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Review

Comparative genomics of MAP kinase and calcium–calcineurin signalling components in plant and human pathogenic fungi

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ABSTRACT

Mitogen-activated protein kinase (MAPK) cascades and the calcium–calcineurin pathway control fundamental aspects of fungal growth, development and reproduction. Core elements of these signalling pathways are required for virulence in a wide array of fungal pathogens of plants and mammals. In this review, we have used the available genome databases to explore the structural conservation of three MAPK cascades and the calcium–calcineurin pathway in ten different fungal species, including model organisms, plant pathogens and human pathogens. While most known pathway components from the model yeast *Saccharomyces cerevisiae* appear to be widely conserved among taxonomically and biologically diverse fungi, some of them were found to be restricted to the *Saccharomycotina*. The presence of multiple paralogues in certain species such as the zygomycete *Rhizopus oryzae* and the incorporation of new functional domains that are lacking in *S. cerevisiae* signalling proteins, most likely reflect functional diversification or adaptation as filamentous fungi have evolved to occupy distinct ecological niches.

1. Introduction

Adaptation to changes in the environment is crucial for viability of all organisms. In fungi, conserved signal transduction pathways control fundamental aspects of growth, development and reproduction. Two important classes of fungal signalling pathways are mitogen-activated protein kinase (MAPK) cascades and the calcium–calcineurin pathway. MAPK cascades are characterized by a three-tiered module comprising a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK) and the MAPK which is activated by dual phosphorylation of conserved threonine and tyrosine residues within the activation loop (Chang and Karin, 2001). The calcium–calcineurin pathway functions via the Ca^{2+}-binding protein calmodulin and the calmodulin-dependent serine–threonine phosphatase, calcineurin (Chin and Means, 2000). There is evidence for crosstalk between the MAPK and the calcium–calcineurin pathways, since the mating MAPK cascade regulates certain upstream components of the calcium–calcineurin pathway (Muller et al., 2003). Fungal MAPK and calcium signalling cascades are triggered by an array of stimuli and target a broad range of downstream effectors such as transcription factors, cytoskeletal proteins, protein kinases and other enzymes, thereby regulating processes such as the cell cycle, reproduction, morphogenesis and stress response (Cyert, 2003; Kraus and Heitman, 2003; Qi and Elion, 2005).

Core elements of MAPK and calcium signalling pathways are required for virulence in a wide array of fungal pathogens of plants and mammals (Kraus and Heitman, 2003; Lee et al., 2003; Lengeler et al., 2000; Zhao et al., 2007). Such a degree of functional conservation is remarkable, considering the taxonomic and biological diversity among these pathogens, but also raises a number of questions regarding the specific role of these pathways in fungal infection. Are virulence defects in signalling mutants simply caused by

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perturbation of general metabolic and developmental processes, or are they related to "true" pathogenicity mechanisms that are specific for host infection? If the latter is true, what are these specific pathogenicity functions and which are the upstream and downstream signalling components that regulate their activity?

The availability of complete genome sequences from an increasing number of pathogenic fungi allows us to approach these questions at the genomic level. Comparative analysis of complete genome sequences from different yeasts and fungi has provided valuable insight into the evolution of genome organisation (Dietrich et al., 2004; Dujon et al., 2004; Kellis et al., 2004), facilitated the identification of regulatory sequences (Cliften et al., 2003) and assisted genome annotation (Dujon et al., 2004). Genome sequences are also valuable tools for the functional analysis of proteins and cellular pathways. At the protein level, comparison of orthologous sequences allows predictions on putative functional domains or key residues, whereas at the pathway level it provides the opportunity to assess the level of evolutionary conservation of specific pathways and to generate new hypotheses for their functional analysis.

