MINIREVIEWS

Mitogen-Activated Protein Kinase Pathways and Fungal Pathogenesis

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In eukaryotic cells, a family of serine/threonine protein kinases known as mitogen-activated protein (MAP) kinases (MAPKs) is involved in the transduction of a variety of extracellular signals and the regulation of different developmental processes. The MAPK is activated by dual phosphorylation of the TXXY motif by MAP kinase (MEK or MAPKK), which is activated in turn by MEK kinase (MEKK or MAPKKK). The sequential activation of the MAPK cascade eventually results in the activation of transcription factors and the expression of specific sets of genes in response to environmental stimuli. In the budding yeast *Saccharomyces cerevisiae*, five MAPK pathways are known to regulate mating, invasive growth, cell wall integrity, hyperosmoregulation, and ascospore formation (50). In the past decade, MAPKs in various plant and human pathogenic fungi have been characterized. In this review, we will compare their functions in different fungal pathogens with a focus on infection-related morphogenesis and virulence.

MAPK PATHWAYS IN *S. CEREVISIAE*

Because of advanced studies with *S. cerevisiae*, we will first present a brief overview of yeast MAPKs (Fig. 1). The pheromone response pathway (for reviews, see the work of Bardwell [9] and of Schwartz and Madhani [132]) is initiated by the binding of pheromone with a G-protein-coupled receptor (GPCR), Ste2 or Ste3, and the dissociation of an inhibitory Gα subunit, Gap1, from stimulatory Gβγ subunits. The liberated Gβγ directly associates with a scaffold protein Ste5 and a p21-activated protein (PAK) kinase, Ste20, and is essential for the activation of the Ste11-Ste7-Fus3/Kss1 cascade. The cyclin-dependent kinase inhibitor Far1 and the Ste12 transcription factor are activated by Fus3 and Kss1 MAPKs for regulating the mating processes. Several elements of the pheromone response pathway are also involved in filamentous growth, which represents invasive growth in haploid and pseudohyphal development in diploid cells. The specificity of Fus3 and Kss1 cascades is regulated by Ste5, the Ste12/Tecl transcription factor complex, and controlled degradation of Tec1 or Ste12 (12, 28, 132). For filamentous growth, the Kss1 pathway is activated by Ras2, Cdc42, 14-3-3 proteins Bmh1 and Bmh2, and Ste50 (9). The Ras2/cyclic AMP (cAMP) signaling also regulates filamentous growth via the GPCR Gpr1 and Gα Gpa2 subunit (114).

The Pck1-Slt2 (Mpk1) cell integrity pathway monitors cell wall integrity and promotes cell wall biosynthesis (for a review, see the work of Heinisch [52]). It is also involved in responses to certain environmental signals, including low osmolality, high temperature, alkaline pH, and nutrient limitations. Extracellular signals are transmitted from surface sensors to Rom2, a guanine exchange factor of the GTP-binding protein Rhod. Rhod then activates Pck1 and the Bck1-Mkk1/Mkk2-Slt2 cascade (Fig. 1). Downstream transcription factors, such as Rlm1, Sbf, and Swi6, are activated by Slt2 to regulate cell wall synthesis and cell cycle (52, 60). The high-osmolality glyceral (HOG) response pathway is required for growth under hyperosmotic conditions (54, 129). The Pbs2 MEK-Hog1 MAPK module can be activated by two upstream branches. One involves MEKKs Ssk2p and Ssk22p and a two-component histidine kinase phospho-relay system comprised of Sln1, Ypd1, and Ssk1. The other involves the activation of Pbs2 by Ste11 (Fig. 1), which functions downstream from Sho1 and Msb2 (112). In addition to its role in osmoregulation, the HOG pathway has recently been implicated in the response to non-osmotic stresses, such as arsenite, lower temperatures, and acid pH (89, 100, 115, 138). The other yeast MAPK is Smk1, a sporulation-specific MAPK activated by intracellular signals (50). It lacks upstream MEK or MEKK and has no homolog in other fungi except for some ascomycetous yeasts, such as *Ashbya gossypii* and *Kluyveromyces lactis*.

**HOMOLOGS OF THE YEAST FUS3/KSS1 MAPKs**

While fungi in the saccharomycetales, such as *A. gossypii*, *K. lactis*, and *Candida albicans*, have both Fus3 and Kss1 homologs, most filamentous fungi, like the archiascomyete *Schizosaccharomyces pombe*, have only one MAPK, which is homologous either to yeast Fus3 or to Kss1.

**Ustilago maydis.** The corn smut fungus *U. maydis* has been extensively studied for signal transduction pathways regulating mating and pathogenesis (for a review, see the work of Kahmann and Kamper [63]). It is a facultative biotrophic pathogen with a haploid, saprophytic yeast phase. Fusion of compatible yeast cells leads to the development of dikaryotic hyphae that can infect corn plants and cause tumors. The cAMP signaling pathway plays a critical role in regulating hyphal growth and pathogenic development. Strains blocked in the cAMP signaling pathway, such as the *gpa3* (Gα subunit), *uac1* (adenylate cyclase), and *adr* (catalytic subunit of PKA) deletion mutants, are nonpathogenic and grow filamentously (10, 63). Mating...
recognition occurs between pheromones and receptors and results in the activation of a downstream MAPK cascade consisting of the MEKK Kpp4 (Ubc4), MEK Fuz7 (Ubc5), and MAPKs Kpp2 (Ubc3) and Kpp6. Fuz7, a homolog of yeast Ste7, is the first element of this cascade to be characterized. It is important for conjugation tube production, filamentous growth, tumor induction, and teliospore formation and germination.

Kpp2 (Ubc3) and Kpp6 are two MAPKs with overlapping functions in mating and plant infection (10, 63). The kpp2 (ubc3) mutant is defective in pheromone responses and the formation of filamentous dikaryons and reduced in virulence. Kpp6 contains an unusual N-terminal domain and plays a more critical role in appressorial penetration than Kpp2. The kpp6 mutant is reduced in virulence and defective in the penetration of plant cuticle (15). The kpp2 kpp6 double mutants are abolished in mating and nonpathogenic on maize plants. Because transformants expressing the kinase dead kpp6 allele and the unphosphorylatable kpp2 allele are abolished in mating and nonpathogenic on maize plants. Because transformants expressing the kinase dead kpp6 allele and the unphosphorylatable kpp2 allele are more severely compromised in pathogenesis than the kpp6 and kpp2 mutants, respectively (106), the inactive kpp6 and kpp2 alleles may have additional inhibitory effects or downstream targets. Crk1 was first identified as a homolog of yeast Ime2 but later described as a novel MAPK in U. maydis that regulates morphogenesis, cell cycle, and plant infection (48). The crk1 mutant is defective in prf1 expression, mating, and plant infection.

The MEKK acting upstream from Fuz7 is Kpp4/Ubc4. Similar to fuz7 mutants, kpp4 deletion mutants are nonpathogenic and defective in the induction of pheromone-responsive genes (63). Deletion of Ubc2 (Ste50 homolog) also impairs pheromone responses and virulence (91). Different from other Ste50 homologs, Ubc2 has two C-terminal Src homology 3 domains that may be involved in protein-protein interactions. Ubc2 may function as an adaptor protein for the Kpp4-Fuz7-Kpp2/Kpp6 cascades. In U. maydis, the Smu1 PAK kinase is not directly involved in the activation of Kpp4 and downstream MAPKs, and it is dispensable for mating, plant infection, or tumor formation (134).

