al. 1998). In contrast, the *uka1* gene has little effect on mating, morphogenesis, or virulence. There is also preliminary evidence for a third PKA catalytic subunit in *U. maydis* (G. Yang and J. Kronstad, unpublished work), suggesting that *U. maydis*, like *Saccharomyces cerevisiae*, has multiple genes that encode the catalytic subunits of PKA.

A gene (*gpa3*) encoding a Gα-subunit and potentially playing a role in both mating and filamentous growth has been identified in *U. maydis* (Regenfelder et al. 1997). A haploid mutant that is defective in *gpa3* grows in elongated filaments, and treatment of this mutant with exogenous cAMP restores the phenotype to yeastlike budding growth (Kahmann and Basse 1997; Regenfelder et al. 1997). The *gpa3* disruption strain is unable to respond to pheromone signaling and does not cause disease symptoms in plants, implying that the *gpa3* gene product may play roles in mating, filamentous growth, and pathogenicity (Regenfelder et al. 1997). The possibility of multiple roles for the *gpa3* product is intriguing in light of the involvement of G-proteins in both nutrient sensing and pheromone signaling in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. A constitutively filamentous mutant of another smut fungus, *Ustilago hordei*, has been identified, and the defective gene (*fil1*) has been shown to also encode a G-protein α-subunit (Lichter and Mills 1997). Overexpression of the *fil1* gene in a wild-type background suppresses the filamentous growth commonly seen in older haploid cultures grown on nutrient-poor medium (Lichter and Mills 1997). In addition, exogenous cAMP restores budding growth to a *fil1* mutant. The *gpa3* and *fil1* genes appear to be close homologues, and these genes probably play similar roles in *U. maydis* and *U. hordei*.

The pheromone-response pathway in *U. maydis* is initiated by binding pheromone to receptor, and subsequent signaling is thought to be transduced via heterotrimeric G-proteins (encoded in part by *gpa3*), leading to higher levels of activated pheromone response factor (Prf1) (Hartmann et al. 1996; Regenfelder et al. 1997). The genes at the *a* and *b* loci contain pheromone response elements thought to be recognized by the HMG domain of Prf1, a protein that regulates the transcription of pheromone-induced genes (Hartmann et al. 1996). *prf1* mutants are sterile because the *a* and *b* genes are not expressed, and inactivation of *prf1* in pathogenic haploid strains (activated at both the *a* and the *b* loci) also abolishes pathogenicity. Pathogenicity in these strains can be rescued by constitutive expression of the *b* genes, which also induces filamentous growth in the absence of the pheromone signal (Hartmann et al. 1996). Thus, *prf1* links pheromone signaling with filamentous growth and pathogenic development (Hartmann et al. 1996).

There is some evidence that the cAMP and mating pathways may be interconnected in *U. maydis*. This is suggested by the fact that mutants defective in the cAMP pathway are attenuated for the mating reaction and virulence (Gold et al. 1997; Dürrenberger et al. 1998). Furthermore, the cAMP and mating pathways apparently share the same Gα-subunit, Gpa3p (Kahmann and Basse 1997; Regenfelder et al. 1997). The two pathways may also be connected at the level of Prf1. Prf1 may be phosphorylated as a result of a MAPK cascade, but components of this cascade have yet to be described (Hartmann et al. 1996). Fuz7, a MAPKK homologue

identified by Banuett and Herskowitz (1994), does not appear to be a component of the pheromone response pathway (Regenfelder et al. 1997). It is also possible that Prf1 activity is directly or indirectly regulated by PKA in a fashion similar to the influence of PKA on *ste11* expression in *Schizosaccharomyces pombe*.

cAMP signaling clearly plays an important role in morphogenesis and virulence in *U. maydis*. Interestingly, it appears that low PKA activity correlates with filamentous growth in *U. maydis*, and this stands in contrast to the situation in *Saccharomyces cerevisiae*, where activated Ras2p is thought to elevate cAMP and promote filamentous growth. The emerging view of cAMP signaling in *U. maydis* also prompts comparisons with the situation in *Schizosaccharomyces pombe*. Both fungi appear to have an HMG family factor (*prf1* in *U. maydis* and *ste11* in *Schizosaccharomyces pombe*), which is important in sexual development; it is clear that this factor responds to cAMP signaling in *Schizosaccharomyces pombe*, and it is possible that a similar situation exists in *U. maydis*. Additional work is needed to establish the involvement of MAPK pathways in sexual development, virulence, and morphogenesis in *U. maydis*.