In this review, we have explored the structural conservation of MAPK cascades and the calcium–calcineurin pathway in ten different fungi, including the model organisms *Saccharomyces cerevisiae, Ashbya gossypii*, *Neurospora crassa* and *Schizosaccharomyces pombe*, as well as three plant pathogens, *Fusarium graminearum, Magnaporthe grisea* and *Ustilago maydis*, the two human pathogens *Aspergillus fumigatus, Candida albicans*, and the opportunistic pathogen *Rhizopus oryzae*. The study included four species of filamentous ascomycetes from the subphylum *Pezizomycotina* (euascomycetes), one from the subphylum *Taphrinomycotina* (archiascomycetes) and three from the subphylum *Saccharomycotina*, as well as one basidiomycete (*U. maydis*) and one zygomycete (*R. oryzae*), thus covering a broad taxonomic range separated by nearly a billion years of evolution. The analysis addresses the conservation of signalling components beyond the core pathway modules, as well as the existence of paralogues in different organisms and the degree of sequence conservation among the components. Besides comparison of primary sequence, analysis of domain composition and predicted protein size was carried out to assess the quality of annotation in the genome databases.

2. Results

2.1. Pathway components included in the analysis

The following signalling pathways were included in the analysis: the Fus3 and Kss1 mating/filamentation MAPK cascade, the Mpk1 cell integrity MAPK cascade, the osmostress Hog1 MAPK cascade, and the calcium–calcineurin pathway. Database resources and bioinformatic analysis tools used in this study are indicated in Supplementary Table 1. Whenever possible, sequences were retrieved by BLAST (Altschul et al., 1997), using the *S. cerevisiae* sequence for query. Where blast searches with the *S. cerevisiae* sequence failed to retrieve a hit in a given species, orthologues from another species included in the analysis were used for blast analysis. Candidate genes were systematically validated by reciprocal blast, and only those that identified the original protein when used in a blast search of the *S. cerevisiae* genome were considered for further analysis. Multiple alignments, as well as calculations of identity scores, were performed with ClustalW at default settings. Validated candidate sequences were examined for potential annotation errors, and if required the annotation was corrected using the prediction software outlined in Supplementary Table 1. For several signalling components, orthologues from the basidiomycete human pathogen *Cryptococcus neoformans* were included in the analysis to confirm and extend results obtained in *U. maydis*.

Fig. 1 presents a schematic overview of the signalling pathways and their components in *S. cerevisiae*. Table 1 shows the number of orthologues for each component identified in the different fungal species. Identity scores of the *S. cerevisiae* protein with the closest orthologue of each species are provided in Supplementary Table 2. For a number of pathway components, reliable orthologues could not be detected in certain species, either because sequence conservation was too low or because they apparently do not exist. In the following sections, the results of the analysis are summarized for each of the pathways studied.

2.2. The Fus3 and Kss1 MAPK pathways

The Fus3 MAPK cascade mating pathway in *S. cerevisiae* has been characterized in detail (Elion, 2000; Gustin et al., 1998; Kurjan, 1993; Wang and Dohlman, 2004). Signalling is initiated when pheromone binds to the cognate cell surface receptors Ste2 or Ste3. Orthologues of Ste2 and Ste3 were identified in all ascomycetes tested in the study, including putative asexual species. Structural domains, such as the seven transmembrane regions, were well conserved although there were considerable variations in protein size. As reported previously (Bolker et al., 1992), the basidiomycete *U. maydis* has no Ste2 orthologues, but instead has two Ste3 orthologues, Pra1 and Pra2, reflecting the fact that basidiomycetes only have type a, but not type α pheromones. Analysis in *C. neoformans* provided similar results, suggesting that the duplication of Ste3-like receptors occurred early in the basidiomycete clade. Neither Ste2 or Ste3 orthologues could be detected in the zygomycete *R. oryzae*. While this may be due to low sequence homology, an alternative explanation is that this type of receptors is not present in the zygomycetes, which employ a structurally distinct type of pheromones, trisporic acid derivatives, for sexual reproduction (Schimek and Wöstemeyer, 2006).

Once pheromone binds to its cognate receptor, it triggers dissociation of the G protein α subunit Gpa1 from the G protein βγ subunits Ste4 and Ste18. Fungal Gα proteins are divided into three groups according to their structure. *S. cerevisiae* Gpa1 and its orthologues in filamentous fungi belong to class I whereas Gpa2 belongs to class III (Li et al., 2007). In contrast, the two Gα proteins in *S. pombe*, Gpa1 and Gpa2, belong to classes II and III, respectively. Due to its close homology with *S. cerevisiae* Gpa1, *S. pombe* Gpa1 was nevertheless included in the analysis. Gpa1 orthologues from *Saccharomycotina* contain a region of approximately 100 amino acids which is absent in the rest of the fungal species studied. All Gpa1 orthologues are predicted to be prenylated on the N-terminal cysteine residue and myristoylated on an N-terminal glycine residue required for the lipid-anchor to the plasma membrane.