Ras2, one of the Ras proteins in U. maydis, has been placed upstream from Kpp4 and Ubc2. Mutation in the ras2 gene suppresses a constitutively filamentous phenotype of the adr1 mutant (80). Expression of a dominant active ras2 allele promotes pseudohyphal growth in a manner dependent on the Ubc4-Fuz7-Ubc3 cascade. In contrast, constitutive activation of Ras1 increases the expression of mfa1 but has no effect on cell morphology and yeast growth (104). Therefore, Ras2 and Ras1 in U. maydis may affect the MAPK and the cAMP-protein kinase A (PKA) pathway, respectively. A potential activator of Ras2 is the Cdc25-like guanine exchange factor protein Sql2 (104). However, unlike the ras2 mutant, the sql2 deletion mutant is defective in plant infection but not in mating. Sql2 may be an in planta-specific activator of Ras2 in response to lipid signals for promoting and maintaining filamentous growth (68). Although Gpa3 is essential for pheromone signaling and pathogenic development, the pheromone receptors Pral and Fral have not been functionally linked with any of the four Gα subunits in U. maydis. The Gβ subunit Bpp1 is involved in the cAMP-PKA pathway, but it is not an effector for the MAPK cascade (105).

The HMG-domain transcription factor Prf1 is required for
mating and plant infection. It has consensus MAPK and PKA phosphorylation sites and interacts with Adr1 as well as MAPK Kpp2 in vivo (62). While the PKA phosphorylation sites of Prf1 are essential for the induced expression of both a and b mating type genes, the MAPK phosphorylation sites are required only for b gene expression. Another HMG-domain protein, Rop1, is involved in mating, filamentous growth, and regulation of prf1 expression in axenic cultures. However, Rop1 is dispensable for conjugation, prf1 expression, and virulence on maize plants (17). In addition, Prf1 is not required for Kpp4 to control conjugation tube formation (106). Acidic pH still induces the yeast-to-hypha transition in haploid cells of the prf1 mutant but not the ubc4 or fuz7 mutant (90). Therefore, an additional transcription factor(s) may function downstream from this MAPK pathway for regulating responses to different signals and filamentous growth.

Magnaporthe grisea. Rice blast caused by M. grisea is one of the most severe fungal diseases of rice throughout the world. The fungus develops specialized infection structures called appressoria and uses the enormous turgor pressure generated in appressoria for plant penetration. While surface recognition and the initiation of appressorium formation are mediated by the cAMP signaling, late stages of appressorium formation and penetration are regulated by the PMK1 pathway. The pmk1 deletion mutant fails to form appressoria but still recognizes hydrophobic surfaces and responds to cAMP (151). PMK1 also is essential for infectious hyphal growth after penetration. In transformants expressing a PMK-green fluorescent protein construct, enhanced green fluorescent protein signals and nuclear localization are observed in appressoria and developing conidia (18).

Several upstream components of the PMK1 pathway, including the MEKK Mst11 and the MEK Mst7 and an Ste50 homolog, Mst50, have been characterized (118, 158). The mst7, mst11, and mst50 mutants fail to form appressoria and are nonpathogenic. Mst50 directly interacts with both Mst7 and Mst11 and may function as an adaptor protein for the Mst11-Mst7-Pmk1 cascade. The direct interaction of Mst7 with Pmk1 is mediated by the docking site and occurs specifically during appressorium formation (159). MST20, a homolog of yeast STE20, is dispensable for PMK1 activation in M. grisea (85). Consistent with this observation, the CDC42 homolog in M. grisea is dispensable for appressorium formation and plant infection (S. Wu and Z. Wang, personal communication). Therefore, unlike the yeast pheromone response pathway, PAK kinase and MgCdc42 are not essential for activating the Pmk1 MAPK cascade in M. grisea.

Mst50 and Mst11 both interact with Ras1 and Ras2, two Ras proteins in M. grisea (118). The ras1 deletion mutant has no defect in plant infection and appressorium formation, but RAS2 appears to be an essential gene. Expression of a dominant active RAS2 allele in the wild-type strain but not in the pmk1 mutant stimulates appressorium formation on nonconductive surfaces (118), indicating that RAS2 functions upstream from the Mst11-Mst7-Pmk1 cascade. In addition, the Ga subunit MagB, Gβ subunit Mgb1, and a negative regulator of G-protein signaling (Rgs1) have been implicated in regulating appressorium formation (86, 87, 110). Exogenous cAMP induces appressorium formation in mgb1 mutants, but these appressoria are morphologically abnormal and nonfunctional. MGB1 may control surface recognition via the cAMP signaling but function through the PMK1 pathway to regulate appressorial penetration and invasive growth.

The M. grisea genome has no recognizable receptor protein kinase genes but contains a large number of GPCR-like genes (75), including putative homologs of Ste2, Ste3, GprD, and Pre-1. Twelve of them form a subfamily containing the CFEM domain, which is unique to fungi (32). In M. grisea, deletion of the yeast Ste2 and Ste3 homologs has no obvious effects on appressorium formation or plant infection (J.-R. Xu, unpublished data), but one putative CFEM-GPCR gene, PTH11, has been implicated in surface recognition via cAMP signaling (35). However, exogenous cAMP restores appressorium formation and pathogenicity in the pth11 mutant, suggesting that PTH11 may be involved in the regulation of cAMP signaling. Also, predicting GPCRs is not reliable, and there is no direct evidence to support the sensory role of Pth11 in M. grisea.

One putative downstream transcription factor regulated by Pmk1 is Mst12 (Ste12 homolog), which is essential for pathogenesis. Appressoria formed by the mst12 mutant have normal appressorium turgor but fail to develop penetration pegs, probably due to actin filament defects in mature appressoria (117). MST12 may regulate genes involved in penetration and infectious growth, but another transcription factor(s) must function downstream of PMK1 for regulating appressorium formation. One of the REMI mutants defective in appressorium formation is disrupted in a homeobox gene, PTH12. However, the pth12 deletion mutant still occasionally forms melanized appressoria and responds to exogenous cAMP for appressorium formation (Y. Peng, personal communication). Several genes regulated by PMK1 have been identified by subtractive hybridization, including GAS1 and GAS2, two homologous genes that are unique to filamentous fungi and specifically expressed during appressorium formation (153).

Other plant pathogenic fungi. (i) Appressorium-forming pathogens. Homologs of FUS3/KSS1 in several other plant pathogenic fungi that form well-developed appressoria, including Cochliobolus heterostrophus, Colletotrichum lagenarium, C. gloeosporioides, and Pyrenophora teres, have been characterized. In all these fungi, PMK1 homologs are essential for appressorium formation (128, 151). Similar to the pmk1 mutant, the P. teres ptk1 and C. lagenarium cmk1 mutants are non-pathogenic and fail to colonize healthy or wounded host tissues. Conidia of the cmk1 mutant fail to germinate on plant and glass surfaces. In contrast, the STE12 homolog is essential for penetration and infectious growth but dispensable for appressorium formation in C. lagenarium (139). In the rice leaf spot pathogen Bipolaris oryzae, BKM1 is required for plant infection and conidiation, but its role in appressorium formation and penetration has not been examined (101).