Magnaporthe grisea

The ascomycete *M. grisea* causes the economically important disease known as rice blast. An important aspect of the pathogenicity of *M. grisea* is the germination of conidial spores and the subsequent formation of infection structures (appressoria) on the leaf surface. The appressoria form infection hyphae that invade host tissues, proliferate, and eventually sporulate to form conidia. A role for cAMP signaling in the infection process has been uncovered, and intriguing connections between possible mating signals and a MAPK pathway are emerging.

Mitchell and Dean (1995) disrupted the *CPKA* gene encoding an *M. grisea* PKA catalytic subunit and found that the resulting mutant was delayed in appressorium formation and was unable to successfully penetrate host tissues. *CPKA* mutants are, however, still able to grow invasively once host tissue barriers have been overcome, such as in infections initiated by abrasion of the leaf surface or injection (Xu et al. 1997). The exact role of *CPKA* during morphogenesis and infection remains to be elucidated. It has been suggested that functional PKA may serve to mobilize stored carbohydrates to form glycerol, resulting in the generation of turgor pressure within the melanized appressoria and subsequent protrusion of infectious hyphae. Mutants defective in *CPKA* display normal behavior for events prior to infectious hyphae formation such as nuclear migration into the appressoria, septa formation, and tight attachment to the leaf surface (Xu et al. 1997). These observations, combined with the finding that *CPKA* mutants remain responsive to the addition of cAMP, suggest the existence of additional genes encoding catalytic subunits of PKA.

Adenylyl cyclase mutants (*mac1*) of *M. grisea* demonstrate reduced conidiation, delayed conidial germination, and decreased vegetative growth (Choi and Dean 1997). *mac1* cells are unable to form mature appressoria on inductive (hydrophobic) surfaces such as onion skins or rice leaves, and are also asymptomatic in spray infection assays (Choi and Dean 1997). The *mac1*-associated phenotypes are rescued by the addition of cAMP. Exogenous cAMP was also able to suppress the inhibitory effect of *Saccharomyces cerevisiae* α-factor on appressorium formation by *M. grisea* (Beckerman et al. 1997). *Saccharomyces cerevisiae* α-factor may inhibit appressorium formation by triggering a pheromone response pathway in *M. grisea* and by preventing cAMP accumulation (Beckerman et al. 1997).

A gene encoding a Gα-subunit, *MAGB*, has been identified in *M. grisea*, and inactivation of this gene leads to reduced vegetative growth, conidiation, and appressorium formation. These phenotypes are also suppressed by exogenous cAMP. The product of the *MAGB* gene is likely to be an upstream regulator of cAMP formation (Liu and Dean 1997). Suppression of the *magB* phenotype by addition of cutin monomers also suggests the involvement of other pathways capable of promoting appressorium formation in response to signals from the surface of the host plant.

cAMP has also been implicated both in the transmission of signals initiated by the recognition of inductive surfaces that trigger appressorium formation and in appressorial development resulting from MAPK activation (Lee and Dean 1994; Talbot et al. 1996; Xu and Hamer 1996). Germination of conidia on noninductive surfaces results in the formation of vegetative mycelium. Recognition of infection-susceptible surfaces involves a thigmotrophic response, mediated in part by hydrophobin proteins (Beckerman and Ebbole 1996; Talbot et al. 1996). Disruption of an *M. grisea* hydrophobin-encoding gene, *MPG1*, results in decreased pathogenicity as a result of reduced appressorium formation and conidia production. Leaf surface recognition may involve an increase in intracellular cAMP levels because addition of cAMP restores appressorium development in *mpg1* cells (Talbot et al. 1996).

An *M. grisea* MAPK involved in appressorium formation has been identified as the product of the *PMK1* gene. Mutants defective in *PMK1* are unable to form appressoria, and cAMP is able to restore early stages of appressorium formation in these mutants (Xu and Hamer 1996). These results indicate a potential connection between a MAPK cascade (represented by *PMK1*) and the cAMP pathway that influences infection structure formation. The *PMK1* gene is not essential for vegetative growth and does not appear to play a role in sexual or asexual reproduction (conidiation). It will be interesting to see if *pmk1* mutants exhibit altered responses to stress.

Other efforts to find mutants defective for appressorium formation and/or conidiation have provided additional evidence for the importance of cAMP in each of these processes (Zhu et al. 1996; Shi et al. 1998). Screens for defects in appressorium formation (*APP*) or conidiation (*CON*) have identified several mutants whose phe-

notypes are either partially or completely suppressed by the addition of cAMP. The mechanism of cAMP involvement in each of the *APP/CON* mutants remains to be elucidated along with the functions of the gene(s) that are defective in these strains.