According to a recent study, *R. oryzae* has four class I Gα proteins, RO3G_01120, RO3G_09475, RO3G_0005 and RO3G_00875 (Li et al., 2007). We detected a new member of class I, RO3G_06003, whose original predicted sequence lacked the characteristic N-terminal region of Gα proteins. The sequence annotation was manually corrected using Fgenesh+ to include the sequence of a predicted overlapping EST, resulting in a predicted polypeptide of 353 amino acids containing all typical features of Gα proteins. In contrast, RO3G_00875 was found to be closer to *S. cerevisiae* Gpa2 and was therefore excluded from this analysis. We also identified new class II and class III Gα proteins RO3G_16598 and RO3G_15639, respectively, both of which had not been described previously.

The heterotrimeric βγ subunits Ste4 and Ste18 dissociate from Gα to transmit the signal to the downstream pathway components (Wang and Dohlman, 2004). Only one orthologue of Ste4 and Ste18 was detected in most fungal species, except for *R. oryzae*, in which four orthologues of each subunit were identified (Table 1). For two putative Gβ subunits, RO3G_06062 and RO3G_08023, the pre-

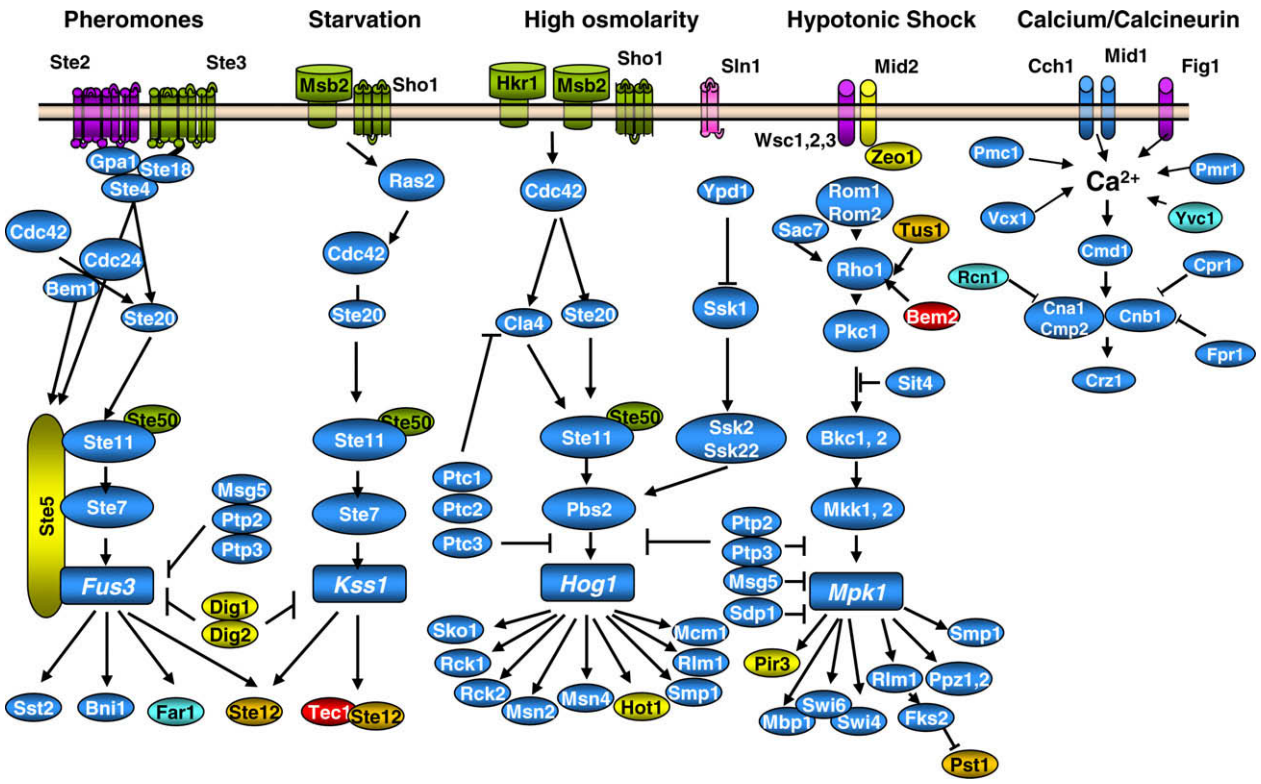

Fig. 1. Schematic view of signalling components included in the study. Colors indicate different degrees of conservation among the fungal species *Saccharomyces cerevisiae, Ashbya gossypii* and *Candida albicans* (hemiascomycetes), *Schizosaccharomyces pombe* (archiascomycetes), *Aspergillus fumigatus, Fusarium graminearum, Magnaporthe grisea and Neurospora crassa* (euascomycetes), *Rhizopus oryzae* (zygomycetes) and *Ustilago maydis* (basidiomycetes): blue, components detected in all species studied; green, all except zygomycetes; orange, all except basidiomycetes; cyan, all except zygomycetes and archiascomycetes; pink, all except zygomycetes, basidiomycetes and archiascomycetes; red, all except euascomycetes; purple, only ascomycetes; yellow: only hemiascomycetes.

dicted sequence in the database lacked the first exon and was corrected manually. Similar to Gα proteins, Gβ subunits from the *Saccharomycotina* differ from those of filamentous fungi in size, due to the presence of additional regions throughout the length of the protein. All Ste18 orthologues contain a predicted prenylation site for lipid-anchoring to the membrane. No clear Ste18 orthologue was found in *S. pombe*, although its annotated genome contains a predicted G protein gamma subunit, Git11, which was included in the analysis.