In C. heterostrophus, CHK1 is important for invasive growth and efficient colonization of leaf tissue after penetration, but the chk1 mutant still forms a few small, restricted lesions on corn leaves. Other phenotypes of the chk1 mutant include reduced aerial hyphae, autolysis, lack of conidiation, and loss of female fertility (83). Genes for cellulohydrolase Cbh1, for endoglucanase Ebg6, for transcription factor Cmr1, and for three enzymes for melanin synthesis are among those known to be regulated by Chk1 (43, 82). Similar to CHK1, the CGB1 (Gβ) gene is essential for appressorium formation, female
fertility, hyphal pigmentation, and full virulence (47). However, the cgb1 mutant still produces conidia and is more severely reduced in virulence than the chk1 mutant. Deletion of CGA1 (Gα) reduces appressorium formation but has no effect on pathogenesis, suggesting that CGB1 and other upstream components, but not CGA1, may function upstream from the Chk1 cascade.

(ii) Non-appressorium-forming foliar pathogens. The PMK1 homologs also are essential for infection in two foliar pathogens of wheat, Mycosphaerella graminicola and Stagonospora nodorum. In M. graminicola, the MgFus3 deletion mutant is nonpathogenic and fails to colonize the mesophyll tissue through stomata (30). MgFUS3 is important for aerial hyphal growth, melanization, and pycnidium formation. Similar to what is seen for the mst20 mutant in M. grisea, deletion of a putative STE20 homolog has no obvious effect on the vegetative growth or virulence of M. graminicola (30). In S. nodorum, mak2 disruption mutants have reduced growth rates and are defective in conidiation, but they have no significant changes in the level of secreted protease activity, osmotic stress response, or melanin synthesis (135). Although mak2 mutants fail to form penetration structures and are essentially nonpathogenic, they are able to enter the leaf via natural openings. However, the infection progress and the ability to cause disease once inside the leaf are compromised in the mak2 mutant, which causes only limited necrosis on leaves (135).

(iii) Root or vascular pathogens. In Fusarium oxysporum f. sp. lycopersici, the fmk1 mutant displays normal growth and conidiation but is nonpathogenic on tomato plants and significantly reduced in the expression of the pectate lyase gene PL1 (37). FMKI is dispensable for conidium germination but is required for the differentiation of penetration hyphae and root attachment. In the vascular wilt pathogen Verticillium dahliae, vmk1 mutants are nonpathogenic on a variety of host plants tested and defective in microsclerotium production (123). Interestingly, PMK1 is also required for root infection in M. grisea, and it can functionally complement the fmk1 mutant (37), suggesting a conserved role for this MAPK pathway in soilborne and foliar pathogens. A FMK1 homolog from Tuber borchii is phosphorylated during plant colonization and partially restores invasive growth of the fmk1 mutant. Therefore, the same MAPK pathway may regulate the establishment of symbiosis and ectomycorrhizae (95).

The fgb1 (Gβ) deletion mutant of F. oxysporum also is reduced virulence, but it has an unaltered Fmk1 phosphorylation level and is defective in hyphal growth (33). Exogenous cAMP reverses part but not all of the fgb1 growth phenotypes. Therefore, Fgb1 may act upstream from the cAMP signaling but not the Fmk1 pathway. In infection assays with an immunodepressed mouse model, the fmk1 fgb1 double mutant but not the fmk1 mutant or the fgb1 mutant is significantly reduced in virulence (121). Fmk1 and Fgb1 appear to be components of distinct signaling pathways with overlapping functions.

(iv) Necrotrophic pathogens. PMKI homologs are also important for pathogenesis in several other necrotrophic ascomycetes (Table 1). In Botrytis cinerea, the bmp1 mutant has a reduced growth rate and is nonpathogenic. Germ tubes of the bmp1 mutant fail to penetrate the host tissue (160). Recently, an independent bmp1 mutant was reported to be defective in conidium germination on hydrophobic surfaces and in responding to carbon sources (39). Differences in conidium germination between two bmp1 mutants may be related to different spore concentrations used or mutant backgrounds. In Alternaria brassicicola, amk1 mutants are nonpathogenic on intact plants but still colonize wounded leaves in the presence of nutrient supplements. Amk1 is essential for the production of mature conidia and up-regulation of several hydrolytic enzyme genes (26). In Cryptonectria parasitica, the cmpk2 deletion mutant is defective in conidiation and pheromone production and has reduced growth rate and canker size (27). Although the activation of CpmK2 is not affected, CpmSTE12 is down-regulated by hypovirus infection (34). Many genes affected by the hypovirus are down-regulated in the cste12 mutant, which is reduced in virulence and female sterile (34). In Sclerotinia sclerotiorum, Smk1 regulates sclerotium development, but its role in plant infection has not been determined (24).

In the wheat scab fungus F. graminearum, the gpmk1 (map1) deletion mutants are reduced in conidiation, female sterile, and nonpathogenic (57, 140). The gpmk1 mutants are impaired in colonizing wheat heads and spreading from inoculated florets to neighboring spikelets. They also fail to infect roots, wounded wheat floral tissues, and tomato fruits but still produce phytotoxic deoxynivalenol. Gpmk1 regulates the early induction of extracellular endoglucanase, xylanolytic, and proteolytic activities and is responsible for the overall induction of secreted lipolytic activities (58). One of the genes regulated by Gpmk1 is FGL1, which encodes a secreted lipase and is an important virulence factor in F. graminearum (144).

(v) Biotrophic pathogens. The cpmk1 mutant of Claviceps purpurea has no defect in vegetative growth or conidiation but is incapable of penetration and nonpathogenic on rye plants (97). Mutants deleted of the CDC42 homolog in C. purpurea also are nonpathogenic, but they still penetrate the styelar tissue. The invasive growth of the Cpcdc42 mutant is arrested at an early infection stage, presumably due to induced plant defense responses (131). Expression of CPMKI in the pmk1 mu-
tant fully complements its defects, indicating that this MAPK pathway is functionally conserved between hemibiotrophic and biotrophic pathogens. In the barley powdery mildew fungus *Blumeria graminis*, both a MAPK and the cAMP signaling are involved in regulating appressorium development (67). In another obligate biotroph, *Puccinia triticina*, the PMAPK1 gene has increased expression levels during urediospore germination and plant infection. When expressed in *U. maydis*, it complements the defect of kpp2 mutants in mating and plant infection (56).

**Model saprophytic filamentous fungi.** In the model filamentous fungus *Neurospora crassa*, mutants deleted of the MEKK NRC-1 and MAPK MAK-2 genes have the same pleiotropic phenotype, including derepressed conidiation, shortened aerial hyphae, lack of hyphal fusion and conidial anastomosis tube, female sterility, and flattened ascospores (72, 116). The pp-1 (Ste12 homolog) deletion mutant has similar defects (84). However, none of the individual GPCR, Ga, Gβ, or Gγ deletion mutants are phenotypically similar to the mak-2 mutant.

Unlike other filamentous fungi, several *Aspergillus* species, including *A. niger*, *A. nidulans*, and *A. fumigatus*, have four MAPK genes (124). The two MAPKs with the TEY dual phosphorylation site, MpkA and MpkB, are homologous to yeast Ste2 and Kss1, respectively. Although MpkB has not been functionally characterized, SteA (Ste12 homolog) is required for the development of ascosogenous hyphae and cleistothecia. The steA deletion mutant is blocked in sexual reproduction but has normal vegetative growth and conidiation (141). However, the steC (Ste11 homolog) deletion mutant has a pleiotropic phenotype, including a reduced growth rate, altered conidiophore morphology, and defects in heterokaryon formation and cleistothecium development (148).

