Medical Mycology Supplement 1 2005, **43**, S83–S86

Taylor & Francis
Taylor & Francis Group

Mitogen activated protein kinases of *Aspergillus fumigatus*

G. S. MAY*, T. XUE*, D. P. KONTOYIANNIS† & M. C. GUSTIN‡

**Division of Pathology and Laboratory Medicine, and †Department of Internal Medicine Specialties, Department of Infectious Diseases, Infection Control and Employee Health, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA, and ‡Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77251, USA*

In microbial eukaryotes, mitogen activated protein kinase (MAPK) pathways play a pivotal role in regulating cellular physiology. In fungi MAPK pathways have established functions in mating-pheromone responses, maintaining cell wall integrity, responding to changes in osmolarity and nutrient sensing. We have been studying MAPK functions in the human pathogenic fungus *Aspergillus fumigatus*. The genome of *A. fumigatus* has four MAPK genes, *sakA/hogA*, *mpkA*, *mpkB* and *mpkC*. Deletion of the *sakA* gene produces a strain that does not correctly regulate conidial germination, sense environmental nitrogen or responds to hypertonic stress. The function of the remaining MAPK genes is still under investigation, but by analogy to work in other filamentous fungi, we speculate as to their possible functions in *A. fumigatus*.

Keywords protein kinase, growth regulation, conidium

Introduction

In eukaryotes, mitogen activated protein kinases (MAPK) regulate cellular physiology in response to environmental change. Environmental changes that activate MAPK pathways in fungi include stresses (increased osmolarity, heat shock, high concentrations of heavy metals, and reactive oxygen species), nutrient limitation, disruption of cell wall integrity, and mating pheromones [1–10]. This central role of MAP kinases makes them potentially interesting targets for development of future antifungal drugs. For example, experiments with plant pathogenic fungi have demonstrated a clear role for MAPK in virulence and recent work has shown that a MAPK signaling pathway is the target of an agricultural antifungal [5,6,11–20]. The role of MAPK signaling pathways in animal fungal pathogens is less clearly established and thus is of critical experimental interest [1,21–26].

MAP kinases are the terminal protein kinase in a kinase cascade that regulates cellular responses to environmental change. These kinases cascades consist of three protein kinases that act in series. The three kinases are a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK) and a MAPK (Fig. 1). When a MAPK cascade is activated, the MAPKKK phosphorylates the MAPKK, which in turn phosphorylates the MAPK. This sequential activation of the kinases in the cascade results in an amplification of the initiating signal. In many MAPK pathways the downstream targets are transcription factors that lead to changes in gene expression [4,9]. The upstream activators of MAPK cascades are signal transduction systems that generally consist of a sensor that signals through another protein kinase that is often a member of the p21 activated kinase family or other protein kinase.

The specific biochemical events that lead to MAPK activation are well understood. When activated, the MAPKKK phosphorylates a serine and a threonine residue in a conserved amino terminal domain of the MAPKK. The active phosphorylated MAPKK then phosphorylates the MAPK on a threonine and a tyrosine. The phosphorylated amino acids are separated by one amino acid and are located in the activation loop in the conserved kinase domain. Once

---

Correspondence: G. S. May, Division of Pathology and Laboratory Medicine, Unit 54, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA. Tel: +1 713 745 1945; Fax: +1 713 792 8460; E-mail: gsmay@mdanderson.org

DOI: 10.1080/13693780400024784

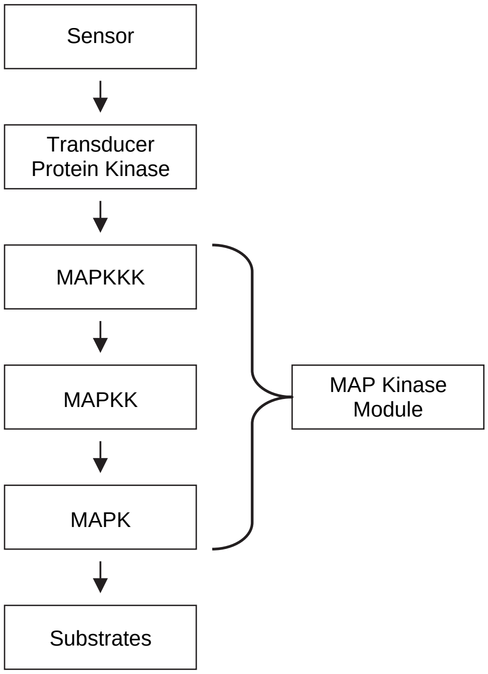

Fig. 1 Generalized MAPK signaling pathway. MAPK signaling pathways consist of a sensor system whose signal is transduced through a protein kinase that leads to activation of a MAP kinase module, resulting in phosphorylation of MAPK substrates.

these two amino acids are phosphorylated the MAPK is fully active. Like activation of MAPK, inactivation of the MAPK is fairly well understood. MAPK activity is negatively regulated by protein phosphatases. Specific protein phosphatases remove the phosphates from the threonine and tyrosine residues in the activation loop, leading to inactivation of the MAPK.