Downstream of the βγ subunits, the signal is transmitted to the guanine nucleotide exchange factor Cdc24 which activates the G protein Cdc42. Both proteins have one clear, and highly conserved orthologue in all fungal species, except for *R. oryzae* which contains two orthologues of each component. Cdc42 activates the PAK-like protein kinase Ste20 and the adaptor protein Ste50, which cooperate in activating the downstream MAPK module. Ste20 orthologues display considerable divergence in size between species. The predicted *S. pombe* and *R. oryzae* proteins are about 300 amino acids shorter than *S. cerevisiae* Ste20, whereas the orthologues from *M. grisea*, *F. graminearum*, *A. fumigatus* and *U. maydis* are approximately 100 residues longer, and that of *C. albicans* is 200 amino acids longer than the *S. cerevisiae* protein. The Ste20 orthologue Smu1 from *U. maydis* which contains a N-terminal Cdc42-binding domain and a C-terminal kinase domain, was previously found to be non-essential for mating and plant infection (Smith et al., 2004).

Orthologues of *S. cerevisiae* Ste50 were detected in all fungal species studied except for *R. oryzae*. Ste50 is an adaptor that links G protein-associated Cdc42–Ste20 complex to the MAPKKK Ste11 through the presence of a Sterile Alpha Motif (SAM) and a Ras Association (RA) domain (Wu et al., 1999). The SAM domain of the *M. grisea* orthologue Mst50 was previously shown to be essential for its interaction with Mst11 and for appressorium formation (Zhao et al., 2005). Interestingly, Ste50 orthologues of the basidiomycetes *U. maydis* (Ubc2) and *C. neoformans* are approximately double in size and contain a Src Homology 3 (SH3) domain which is lacking in the ascomycete Ste50 proteins. Deletion of the *U. maydis* orthologue Ubc2 was found to impair pheromone responses and virulence. Interestingly, the SH3 domains of Ubc2 were apparently not involved in morphogenesis, but clearly required for pathogenicity, suggesting that they are required for some, but not all signalling outputs of the pathway (Mayorga and Gold, 2001).