*Candida albicans*. *C. albicans* causes various forms of candidiasis, particularly in immunocompromised patients. The reversible dimorphic transition between the yeast form and the hyphal form is important for its virulence and can be triggered by various signals in vitro, including high temperature, neutral pH, and serum. *C. albicans* also occasionally undergoes switching between white-phase cells that form dome-shaped, white colonies and opaque-phase cells that are more elongated and form flatter, darker colonies on solid agar. Signal transduction pathways regulating filamentous growth and the white-opaque switching have been well studied for *C. albicans* (for reviews, see the work of Alonso-Monge et al. [2], of Bennett and Johnson [11], and of Chen et al. [25]).

The Cek1 (Kss1 homolog) MAPK cascade has been well characterized for its role in the yeast-hypha transition and virulence (2, 11). Homozygous cek1 mutants are defective in transition from unicellular budding growth to invasive hyphal growth on Spider or synthetic low-ammonium-dextrose (SLAD) medium and are significantly attenuated in virulence in murine models for systemic or superficial candidiasis. All the major components of the yeast pheromone response pathway except Ste5 have been identified in *C. albicans* (11). The PAK kinase Cst20, MEK Hst7, Cph1 (Ste12 homolog), CaTec1, CaRas1, Cdc42, and its exchange factor Cdc24 are also required for hyphal morphogenesis, invasive hyphal growth, and virulence. The defect of the CaRas1 deletion mutant in morphological transition is suppressed by exogenous cAMP or overexpression of CEK1 (78), indicating that CaRas1 functions in both signaling pathways.

All the mutants blocked in the CEK1 pathway, however, are still capable of filamentous growth in response to serum. Multiple signaling pathways, including the cAMP-dependent protein kinase pathway via Efg1, a pH-responsive pathway through Rim101, and Tup1-mediated repression through Rfg1 and Nrg1, are known to regulate hyphal development and infection processes in *C. albicans* (2, 61). Cph1 and Efg1 have overlapping functions for induced expression of genes responsive to serum, and the homoyogous cph1 efg1 double mutant fails to develop hyphae or pseudohyphae in response to many stimuli (16, 88). Unlike Tec1 in yeast, CaTec1 is regulated by Efg1 and the basic helix-loop-helix transcription factor Cph2 but not by Cph1 in *C. albicans* (77). Recently, the Mep2 amnomium permease has been shown to mediate the induction of filamentous growth in response to nitrogen starvation by its interaction with CaRas1 (13).

Although *C. albicans* is a diploid fungus traditionally classified as asexual, mating between genetically modified strains and a parascexual cycle have been observed. CEK2 (25) and several components of the CEK1 pathway, including STE2, GPA2, CST20, HST7, CEK1, and CPH1 (2), are involved in mating responses. CEK2 encodes a MAPK highly similar to that encoded by FUS3. It is dispensable for filamentous growth on artificial media but has overlapping functions with CEK1 in mating. While the cek1 and cek2 deletion mutants are reduced in mating efficiency, cek1 cek2 double mutants, like the hst7 and cph1 mutants, are completely blocked in mating (25). For upstream components, the GPCR gene STE2 is essential for mating responses to the alpha pheromone, and the Gpa2 Gα subunit is involved in relaying nutrient signals to mating. In *S. cerevisiae*, Mcm1 is involved in cell type-specific transcription and pheromone response. Overexpression of CaMCM1 induces the expression of a hypha-specific gene HWP1 and hyphal development, but its function in mating is not clear in the case of *C. albicans* (126). The Cph1-mediated MAPK pathway is involved in the regulation of white-opaque switching, which is controlled by the mating type locus (2, 98). Unlike the yeast pheromone response pathway, Cek1 appears to be activated by quorum sensing and other environmental signals (125, 130). Farnesol, a quorum-sensing molecule, reduces the transcription levels of HST7 and CPH1 in *C. albicans*. Diluting stationary-phase cells in fresh rich medium also stimulates the phosphorylation of Cek1. In *C. albicans*, Sho1 is essential for Cek1 activation under different conditions that require active cell growth and cell wall remodeling (125). The sho1 mutant is sensitive to oxidative stress and cell wall-interfering compounds (Congo red and calcofluor white) and is defective in morphogenesis on SLAD and Spider media, which stimulate hyphal growth (125). These results reveal a role for Sho1 in linking oxidative stress, cell wall biogenesis, and morphogenesis in *C. albicans*.

**Cryptococcus neoformans*. *C. neoformans* is the causal agent of cryptococcal meningoencephalitis. Mating between *MATα* and *MATa* cells on a nutrient starvation medium leads to the formation of dikaryotic filaments. The mating type locus contains mating type-specific Ste11 and Ste12 homologs, but the Cpk1 MAPK and Ste7 homolog are not linked to the mating type locus (31). The cpk1 and ste7 deletion mutants of both
matting types are severely impaired in mating but not completely sterile. The \( \text{cpk1a}, \text{ste7a}, \) and \( \text{ste11a} \) mutants are also blocked in haploid fruiting (29, 31). However, the \( \text{ste11a}, \text{ste7}, \) and \( \text{cpk1} \) mutants are as virulent as the wild-type strains in a disseminated mouse model, indicating that the \( \text{CPK1} \) MAPK pathway is important for mating and haploid fruiting but dispensable for virulence.

While the \( \text{Gpa1} \) Go subunit plays a critical role in the cAMP signaling, the \( \text{Gpb1} \) G\( \beta \) subunit functions upstream from the \( \text{CPK1} \) cascade. The mating defect of the \( \text{gpb1} \) mutant is suppressed by overexpression of \( \text{CPK1} \) but not exogenous cAMP (146). The \( \text{gpb1} \) mutant also is defective in haploid fruiting, which can be suppressed by the overexpression of \( \text{Ste12a} \). Therefore, mating and haploid fruiting in \( \text{C. neoformans} \) are mediated by \( \text{GPB1} \) via the \( \text{CPK1} \) MAPK cascade (146). A Ras homolog has also been identified as an upstream component of this pathway. The \( \text{Ras1} \) protein is unstable but unable to grow at \( 37^\circ \text{C}, \) defective in mating, and avirulent in a rabbit model of cryptococcal meningitis. It has no defects in melanin and capsule production, which are two events regulated by \( \text{Gpa1}-\text{cAMP} \) signaling (4, 147). Expression of a dominant active \( \text{RAS1} \) allele enhances haploid fruiting in serotype A strain H99 but not in the \( \text{ste12a} \) mutant (3, 156). Although the mating defect of the \( \text{gpb1} \) mutant is not recovered by the \( \text{RAS1}^{\text{Q67L}} \) allele, expression of a dominant \( \text{GPB1} \) allele rescues the mating defect of the \( \text{ras1} \) mutant, indicating that \( \text{RAS1} \) functions upstream of \( \text{GPB1} \) in the mating process (4, 81).

Several GPCR genes in \( \text{C. neoformans} \), including \( \text{Ste3a}, \text{Ste3a}, \) and \( \text{Gpr4} \), have also been characterized. The \( \text{Ste3} \) homologs are important for mating and have a mating type-specific role in virulence (20). Gpr4 is dispensable for infection, but it may function as an amino acid sensor and interact with \( \text{Gpa1} \) for regulating the cAMP-PKA pathway (154).