In fungi MAPK pathways function to regulate the mating-pheromone responses, dimorphic growth transitions, growth following hypertonic shock, growth in response to nutrient availability and cell integrity [4,6–9,21,25,27,28]. The role that these MAPK pathways play in regulating cellular physiology in fungi is best understood in budding and fission yeasts [4,9]. While budding and fission yeast are excellent models for making fundamental discoveries, they are not good models for understanding the functions of the MAPK pathways in other fungi. These two simple model organisms have very simple lifestyles compared to many other fungi. For example, true hyphal growth that allows filamentous fungi to penetrate substrates in a search for nutrients is not exhibited by either of these model organisms. Fungi of the genus *Aspergillus* produce a number of metabolites called mycotoxins that are made only under specific physiological conditions. How MAPK signaling pathways may contribute to regulating the more complex lifestyles of filamentous or dimorphic fungi is important to understand. Thus there are numerous reasons to investigate the role of MAPK signaling pathways in fungi other than the model yeasts. Our work has focused on the functions of the MAPK pathways of *A. fumigatus* [27].

Results and discussion

MAP kinase genes of A. fumigatus

The complete genome sequence of *A. fumigatus* has recently been determined, thus providing us with the ability to identify genes encoding the MAP kinases and other proteins of these signaling pathways. In addition, the genome sequences of *Aspergillus nidulans* and *A. oryzae* have also been determined and permit a comparative analysis of genes encoding constituents of MAPK signaling pathways in these related fungi. The genomes of each of these three fungi contain four genes encoding MAP kinases, and comparisons of the amino acid sequence indicate close relationships among the kinases. In *A. fumigatus* the four predicted MAP kinase proteins, SakA/HogA, MpkA, MpkB and MpkC, exhibit a clear pattern of relatedness (Table 1). SakA and MpkC form a pair, being 68% identical, and MpkA and MpkB form a pair, being 56% identical. In contrast, all other pair-wise comparisons result in approximately 45% identity.

SakA

SakA is most closely related to the high osmolarity glycerol (Hog) or stress activated MAP kinase proteins found in other eukaryotes [6,7,27]. We have shown that a *sakA* deletion mutant has several phenotypes that are common to other fungi mutant for this MAPK [27]. Germlings of the deletion mutant arrest growth in response to hypertonic conditions, a phenotype similar to that seen in budding yeast [2]. Interestingly, conidia under hypertonic stress will germinate and grow, although they develop more slowly than the parental wild type strain. This suggests that the signaling pathway is not active in metabolically dormant conidia

Table 1 Percent amino acid identities between the four MAPK proteins predicted from the *Aspergillus fumigatus* genome sequence

	SakA	MpkA	MpkB	MpkC
SakA	–			
MpkA	43%	–		
MpkB	48%	56%	–	
MpkC	68%	44%	46%	–

or is used for other purposes, but that the actively growing mutant responds to the hypertonic stress by arresting its growth and failing to reinitiate growth. The failure to begin growing following a hypertonic shock is a characteristic feature of mutants in this MAPK [2]. Specific transcriptional responses of genes that contribute to regulation of this signaling pathway are highly conserved in fungi [2,6,7,27]. The surprising and novel findings were that SakA also regulates conidial germination in response to the nitrogen source in the medium and that *sakA* messenger RNA accumulates in response to starvation for nitrogen or carbon sources [27]. These results implicate the SakA MAP kinase signaling pathway in negative regulation of conidial germination, in response to environmental nitrogen sources and nitrogen and carbon source starvation in growing hyphae. Similarly, the *Schizosaccharomyces pombe* homologue of SakA, called Spc1/Sty1, is activated by nitrogen limitation and stimulates mating and the production of spores through meiosis [28]. Thus, SakA and its relatives in other fungi also appear to signal transitions to a new life stage in response to specific types of starvation conditions.

MpkC

The MpkC MAPK that is most similar to SakA in primary sequence is functionally uncharacterized. Genes encoding orthologous proteins are found in the *A. nidulans* and *A. oryzae* genomes but nothing is known about their functions. Our preliminary study of an MpkC deletion mutant indicates that, like SakA, MpkC also has functions in regulating conidial germination. Additional studies are required to determine if MpkC and SakA have overlapping or redundant activities. It will also be necessary to construct *sakA* and *mpkC* double mutants to evaluate this possibility. This is similar to the situation in budding yeast where *FUS3* and *KSS1* have been shown to have overlapping functions in the mating-pheromone response pathway [4,9].