Bem1 is a SH3-domain protein that links the Ste5–MAPK cascade complex to upstream activators and specific downstream substrates, thus enabling efficient circuitry for G1 arrest and mating (Lyons et al., 1996). Bem1 orthologues are well conserved in the fungal species studied. *R. oryzae* has two Bem1 orthologues, one of which (RO3G_02285) contains a Rho-GDI domain that is lacking in the other Bem1 proteins. On the other hand, a conserved PB1 domain associated with heterodimer formation is lacking in the Bem1 orthologues of *C. albicans, R. oryzae* and *U. maydis.*

The MAPK module of the *S. cerevisiae* pheromone response pathway is composed of MAPKKK Ste11, MAPKK Ste7 and MAPK Fus3 (Wang and Dohlman, 2004). Ste11 functions in the Fus3 and Kss1 cascade, as well as in the Hog1 pathway by phosphorylating MAPKKs Ste7 and Pbs2, respectively. It contains a sterile alpha motif (SAM) domain involved in interaction with Ste50 (Grimshaw et al., 2004), which is conserved in all fungal orthologues except that of *A. gossypii*. In addition, Ste11 proteins from *F. graminearum, M. grisea, N. crassa* and *R. oryzae* contain a Ras association (RA) domain which is lacking in the other Ste11 orthologues. In *S. cerevisiae*, the RA domain of Ste50, an interaction partner of Ste11, is essential for tethering Ste11 to the plasma membrane through

Table 1
Signalling pathway components included in the study and number of orthologues identified in different fungal species.

Protein	Function	*Saccharomyces cerevisiae*	*Ashbya gossypii*	*Candida albicans*	*Schizosaccharomyces pombe*	*Aspergillus fumigatus*	*Fusarium graminearum*	*Magnaporthe grisea*	*Neurosapora crassa*	*Rhizopus oryzae*	*Ustilago maydis*
Fus3 and Kss1 MAPK pathway											
Ste2	α-Factor pheromone receptor	1	1	1	1	1	1	1	1	0	0
Ste3	a-Factor receptor	1	1	1	1	1	1	1	1	0	2
Gpa1	Guanine nucleotide-binding protein α subunit	1	1	1	1	1	1	1	1	4	1
Ste4	Guanine nucleotide-binding protein β subunit	1	1	1	1	1	1	1	1	4	1
Ste18	Guanine nucleotide-binding protein γ subunit	1	1	1	1	1	1	1	1	4	1
Cdc24	Guanine nucleotide exchange factor	1	1	1	1	1	1	1	1	2	1
Cdc42	Small rho-like GTPase	1	1	1	1	1	1	1	1	2	1
Bem1	SH3-domain protein	1	1	1	1	1	1	1	1	2	1
Ras2	GTP-binding protein	1	1	1	1	1	1	1	1	1	1
Ste20	PAK (p21-activated kinase)	1	1	1	1	1	1	1	1	2	1
Ste50	Protein kinase regulator	1	1	1	1	1	1	1	1	0	1
Ste11	MAP kinase kinase kinase	1	1	1	1	1	1	1	1	1	1
Ste7	MAP kinase kinase	1	1	1	1	1	1	1	1	1	1
Ste5	Pheromone-response scaffold protein	1	1	0	0	0	0	0	0	0	0
Fus3/ Kss1[a]	MAP Kinase	2	2	2	1	1	1	1	1	2	2
Ste12	Transcription factor	1	1	1	0	1	1	1	1	1	0
Bni1	Formin	1	1	1	1	1	1	1	1	1	1
Tec1	TEA/ATTS DNA-binding domain transcription factor	1	1	1	0	1	0	0	0	2	1
Sst2	Regulator of G protein signalling	1	1	1	1	1	1	1	1	2	1
Far1	Cyclin-dependent kinase inhibitor	1	1	1	0	1	1	1	1	0	1
Msg5/ Sdp1[a]	MAPK phosphatase	2	1	1	1	1	1	0	1	2	2
Dig1	Regulatory protein	1	1	0	0	0	0	0	0	0	0
Dig2	Regulatory protein	1	0	0	0	0	0	0	0	0	0
Ptp2/3[a]	Tyrosine-protein phosphatase 3	2	2	2	2	1	1	1	1	2	1
Hog1 MAPK pathway											
Msb2/ Hkr1[a]	Mucin family member	2	2	1	0	1	1	1	1	0	1
Sho1	Transmembrane osmosensor	1	1	1	0	1	1	1	1	0	1
Sln1	Osmosensing histidine protein kinase	1	1	1	0	1	1	1	1	0	0
Ypd1	Phosphorelay intermediate protein	1	1	1	1	1	1	1	1	1	1
Cdc42	Small rho-like GTPase	1	1	1	1	1	1	1	1	2	1
Ste20	PAK (p21-activated kinase)	1	1	1	1	1	1	1	1	2	1
Cla4	PAK (p21-activated kinase)	1	1	1	1	1	1	1	1	1	1
Ssk1	Cytoplasmic response regulator	1	1	1	1	1	1	1	1	1	1
Ste50	Protein kinase regulator	1	1	1	1	1	1	1	1	0	1
Ste11	MAP kinase kinase kinase	1	1	1	1	1	1	1	1	1	1
Ssk2/22[a]	MAP kinase kinase kinase	2	1	1	2	1	1	1	1	1	1
Pbs2	MAP kinase kinase	1	1	1	1	1	1	1	1	1	1
Hog1	MAP kinase	1	1	1	1	2	1	1	1	1	1
Rck1/2[a]	Serine–threonine protein kinase	2	1	1	2	1	1	1	1	1	1
Sko1	Basic leucine zipper (bZIP) transcription factor	1	1	1	1	1	1	1	1	1	1
Msn2/4[a]	Zinc finger transcription factor	2	1	1	1	1	1	1	1	1	1
Hot1	Transcription factor	1	1	1	0	0	0	0	0	0	0
Smp1/ Rlm1[a]	MADS-box transcription factor	2	1	1	1	1	1	1	1	2	1
Mcm1	MADS-box transcription factor	1	1	1	1	1	1	1	1	2	1