\( \text{Ste12a} \) may function downstream of \( \text{Cpk1} \) because overexpression of \( \text{Ste12a} \) suppresses the defects of the \( \text{cpk1} \) mutant in mating and haploid fruiting. However, overexpression of \( \text{Ste11a} \) or \( \text{Cpk1} \) can restore haploid fruiting in the \( \text{ste12a} \) mutant, suggesting that \( \text{Ste12a} \) does not function downstream from \( \text{Cpk1} \) in a strictly linear pathway (31). Interestingly, \( \text{Ste12a} \) and \( \text{Ste12a} \) are important for virulence in a serotype-specific manner. Unlike the \( \text{ste11a}, \text{ste7}, \) and \( \text{cpk1} \) mutants, the \( \text{ste12} \) mutants in serotype D are significantly reduced in virulence (22, 156). In contrast, the PAK kinase \( \text{Ste20a} \) contributes to virulence in serotype A but not in serotype D strains (145).

**Other human pathogenic fungi.** The \( \text{FUS3/KSS1} \) homologs have been identified in several other human pathogens, including \( \text{A. fumigatus}, \text{Penicillium marneffei}, \) and \( \text{Pneumocystis carinii} \) (44). However, their function in pathogenesis has not been characterized. In \( \text{P. carinii} \), phosphorylation assays and expression analyses of a few candidate components of the \( \text{PCM} \) MAPK pathway, including \( \text{PcSte11}, \text{PcSte20}, \) and \( \text{PcSte3} \), have suggested that this MAPK pathway may play regulatory roles in the life cycle and infection processes of this opportunistic pathogen (143). In \( \text{P. marneffei}, \text{SdA} \) (Ste12 homolog) is dispensable for asexual development and dimorphic switching but can complement the sexual reproduction defect of the \( \text{A. nidulans steA} \) mutant (14).

### TABLE 2. **SLT2 homologs in pathogenic fungi**

| Fungal pathogen | MAPK | Major functions |
|-----------------|------|-----------------|
| \( M. grisea \) | \( \text{Mps1} \) | Pathogenicity, penetration, conidiation, cell wall integrity |
| \( C. lagenarium \) | \( \text{Maf1} \) | Pathogenicity, appressorium formation, conidiation |
| \( C. purpurea \) | \( \text{Cpmk2} \) | Pathogenicity, penetration, conidiation, cell wall integrity |
| \( F. graminearum \) | \( \text{Mgv1} \) | Pathogenicity, hyphal fusion, cell wall integrity |
| \( M. graminicola \) | \( \text{Mgs1} \) | Pathogenicity, infectious growth |
| \( B. cinerea \) | \( \text{Bmp3} \) | Pathogenicity, penetration, macro- and microconidiation |
| \( C. albicans \) | \( \text{Mkc1} \) | Virulence, cell wall biogenesis, stress response |
| \( \text{C. neoforms} \) | \( \text{Mpk1} \) | Virulence, cell wall biogenesis, stress response |

**CELL WALL INTEGRITY PATHWAY**

Several orthologs of yeast \( \text{SLT2} \) in plant and human pathogens have been characterized. In general, this MAPK pathway is important for pathogenesis and cell wall integrity.

**Plant pathogenic fungi.** The \( \text{MPS1} \) MAPK is essential for conidiation, appressorial penetration, and plant infection in \( M. grisea \) (152). The \( \text{mps1} \) deletion mutant is significantly reduced in aerial hyphal growth and conidiation, but it displays no obvious changes in the growth rate. Unlike \( \text{Pmk1}, \text{Mps1} \) is dispensable for appressorium formation. Appressoria formed by the \( \text{mps1} \) mutant fail to penetrate and develop infectious hyphae but still elicit plant defense responses. Vegetative hyphae of the mutant have a weakened cell wall, undergo autolysis in aging colonies, and are hypersensitive to cell wall-lytic enzymes (152). The \( M. grisea \) genome contains distinct homologs of many components of the yeast \( \text{Pck1-Slt2} \) pathway (32), including \( \text{Pck}, \text{Bck1}, \text{Mmk2}, \text{Rom2}, \text{Rlm1}, \) and \( \text{Swi6} \). However, it lacks significant homologs of the receptor genes of the yeast \( \text{Pck1-Slt2} \) pathway except for one gene with limited homology to \( \text{Wsc1} \) (e-value, 1e-4). Receptors may not be well conserved, and \( M. grisea \) may have novel receptors for recognizing plant or environmental signals.

Functional characterization of the \( \text{SLT2} \) homolog in several other plant pathogenic fungi (Table 2) has indicated that this MAPK is well conserved among fungal pathogens and plays important roles during plant infection. In \( C. lagenarium, \text{MAF1} \) is required for the early stages of appressorium formation (70). Elongated germ tubes of the \( \text{maf1} \) mutant fail to form appressoria. In \( C. purpurea, \text{CPMK2} \) also is necessary for penetration, and the \( \text{cpmk2} \) mutant retains only a limited ability to colonize host tissues (96). In \( M. graminicola, \text{Mgslt2} \) mutants are normal in penetration of stomata but fail to colonize and grow invasively in plants (92). The \( \text{bmp3} \) mutant of \( B. cinerea \) is defective in penetrating dead onion epidermal cells and developing necrotic lesions (127). In \( F. graminearum, \text{mpg1} \) mutant is reduced in deoxynivalenol accumulation (55) and hypersensitive to plant defensin MsDef1 (122). \( \text{Mgvl} \) also is essential for hyphal fusion and heterokaryon formation (55).

In contrast to its conserved role in pathogenesis, the function of this MAPK in cell wall integrity, conidiation, and stress responses varies among fungal pathogens. Similar to the \( \text{mps1} \) mutant, the \( \text{cpmk2} \) and \( \text{mpg1} \) mutants in \( C. purpurea \) and \( F. graminearum \) have weakened cell walls and increased susceptibility to cell wall-lytic enzymes and certain compounds that interfere with the cell wall, such as nikkomycin \( Z \) (a chitin
synthase inhibitor) and calcofluor white (55, 96). In contrast, deletion of the SLT2 ortholog has no obvious effect on sensitivities to cell wall-lytic enzymes and inhibitors in M. graminicola and B. cinerea (127). Interestingly, the Msf12 mutant is hypersensitive to several azole fungicides, including miconazole and cyproconazole. The bmp3 mutant has increased sensitivity to paraquat and the phenylpyrrole fungicide fluoroquinoloxin, but it is not hypersensitive to the azoles, elevated temperatures, or \( \text{H}_2\text{O}_2 \) (127).

The SLT2 orthologs are also required for conidiation in \( \text{C. lagenarium} \) and \( \text{C. purpurea} \). However, conidiation is normal in the \( \text{mvg1} \) mutant of \( \text{F. graminearum} \) and the \( \text{mgs1t2} \) mutant of \( \text{M. graminicola} \) (Table 2). The bmp3 mutant of \( \text{B. cinerea} \) produces fewer macroconidia but more microconidia than the wild type (127). It also is defective in sclerotium formation and germ tube responses to surfaces. Unlike the \( \text{pmk1} \), \( \text{mfl1} \), and \( \text{mgs1t2} \) mutants, the \( \text{mvg1} \) and \( \text{bmp3} \) mutants exhibit reduced growth rates on solid media. However, vegetative growth in liquid cultures is normal in the \( \text{mvg1} \) mutant. The pleiotropic phenotypes observed for these mutants indicate that this MAPK pathway may regulate various growth or differentiation processes in different plant pathogenic fungi.