MpkB

Like MpkC, there have been no studies of the functions of MpkB. This kinase is most similar to the budding yeast Fus3p and Kss1p MAP kinases. Therefore it is predicted to function in growth regulatory responses related to pheromone signaling and mating. This is a surprising possibility since *A. fumigatus* is not known to have a sexual cycle. Nevertheless, several genes associated with the sexual cycle in other fungi have been identified in the *A. fumigatus* genome sequence [29], raising the possibility that sexual development may be induced under special circumstances. Another possibility is that a mating pathway may actually mediate pathogenic development, as it does in *Ustilago maydis* [30]. It is also interesting to speculate that MpkB may have overlapping or redundant functions with MpkA, the other MAPK most similar in its protein sequence.

MpkA

MpkA functions have not been studied in *A. fumigatus,* but in *A. nidulans* deletion of *mpkA* affects conidial germination and hyphal growth [31]. The defects observed in the deletion mutant are partially overcome by growth on high osmolarity complex media. MpkA is most similar in sequence and activity to MAP kinases in yeast and other fungi that function in the cell wall integrity pathway. The similarity of the MAPK gene complexes in *A. nidulans* and *A. fumigatus* suggests that the MpkA homologue in *A. fumigatus* will have similar functions to those found in *A. nidulans*.

Given the central role of MAPK signaling pathways in governing cellular physiology, these are potential targets for future antifungals. This is supported by recent work in the plant pathogenic fungus *Colletotrichum lagenarium* [16]. In this fungus, the fungicide fludioxonil leads to hyperactivation of the MAPK Osc1, the ortholog of SakA. Thus, constitutive activation of the osmotic stress response MAPK signaling pathway in this fungus results in the inability to infect the host. Inactivation of the osmotic stress response pathway in many plant pathogenic fungi has also been shown to lead to loss of virulence, further emphasizing the need to carefully regulate this MAPK pathway. We would predict that the development of antifungal compounds that inhibit or constitutively activate other MAPK pathways would similarly affect fungal virulence, as they impact upon fundamental homeostatic systems regulating fungal cellular physiology. While there has been considerable work on MAPK signaling pathways in a number of yeast and filamentous fungi there is still much to be learned about their roles in the medically important fungi. Work in this area not only contributes to our general knowledge of the how MAP kinases regulate fungal physiology but may lead to the development of new classes of antifungal drugs.

Acknowledgements

This research was supported by grants from the National Institutes of Health AI051144, AI052236, AI052236 and N01-AI-30041 (GSM). We also want to acknowledge that preliminary sequence data were

obtained from The Institute for Genomic Research website at http://www.tigr.org.

References

1 Alonso-Monge R, Navarro-Garcia F, Roman E, *et al*. The Hog1 mitogen-activated protein kinase is essential in the oxidative stress response and chlamydospore formation in *Candida albicans*. *Eukaryot Cell* 2003; **2**: 351–361.

2 Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC. An osmosensing signal transduction pathway in yeast. *Science* 1993; **259**: 1760–1763.

3 Glass NL, Rasmussen C, Roca MG, Read ND. Hyphal homing, fusion and mycelial interconnectedness. *Trends Microbiol* 2004; **12**: 135–141.

4 Gustin MC, Albertyn J, Alexander M, Davenport K. MAP kinase pathways in the yeast *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 1998; **62**: 1264–1300.

5 Hamer JE, Talbot NJ. Infection-related development in the rice blast fungus *Magnaporthe grisea*. *Curr Opin Microbiol* 1998; **1**: 693–697.

6 Han KH, Prade RA. Osmotic stress-coupled maintenance of polar growth in *Aspergillus nidulans*. *Mol Microbiol* 2002; **43**: 1065–1078.

7 Kawasaki L, Sanchez O, Shiozaki K, Aguirre J. SakA MAP kinase is involved in stress signal transduction, sexual development and spore viability in *Aspergillus nidulans*. *Mol Microbiol* 2002; **45**: 1153–1163.

8 Mayorga ME, Gold SE. A MAP kinase encoded by the ubc3 gene of *Ustilago maydis* is required for filamentous growth and full virulence. *Mol Microbiol* 1999; **34**: 485–497.

9 Millar JB. Stress-activated MAP kinase (mitogen-activated protein kinase) pathways of budding and fission yeasts. *Biochem Soc Symp* 1999; **64**: 49–62.