Ptc1	Protein phosphatase 2C homolog 1	1	1	1	1	1	1	1	1	1	1
Ptc2/3[a]	Protein phosphatase 2C homolog 2	2	1	1	2	1	1	1	1	1	1
Mpk1 MAPK pathway											
Wsc1	Plasma membrane sensor	1	1	1	1	1	1	1	0	0	0
Wsc2/3[a]	Plasma membrane sensor	2	1	1	1	1	1	1	1	0	0
Mid2/ Mtl1[*]	Plasma membrane sensor	2	1	2	0	0	0	0	0	0	0
Zeo1	Peripheral membrane protein	1	0	0	1	0	0	0	0	0	0
Rom1/2[a]	Guanine nucleotide exchange factor	2	1	1	2	1	1	1	1	2	1
Tus1	Guanine nucleotide exchange factor	1	1	1	1	1	1	1	1	1	0
Sac7	GTPase activating protein	1	1	1	1	1	1	1	1	1	1
Bem2	GTPase activating protein	1	1	1	0	0	0	0	0	2	1
Rho1	GTP-binding protein	1	1	1	2	1	1	1	1	1	1
Pkc1	Protein kinase C	1	1	1	2	1	1	1	1	2	1
Sit4	Type 2A-related serine–threonine phosphatase	1	1	1	1	1	1	1	1	2	1
Bck1	MAP kinase kinase kinase	1	1	1	1	1	1	1	1	1	1
Bck2	MAP kinase kinase kinase	1	1	0	0	0	0	0	0	0	0
Mkk1/2[a]	MAP kinase kinase	2	1	1	1	1	1	1	1	1	1
Mpk1	MAP kinase	1	1	1	1	1	1	1	1	1	1
Swi4/ Mbp1[a]	DNA-binding component of the SBF complex	2	2	1	2	1	1	1	1	2	1
Swi6	Transcription cofactor	1	1	1	1	1	1	1	1	1	1
Smp1/ Rlm1[a]	MADS-box transcription factor	2	1	1	1	1	1	1	1	2	1
Fks2	Catalytic subunit of β-1,3-glucan synthase	1	1	1	4	1	1	1	1	1	1
Pst1	Cell wall protein	1	1	1	2	1	1	1	1	1	0
Ppz1/2[a]	Protein Phosphatase Z	2	1	1	1	1	1	1	1	2	1
Pir3	Protein containing internal repeats	1	1	1	0	0	0	0	0	0	0
Msg5/ Sdp1[a]	MAPK phosphatase	2	1	1	1	1	1	0	1	2	2
Ptp2/3[a]	Tyrosine-protein phosphatase 3	2	2	2	2	1	1	1	1	2	1
Ca^{2+}–calmodulin–calcineurin pathway											
Cch1	Probable calcium-channel protein	1	1	1	1	1	1	1	1	2	1
Mid1	Putative stretch-activated Ca^{2+} channel component	1	1	1	1	1	1	1	1	1	1
Fig1	Integral membrane protein required for efficient mating	1	1	1	1	1	1	1	1	0	0
Cmd1	Calmodulin	1	1	1	2	1	1	1	1	4	1
Cna1/ Cmp2[a]	Calcineurin subunit A	2	1	1	1	1	1	1	1	2	1
Cnb1	Calcineurin subunit B	1	1	1	1	1	1	1	1	1	1
Pmc1	Calcium-transporting ATPase 2	1	1	1	1	3	5	3	2	5	1
Pmr1	Calcium-transporting ATPase 1	1	1	1	1	2	2	2	2	2	2
Vcx1	Vacuolar calcium ion transporter	1	1	1	1	4	4	5	5	5	2
Yvc1	Vacuolar cation channel	1	0	1	0	1	1	1	1	0	1
Fpr1	Peptidyl–prolyl cis–trans isomerase	1	1	1	1	1	1	1	1	1	1
Cpr1	Peptidyl–prolyl cis–trans isomerase	1	1	1	1	1	1	1	1	1	1
Rcn1	Calcineurin inhibitor	1	1	1	1	1	1	1	1	0	1
Crz1	Transcriptional regulator	1	1	1	2	1	1	1	1	2	1