**Model saprophytic filamentous fungi.** In \( \text{A. nidulans} \), MpkA plays an important role in conidium germination and hyphal tip growth. The \( \text{mpkA} \) mutant has a reduced growth rate, and its hyphal tips tend to swell (19). Conidia often grow isotropically and fail to produce germ tubes under normal culture conditions. In \( \text{Podospora anserina} \), crippled growth is an epigenetic cell degeneration phenomenon caused by \( \text{C} \), a cytoplasmic and infectious hereditary unit that resembles a prion. \( \text{PaASK1}, \text{a BCK1} \) homolog, is required for \( \text{C} \) production. The \( \text{paask1} \) mutant is defective in hyphal pigmentation, differentiation of aerial hyphae, and fruiting body development. Mutants deleted of the downstream MEK \( \text{PaMkk1} \) or MAPK \( \text{PaMpk1} \) have the same phenotype as the \( \text{paask1} \) mutant (65). Overexpression of \( \text{PaASK1} \) or \( \text{PaMPK1} \) facilitates \( \text{C} \) propagation and enhances crippled growth in wild-type strains. The activation and nuclear localization of \( \text{PaMPK1} \) mutants appear to be correlated with the presence of the \( \text{C} \) element in vegetative hyphae (65). The autolysis and pigmentation defects of the \( \text{mps1} \) mutants of \( \text{M. grisea} \) and \( \text{C. heterostrophus} \) may be related to cell degeneration.

**Human pathogens.** In \( \text{C. albicans} \), Mk1 (Slt2 homolog) is involved in regulating cell wall integrity and required for growth at elevated temperatures (109). Homozygous \( \text{mck1} \) deletion mutants exhibit increased susceptibility to caffeine, gluconolactone, and some inhibitors of cell wall synthesis. Growth rates and cell viabilities of \( \text{mck1} \) mutants are reduced in cultures grown at 42°C. In addition, Mk1 has been implicated in morphological transitions and pathogenesis (36, 107). The \( \text{mck1} \) mutants are also reduced in invasive growth on Spider medium and produce shorter hyphal cells (for a review, see the work of Alonso-Monge et al. [2]). In infection assays with a murine model, \( \text{mck1} \) mutants are attenuated in virulence. The \( \text{mck1} \) mutants have increased sensitivities to nitric oxide (NO) in vitro assays and reduced abilities to inhibit NO production by macrophages (99).

The Mk1 pathway is also involved in responses to other stresses. The activation of Mk1 is triggered by various oxidants, certain osmotic stresses, antifungal drugs targeted at cell wall and membrane synthases, calcium ion, and low-temperature shock (108). The phosphorylation of Mk1 in response to oxidative stress is partially dependent on the \( \text{CaHOG1} \) pathway (5), suggesting cross talk between these two pathways. The activation of Mk1 by oxidative stress is blocked in the \( \text{capk1} \) mutant (108), indicating that \( \text{C. albicans} \) has a similar PKC-Slt2 cell wall integrity pathway. The \( \text{capk1} \) deletion mutants are normal in the yeast-to-hypha transition but have an osmotically remediable cell lysis defect. Cercosporamide, a selective inhibitor of Pkc1, has synergistic fungicidal effects with caspofungin (a β-1,3-glucan synthase inhibitor) in \( \text{C. albicans} \) (136). In \( \text{S. cerevisiae} \), Wsc1 acts as the dedicated sensor for caspofungin-induced cell wall damage to activate the Pkc1-Slt2 pathway. Short exposure to caspofungin results in the up-regulation of \( \text{MKC1} \), and the \( \text{mkc1} \) mutant has increased sensitivity to caspofungin (149). Mk1 is also activated by physical contact in \( \text{C. albicans} \). The \( \text{mkc1} \) mutants are defective in invasive hyphal growth on YPS agar and normal biofilm formation, two contact-dependent responses (76).

In \( \text{C. neoformans} \), the \( \text{MPK1} \) MAPK gene is required for growth at elevated temperatures and the maintenance of cell wall integrity (73). The growth defect of the \( \text{mpk1} \) deletion mutant at 37°C can be remedied by an osmotic stabilizer such as 1 M sorbitol. Phosphorylation of Mpk1 is induced by antifungal compounds nikkomycin Z and caspofungin. The \( \text{mpk1} \) mutant is significantly attenuated in virulence and hypersensitive to fluoroquinoloxin or cell wall synthesis inhibitors, but it is normal in the production of melanin and capsule (69, 73). The growth defect at 37°C and weakened cell wall of the \( \text{mpk1} \) mutant may be responsible for its reduced virulence.

Several other components of the putative Pck1-Mpk1 pathway in \( \text{C. neoformans} \), including homologs of yeast Rhol, Bck1, Mkk2, Lrg1, Rom2, Rom20, and Rom21, have been characterized, but their functions in virulence remain to be examined (21, 49). Although both Bck1 and Mkk2 homologs are critical for maintaining cell wall integrity and growth at elevated temperatures, the \( \text{mkk2} \) but not the \( \text{bck1} \) deletion mutant is reduced in melanin production. Inhibition of Pck1 abolishes melanin synthesis in \( \text{C. neoformans} \) (53). Deletion of the phosphatase \( \text{Ppg1} \) also reduces melanin production, but \( \text{ppg1} \) mutants have additional defects, such as reduced capsule size. While \( \text{ROM2} \) is dispensable, \( \text{Lrg1} \) and two other genes (\( \text{SSD1} \) and \( \text{PUD4} \)) are important for cell wall integrity in \( \text{C. neoformans} \), which lacks homologs of several yeast membrane-associated stress sensors (49). Therefore, the regulatory mechanisms for maintaining cell wall integrity in \( \text{S. cerevisiae} \) and \( \text{C. neoformans} \) appear to be different. The \( \text{C. neoformans} \) genome also lacks significant homologs of several yeast membrane-associated stress sensors, including \( \text{Slg1}, \text{Wsc2}, \) and \( \text{Mid2} \) (49).

The \( \text{Slt2} \) homolog has been identified but not functionally characterized for several other human pathogens, including \( \text{A. fumigatus} \), \( \text{P. carinii} \), \( \text{P. marneffei} \), \( \text{Histoplasma capsulatum} \), \( \text{Blastomyces dermatitidis} \), and \( \text{Coccidioides posadasii} \). In \( \text{P. carinii} \), expression of the \( \text{MKP1} \) MAPK gene and \( \text{PeBCK1} \) can partially complement the yeast \( \text{slt2} \) and \( \text{bck1} \) mutants, respectively (45, 137), but their functions in \( \text{P. carinii} \) are not clear. Mpk1 contains a unique phosphorylation motif (TEY MTEY). Dual phosphorylation of Mpk1 at T186 and Y188 is required for its kinase activity but not for its ability to partially


TABLE 3. HOG1 homologs in pathogenic fungi

| Fungal pathogen          | MAPK     | Major function(s)                        |
|--------------------------|----------|------------------------------------------|
| M. grisea                | Osm1     | Osmoregulation and stress response       |
| C. lagenarium            | Osc1     | Hypersoromotic stress response, sensitivity to fludioxonil |
| B. oryzae                | Srm1     | Osmoregulation and stress response       |
| C. parastica             | Cpmk1    | Virulence, pigmentation, conidiation, laccase production |
| M. graminicola           | MgHog1    | Pathogenicity, osmoregulation, stress response, transition from yeast-like growth to filamentous growth |
| B. cinerea               | BcSak1    | Pathogenicity, osmoregulation, stress response, macroconidiation, appressorium formation |
| C. albicans              | CaHog1    | Virulence, stress response, cell wall biosynthesis, morphology |
| C. neoformans            | Hog1     | Virulence, stress response               |
| A. fumigatus             | SakA     | Stress response                          |


**OSMOREGULATION/STRESS RESPONSE PATHWAY**

Hog1 and its homologs in filamentous fungi have the TGY dual phosphorylation site, a hallmark of stress-activated MAPKs. While Hog1 has limited functions besides osmoregulation in S. cerevisiae, its homolog in pathogens is involved in pathogenesis and response to various stresses.