10 Pandey A, Roca MG, Read ND, Glass NL. Role of a mitogen-activated protein kinase pathway during conidial germination and hyphal fusion in *Neurospora crassa*. *Eukaryotic Cell* 2004; **3**: 348–358.

11 Brachmann A, Schirawski J, Muller P, Kahmann R. An unusual MAP kinase is required for efficient penetration of the plant surface by *Ustilago maydis*. *Embo J* 2003; **22**: 2199–2210.

12 Hou ZM, Xue CY, Peng YL, *et al*. A mitogen-activated protein kinase gene (MGV1) in *Fusarium graminearum* is required for female fertility, heterokaryon formation, and plant infection. *Mol Plant Microbe In* 2002; **15**: 1119–1127.

13 Jenczmionka NJ, Maier FJ, Losch AP, Schafer W. Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. *Curr Genet* 2003; **43**: 87–95.

14 Kinane J, Oliver RP. Evidence that the appressorial development in barley powdery mildew is controlled by MAP kinase activity in conjunction with the cAMP pathway. *Fungal Genet Biol* 2003; **39**: 94–102.

15 Kojima K, Kikuchi T, Takano Y, Oshiro E, Okuno T. The mitogen-activated protein kinase gene MAF1 is essential for the early differentiation phase of appressorium formation in *Colletotrichum lagenarium*. *Mol Plant Microbe In* 2002; **15**: 1268–1276.

16 Kojima K, Takano Y, Yoshimi A, *et al*. Fungicide activity through activation of a fungal signalling pathway. *Mol Microbiol* 2004; **53**: 1785–1796.

17 Muller P, Aichinger C, Feldbrugge M, Kahmann R. The MAP kinase kpp2 regulates mating and pathogenic development in *Ustilago maydis*. *Mol Microbiol* 1999; **34**: 1007–1017.

18 Takano Y, Kikuchi T, Kubo Y, *et al*. The *Colletotrichum lagenarium* MAP kinase gene CMK1 regulates diverse aspects of fungal pathogenesis. *Mol Plant Microbe Interact* 2000; **13**: 374–383.

19 Urban M, Mott E, Farley T, Hammond-Kosack K. The *Fusarium graminearum* MAP1 gene is essential for pathogenicity and development of perithecia. *Mol Plant Pathol* 2003; **4**: 347–359.

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

21 Alonso-Monge R, Navarro-Garcia F, Molero G, *et al*. Role of the mitogen-activated protein kinase hog1p in morphogenesis and virulence of *Candida albicans*. *J Bacteriol* 1999; **181**: 3058–3068.

22 Davidson RC, Nicholls CB, Cox GM, Perfect JR, Heitman J. A MAP kinase cascade composed of cell type specific and non-specific elements controls mating and differentiation of the fungal pathogen *Cryptococcus neoformans*. *Mol Microbiol* 2003; **49**: 469–485.

23 Kraus PR, Fox DS, Cox GM, Heitman J. The *Cryptococcus neoformans* MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. *Mol Microbiol* 2003; **48**: 1377–1387.

24 Navarro-Garcia F, Sanchez M, Pla J, Nombela C. Functional characterization of the MKC1 gene of *Candida albicans*, which encodes a mitogen-activated protein kinase homolog related to cell integrity. *Mol Cell Biol* 1995; **15**: 2197–2206.

25 Navarro-Garcia F, Alonso-Monge R, Rico H, *et al*. A role for the MAP kinase gene MKC1 in cell wall construction and morphological transitions in *Candida albicans*. *Microbiology* 1998; **144**: 411–424.

26 Navarro-Garcia F, Alonso-Monge R, Nombela C, Pla J. The Hog1 MAP kinase is activated by oxidative stress in the pathogenic fungus *Candida albicans*. *Yeast* 2003; **20**: S194–S194.

27 Xue T, Nguyen CK, Romans A, May GS. A mitogen-activated protein kinase that senses nitrogen regulates conidial germination and growth in *Aspergillus fumigatus*. *Eukaryot Cell* 2004; **3**: 557–560.

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

29 Dyer PS, Paoletti M, Archer DB. Genomics reveals sexual secrets of *Aspergillus*. *Microbiol-Sgm* 2003; **149**: 2301–2303.

30 Muller P, Weinzierl G, Brachmann A, Feldbrugge M, Kahmann R. Mating and pathogenic development of the smut fungus *Ustilago maydis* are regulated by one mitogen-activated protein kinase cascade. *Eukaryotic Cell* 2003; **2**: 1187–1199.

31 Bussink HJ, Osmani SA. A mitogen-activated protein kinase (MPKA) is involved in polarized growth in the filamentous fungus, *Aspergillus nidulans*. *FEMS Microbiol Lett* 1999; **173**: 117–125.