[a] Two paralogues present in *Saccharomyces cerevisiae*.

association of Ste50 with Cdc42 (Truckses et al., 2006). The presence of a RA domain in Ste11 in filamentous species suggests, that the MAPKKK in these fungi could localize to the plasma membrane by directly binding Cdc42. Orthologues of the MAPKK Ste7 were detected in all species. Fuz7 and Mst7 were previously shown to be required for mating and virulence in *U. maydis* (Banuett and Herskowitz, 1994) and *M. grisea* (Zhao et al., 2005).

In *S. cerevisiae,* two MAPKs regulate distinct signalling outputs downstream of Ste7. One of them, Fus3, is essential for mating, whereas the other, Kss1, controls invasive growth and pseudohyphal development (Madhani et al., 1997). In contrast to Fus3, Kss1 can also be activated by Ste7 that is not bound to the Ste5 scaffold (Elion, 1998). Fus3 and Kss1 orthologues play crucial roles during infection in many plant pathogenic fungi including the three phytopathogens surveyed in this study, *M. grisea* (Xu and Hamer, 1996), *F. graminearum* (Jenczmionka et al., 2003) and *U. maydis* (Brachmann et al., 2003; Mayorga and Gold, 1999; Muller et al., 1999). Several species analyzed here have two orthologues of Fus3 and Kss1, including the close relative of *S. cerevisiae*, *A. gossypii*. In *C. albicans*, one of the two MAPKs, Cek2, clusters close to the *Saccharomycotina* sequences whereas the second MAPK, Cek1, which is involved in yeast-hyphal switching, mating efficiency and virulence (Csank et al., 1998; Chen et al., 2002), is more closely related to MAPK orthologues from filamentous Ascomycetes. In *U. maydis,* Kpp2 (Ubc3) and Kpp6 are two orthologues with overlapping functions in mating and plant infection, but Kpp6, which contains an unusual N-terminal domain, appears to be more specific for host penetration (Brachmann et al., 2003). The zygomycete *R. oryzae* also has two orthologues of Fus3 and Kss1 whose functions remain to be determined.

The scaffold protein Ste5 plays an essential role in *S. cerevisiae* pheromone signalling by recruiting the Ste11–Ste7–Fus3 complex to the plasma membrane (Pryciak and Huntress, 1998) and stimulating phosphorelay by proximity effects, oligomerization, and conformational changes (Qi and Elion, 2005). Our analysis failed to detect Ste5 orthologues in any of the fungal species studied except *A. gossypii*. It is possible that other, hitherto unknown, signalling components may carry out the scaffold function in this MAPK pathway.