**Plant pathogenic fungi.** Unlike what is seen for the other two MAPKs, the role of HOG1 homologs in pathogenesis differs drastically among plant pathogens, probably due to differences in host defensive responses or infection mechanisms. In M. grisea, OSM1 is dispensable for plant infection (38). Although the osm1 mutant is hypersensitive to desiccation and hypersoromotic stress, it has no defect in conidiation and appressorium function. In the presence of 0.4 M NaCl, conidia of the osm1 mutant form multiple appressoria, suggesting that OSM1 suppresses inappropriate activation of the PMK1 pathway under hypersoromotic conditions. Deletion of histidine kinase gene HIK1, homologous to OS-1, also has no effect on appressorium function and virulence (103). Unlike the N. crassa os-1 mutant, the hik1 mutant is more sensitive to high concentrations of sugars but not salts, suggesting that M. grisea can distinguish between hypersoromotic stresses caused by these compounds.

Mutants deleted of the HOG1 homolog in C. parastica, Bipolaris oryzae, C. lagenarium, and M. graminicola also are sensitive to hypersoromotic stresses (71, 93, 102, 119). While the osc1 and smr1 deletion mutants of C. lagenarium and B. oryzae are fully pathogenic, the M. graminicola mhgog1 mutant and the B. cinerea bcsak1 mutant are nonpathogenic (Table 3). The mhgog1 mutant fails to switch to filamentous growth on water agar plates and is defective in melanization and formation of infectious germ tubes (93). In B. cinerea, macroconidiation but not microconidiation is regulated by BcSak1. The bcsak1 mutant is derepressed in sclerotium formation but blocked in appressorium formation and plant penetration (133). In C. parastica, cpmk1 mutants are reduced in virulence and form cankers smaller than those formed by the wild-type strain (119). In several plant pathogenic fungi, this MAPK pathway also regulates responses to oxidative stress and UV irradiation (71, 102). In general, deletion of the Hog1 homolog increases the sensitivity to various oxidants.

In several filamentous fungi, including N. crassa, C. lagenarium, and M. grisea, mutants blocked in the HOG pathway are resistant to phenylpyrrole, dicarboximide, and aromatic hydrocarbon fungicides (71, 103, 157). Treatments with these fungicides stimulate the activation of the Hog1 homolog, glycercol accumulation, and cell swelling or bursting, indicating that fungicidal effects may result from the overstimulation of the osmoregulation pathway. In B. cinerea, the bcsak1 mutant is resistant to dicarboximide but still sensitive to phenylpyrrole or aromatic hydrocarbon fungicides. In contrast, the deletion of BOS1 (OS-1 homolog) confers resistance to all these fungicides. The bos1 mutant also differs from the sak1 mutant in virulence and appressorium formation (133, 142). Phenotypic differences between the bos1 and bcsak1 mutants may be related to the isolates used in these studies, but it is also possible that the Hog pathway in B. cinerea is more complex than that of other fungi.

**Model saprophytic filamentous fungi.** In N. crassa, the Os-2 MAPK cascade plays an important role in osmoregulation, fungicide resistance, and response to oxidative stress (111, 157). Unlike Os-4 (MEK) and Os-5 (MEKK), Os-1 is required only for Os-2 activation by fludioxonil or low osmotic stress. Phosphorylation of Os-2 is still detectable in the os-1 mutant by hyperosmolarity or heat shock (111), suggesting the involvement of other osmosensors. For the two putative response regulators, only Rrg-1 functions upstream from the Os-2 cascade for osmoregulation (59). Rrg-2 is dispensable for osmoregulation but is involved in response to oxidative stress (8).

In A. nidulans, SakA (HogA) is involved in the repression of sexual development, survival of conidia, and response to hyperosmotic and oxidative stresses (51, 64). Under hyperosmotic conditions, reduced growth rate, hyperbranching, abnormal nuclear distribution, and lack of septation near hyphal tips are observed in the sakA mutant incubated at 30°C. However, the same growth and branching defects are not detectable when the mutant is cultured at 37°C. A. nidulans must have an additional gene(s) involved in regulating response to osmotic stress. One candidate is MpkC, which is also activated by Pbs2. Although the deletion of MpkC has no obvious effect on stress response, the sakA mpkC double mutant may be nonviable (46).

**Human pathogens.** The HOG1 homologs have been implicated in responses to oxidative and hyperosmotic stresses in a few human pathogens. In C. albicans, CaHog1 is activated by various stress conditions, including high osmolality, salts, oxidants, heavy metals, farnesol, and UV irradiation (for a review, see the work of Alonso-Monge et al. [2]). Several upstream components of the CaHog1 pathway have also been characterized, including PBS2, SSK1, and SHO1 (5, 23, 125). Activation of Hog1 by oxidative stress is Pbs2 dependent and is mediated by the Ssk1 branch. Because ssk1 sho1 double mutants still respond to hyperosmotic stress and grow on high-osmolality media, additional upstream input may exist in C. albicans for CaHog1 activation. Among three putative C. albicans histidine kinase genes, NIK1, CHK1, and SLN1, that have been characterized, none has a clearly defined role in CaHog1 activation (2, 74). Although the deletion of SLN1 results in the constitutive activation of CaHog1 and a lower growth rate, the shn

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|--------------------------|----------|------------------------------------------|
| M. grisea                | Osm1     | Osmoregulation and stress response       |
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| M. graminicola           | MgHog1    | Pathogenicity, osmoregulation, stress response, transition from yeast-like growth to filamentous growth |
| B. cinerea               | BcSak1    | Pathogenicity, osmoregulation, stress response, macroconidiation, appressorium formation |
| C. albicans              | CaHog1    | Virulence, stress response, cell wall biosynthesis, morphology |
| C. neoformans            | Hog1     | Virulence, stress response               |
| A. fumigatus             | SakA     | Stress response                          |
mutant, similar to the nik1 mutant, is viable when grown under hyperosmotic conditions. Chk1 regulates responses to oxidative stress and but is dispensable for Hog1 activation.

The CaHOG1 pathway also plays a role in cell wall biosynthesis and integrity. The cahog1 and capbs2 deletion mutants have increased susceptibility to β-1,3-glucanases and are defective in chlamydospore formation (1, 42). Approximately 25% of genes with altered expression levels in the ssk1 mutant are related to cell wall and stress adaptation functions, including CHK1, HSP12, AHP1, and FLO1 (23). In addition, the CaHOG1 pathway has a repressive effect on filamentous growth, and it is important for pathogenesis (2). The cahog1 and capbs2 mutants are derepressed in hyphal formation, resistant to iprodione and fluoxidonil, and reduced in virulence, probably due to the increased sensitivity to oxidative stress generated by the host immune cells. The ssk1 mutation is hypersensitive to oxidative stress and human neutrophils and avirulent in an invasive murine model (94). Although their molecular mechanisms remain to be determined, the NIK1, CHK1, and SLN1 histidine kinase genes all are required for virulence and cell wall integrity in C. albicans (for a review, see the work of Kruppa and Calderone [74]).