In summary, an involvement of cAMP signaling in infection structure formation has been characterized in some detail in *M. grisea*. There are suggestions that a relationship exists between cAMP signaling, response to a pheromone (although from a different species), and a MAPK. The information on signaling in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* may provide useful insights that will aid in directing additional work on *M. grisea*. Signaling via cAMP may be a general aspect of the virulence of fungal pathogens towards plants [for a recent review, see Kronstad (1997)]. For example, defects in cAMP signaling impair the ability of the fungus *Cryphonectria parasitica* to cause chestnut blight disease (Gao and Nuss 1996).

Cryptococcus neoformans

The basidiomycete *C. neoformans* is an opportunistic pathogen of immunocompromised individuals such as AIDS patients and people receiving immunosuppressive therapy [reviewed by Buchanan and Murphy (1998)]. *C. neoformans* has a haploid yeast stage in which cells display either of two mating types, *MATa* or *MATa* . Cells of opposite mating type can fuse and form a dikaryotic, filamentous cell type that sporulates to produce basidiospores [reviewed in Buchanan and Murphy (1998)]. Some haploid *MATa* strains can produce invasive hyphae and aerial mycelium that can give rise to haploid spores under culture conditions of low nitrogen, low glucose, and relative dryness (Wickes et al. 1996). Although it is clear that dimorphism is not directly involved in infection, sporulation may play a role in the dissemination of the fungus or in resistance to environmental factors.

The evidence that cAMP signaling is important in mating and virulence in *C. neoformans* comes from the analysis of the *GPA1* gene encoding a G-protein α-subunit (Alspaugh et al. 1997). This gene shows high sequence similarity with *gpa3*, the *U. maydis* Gα-encoding gene described earlier (Regenfelder et al. 1997). *GPA1* was disrupted in a virulent strain of *C. neoformans*, resulting in a *gpa1* mutant that is defective in mating and in the production of two known virulence factors, capsule and melanin (Alspaugh et al. 1997). Indeed, the *gpa1* mutant is also attenuated for virulence in a rabbit model. Capsule formation, melanin production, and the ability to mate were restored by the addition of cAMP, indicating that *GPA1* is involved in the regulation of the cAMP pathway in C. neoformans and that cAMP is involved in the regulation of at least two virulence factors (Alspaugh et al. 1997). An additional component of the cAMP pathway, the gene for adenylyl cyclase (*CAC1*), has also been isolated from *C. neoformans*. Sequencing of the catalytic domain of *CAC1* revealed high sequence similarity to the same region in the *U. maydis uac1* gene (51% identity at the amino acid level; D. Funnell and J. Kronstad, unpublished work).

C. neoformans provides an interesting opportunity to explore the regulation of expression of virulence factors via cAMP signaling. In particular, the components of the pathway will be valuable tools for identifying and characterizing environmental and host factors that influence signaling through the pathway. Other fungal pathogens of humans have been tested for influence of cAMP on various processes such as morphogenesis. For example, cAMP has been implicated in the dimorphic switch that occurs in the opportunistic fungal pathogen *Candida albicans* [e.g., see Niimi (1996)].

Concluding remarks

Saccharomyces cerevisiae and *Schizosaccharomyces pombe* have provided an inventory of the processes controlled by cAMP signaling in yeasts and a signaling road map that can be applied to other fungi, including pathogens of plants and animals. In particular, the analysis of filamentous growth in *Saccharomyces cerevisiae* serves as an excellent model for fungal morphogenesis with clear application to many fungal pathogens that exhibit dimorphic growth (e.g., *C. albicans*). Detailed information has been collected on the role of cAMP in morphogenesis in *Saccharomyces cerevisiae*, and work in the last two years has yielded both information on potential signals, receptors, and downstream targets and insight into interactions between the cAMP pathway and MAPK pathways. The intracacies of the connections between signaling pathways is also apparent from the genetic analysis of sexual development, response to stress, control of entry into stationary phase, and carbon regulation in *Schizosaccharomyces pombe*.

The analysis of cAMP signaling in other fungi is starting to reveal patterns that are reminiscent of those in the yeasts. In this review, we have focused on selected fungal pathogens of plants and animals to highlight the evidence for a role for cAMP in the processes of virulence, morphogenesis, and sexual development. From the limited work performed to date, it is possible to see glimmers of the patterns already revealed by *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. We anticipate that additional novel aspects of cAMP signaling with relevance to pathogenicity will be found upon more detailed analysis of these fungi.

Acknowledgements We would like to thank S. Garrett and B. Errede for communicating recent results and for helpful discussions. Work in our laboratory is funded by the Natural Sciences and Engineering Research Council and the Medical Research Council of Canada. A. De Maria is a postdoctoral fellow from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). We apologize to those authors whose work we could not cite and discuss in detail because of space limitations.

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