In C. neoformans, Pbs2 and Hog1 are functionally conserved for regulating responses to UV radiation and hyperosmotic stress in both a highly virulent serotype A stain, H99, and a less virulent serotype D strain, JEC21. However, their functions in responses to elevated temperature (40°C) and oxidative stress appear to be different (6). While the hog1 mutant of H99 is hypersensitive to H2O2, the JEC21 hog1 mutant is resistant. In H99 but not in JEC21, the Hog1 MAPK cascade negatively regulates the mating processes and production of melanin and capsule. The mating ability and production of mating pheromone, capsule, and melanin are increased in the hog1 and pbs2 mutants of H99. In H99, fluoxidonil treatment activates the Hog1 pathway and causes growth arrest, glycerol accumulation, and cell swelling. The hog1 and pbs2 mutants are resistant to fluoxidonil (69). Hog1 is constitutively phosphorylated in H99 under normal conditions and rapidly dephosphorylated after exposure to 1 M NaCl. In JEC21 and a few other serotype D strains tested, like Hog1 in yeast, Hog1 in C. neoformans is rapidly phosphorylated in response to hyperosmotic stress. Nuclear localization of Hog1 is associated with its activation in H99 but less dependent on its phosphorylation in JEC21. The unique activation pattern of Hog1 is widespread in serotype A strains and in some clinical serotype D isolates, suggesting that C. neoformans may have adapted this pathway to control differentiation and virulence at the subspecies level, probably specific to environmental niches.

The hog1 and pbs2 mutants have attenuated virulence in infection assays with a murine cryptococcosis model. The pbs2 mutant is less virulent than the hog1 mutant, suggesting that Pbs2 has additional downstream targets that contribute to virulence. Similar to the hog1 and pbs2 mutants, deletion of a response regulator homologous to Ssk1 results in resistance to fluoxidonil, enhanced mating efficiency, and increased sensitivity to various stresses (7). The ssk1 mutant also produces more melanin and capsule. However, the ssk1 mutant is less sensitive to hyperosmotic stress than the pbs2 and hog1 mutants. Although Ssk1 is required for Hog1 phosphorylation under normal conditions and in response to fluoxidonil, Hog1 is still activated by high osmolarity in the ssk1 mutant. Therefore, Ssk1 is a major but not the only response regulator of the Pbs2-Hog1 pathway and is important but not essential for osmoregulation. A second response regulator is an Skn7 homolog, which governs resistance to oxidants and Na+ ions but may be not functionally related to the Hog1 pathway (150).

C. neoformans has seven putative histidine kinase genes (TCO1 to TCO7). One of them (TCO6) appears to be essential, but deletion of any other TCO genes individually does not cause hypersensitivity to high osmolarity or UV irradiation (7). Tco1 likely is a key sensor for negative regulation of melanin synthesis via the Hog1 pathway but has no effect on capsule production. Tco2 may play a role in Hog1 activation in response to oxidative stress. The tco2 mutant has increased sensitivity to oxidative stress but is less sensitive than the hog1 or pbs2 mutant. Tco2 also has overlapping functions with Tco1 in mediating fluoxidonil sensitivity and Hog1 dephosphorylation in response to methylglyoxal (7). The tco1 tco2 double mutant but not the tco1 mutant or the tco2 mutant is as sensitive to hyperosmolarity as the hog1 mutant or the pbs2 mutant. Therefore, the Hog1 MAPK pathway may have multiple upstream sensors with shared and distinct functions.

In A. fumigatus, SakA and MpkC MAPKs both have the TGY phosphorylation motif, but they differ in functions. SakA is required for responses to heat shock, hyperosmotic, and oxidative stresses. It is also involved in the negative regulation of conidium germination under nitrogen- or carbon-deficient conditions (41, 155). In contrast, MpkC is dispensable for stress responses and conidium germination, but it may be involved in nutrient sensing, because the mpkC deletion mutant is defective in hyphal growth and conidium germination with sorbitol or mannitol as the sole carbon source. The expression pattern of mpkC also differs from that of sakA under various growth conditions, and only the sakA mutant is resistant to fluoxidonil (66, 124). For putative upstream sensors, an Sln1 homolog, tcsB, and the OS-1 homolog fos-I are dispensable for the response to hyperosmotic or oxidative stress (41, 120), but fos-I may play a role in cell wall assembly and conidiophore development.

CONCLUDING REMARKS

The Fus3/Kss1 homolog is more extensively studied than the other two MAPKs in fungal pathogens. In general, this well-conserved MAPK pathway is essential for regulating plant infection processes in phytopathogenic fungi, but it plays a lesser or no role in the virulence of human pathogens (Fig. 2). In the multihost pathogen F. oxysporum, the fmk1 mutant is nonpathogenic on plants but fully pathogenic in the murine model (113). In plant pathogens, this MAPK pathway may regulate the penetration of host physical barriers, such as cuticle and cell wall, which are not encountered by human pathogens. The Slt2 homologs also are essential for plant infection, but the functions of the HOG1 pathway vary among phytopathogenic fungi. In human pathogens, both the HOG1 and cell wall integrity pathways play important roles in virulence, probably for adaptation to physiological conditions and immune responses in the host. Interestingly, three classes of ex-
standing fungicides interfere with the HOG pathway in several fungi. Components of this pathway may be suitable as targets for developing new fungicides.

Although the MEKK-MEK-MAPK cascades are conserved, the upstream signal inputs and downstream transcription factors in pathogens are different from those of *S. cerevisiae*. Fungal pathogens may have novel receptors for sensing host and environmental signals to regulate penetration and infectious growth. Although Ras proteins have been shown to activate downstream MAPKs in several fungi, sequenced fungal genomes lack significant homologs of receptor kinase genes that function upstream from Ras in mammalian cells. Related to this subject, pathway specificity in fungal pathogens is not well studied. To date, no Ste5 homolog or Ste5-like scaffold protein in pathogenic fungi has been identified. With genomic resources becoming available for more and more fungi, comparative and functional genomic analyses will be useful to identify the missing regulatory and structural components of these MAPK pathways and their downstream targets or network of transcription factors.

Another interesting area is the interaction between the MAPK cascades and other signaling pathways. Several studies have indicated that cross talk occurs among MAPK pathways in fungal pathogens (42, 43, 69). However, the exact molecular mechanisms regulating their interaction are not clear, and the relationship between these pathways during infection may be more complex than what has been observed in *in vitro* cultures. MAPKs are also known to interact with the cAMP signaling network of *C. albicans* [43]. The roles of these genes in fungal pathogenesis are shaded in black for essential, gray for important but not essential, and white for dispensable.

**Acknowledgments**

We thank Paul Tudzynski, Youliang Peng, and Antonio Di Pietro for communicating unpublished results. We also thank Larry Dunkle and Stephen Goodwin for critical reading of the manuscript.

This is journal article no. 18184 of the Purdue University Agricultural Experiment Station.

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