Phosphorylated Fus3 in *S. cerevisiae* activates downstream effectors such as Ste12, Far1 or Sst2, leading to cell cycle arrest, polarized growth and formation of specialized fusion tubes called shmoos (Elion et al., 1993). Ste12 is a key transcription factor downstream of the pheromone-response cascade, which binds to pheromone response elements (PREs) in the upstream activating sequences of its target genes and, in cooperation with Tec1, also regulates genes involved in invasive growth (Madhani and Fink, 1997). A single Ste12 orthologue was detected in all fungal species examined, except *S. pombe* and *U. maydis*. Lack of Ste12 in *U. maydis* appears to be characteristic for this species rather than for the basidiomycete group, since *C. neoformans* does contain mating type-specific Ste12 orthologues (Wickes et al., 1997). In addition to the characteristic Ste-like homeodomain in the N-terminal region of the protein, Ste12 orthologues from filamentous fungi contain two C-terminal C_2H_2 zinc finger motifs which are lacking in the *Saccharomycotina* (Fig. 2A). The role of the zinc finger domain in Ste12 function is poorly understood. In *M. grisea*, both the Ste-like region and the zinc finger region of Mst12 were required for invasive growth and virulence on rice plants (Park et al., 2004).

Far1 mediates the cell cycle arrest in response to pheromone (Peter et al., 1993), and specifies direction of polarized growth during mating by linking the heterotrimeric G $\beta\gamma$ subunits to the polarity establishment machinery (Butty et al., 1998). The Far1 protein contains a C_3HC_4-type ring zinc finger domain with a predicted role in the ubiquitination pathway. Far1 was recently shown to act as a dosage-dependent regulator of the pheromone response during mating in *C. albicans* (Cote and Whiteway, 2008). Far1 orthologues were found in all species except *S. pombe* and *R. oryzae*. In spite of sharing a low degree of sequence conservation (10–20%), all predicted Far1 proteins have the characteristic ring zinc finger domain. In addition, Far1 orthologues from filamentous fungi, but not from *Saccharomycotina,* contain a pleckstrin homology domain and a von Willebrand factor type A (VWA) domain, indicative of a possible involvement in multiprotein complexes (Fig. 2B). The role of Far1 proteins in fungal pathogenicity has not been addressed experimentally so far.

Sst2 is a GTPase-activating regulator of G protein signalling (RGS) for Gpa1, which regulates pheromone desensitization and prevents receptor-independent signalling of the mating pathway (Dohlman et al., 1996). Orthologues of Sst2 were identified in all species examined, including two orthologues in *R. oryzae*. Annotation errors in the predicted protein database sequences of *F. graminearum* and *M. grisea* were corrected manually. *S. pombe* Sst2 is significantly shorter (480 aa) than the rest of the orthologues (650–780 aa). All Sst2 proteins share the RGS domain, but the predicted *A. gossypii* and *C. albicans* proteins lack a conserved DEP-like segment (residues 50–135) which is required for binding to the cognate G protein-coupled receptor Ste2 (Ballon et al., 2006).

The filamentation and pseudohyphal growth pathway in *S. cerevisiae* is activated by a mucin-like protein, Msb2, consisting of a N-terminal signal peptide, an extracellular serine–threonine-rich repeat region predicted to be highly O-glycosylated, a single transmembrane domain and a short cytoplasmic tail interacting with the downstream component Cdc42 (Cullen et al., 2004). Recently a second mucin-like protein, Hkr1, was shown to function together with Msb2 as an osmosensor in the *S. cerevisiae* Hog1 pathway (Tatebayashi et al., 2007). We detected single orthologues of Msb2, but not Hkr1, in all species studied except for *S. pombe* and *R. oryzae*. Only *A. gossypii* has also an orthologue of Hkr1. All predicted Msb2 proteins contain a putative signal peptide and transmembrane region. While the exact amino acid repeats in the extracellular region are not present in some of the fungal orthologues, the high content of putatively glycosylated serine and threonine residues in this region is maintained, suggesting a conserved role of O-glycosylation in Msb2 function. Similarly, the amino acid sequence of the short cytoplasmatic tail is well conserved among filamentous ascomycetes, indicating an important role of this domain in intracellular signalling.

Another component required specifically for the filamentation and invasive growth pathway upstream of Cdc42, Ste20 and Kss1 is the small GTP-binding protein Ras2 (Mosch et al., 1996). In *C. albicans*, Ras links cellular morphogenesis to virulence by regulating the MAPK and cAMP signalling pathways (Leberer et al., 2001). In *U. maydis*, expression of a dominant active allele of the *ras2* orthologue promoted pseudohyphal growth in a manner dependent on the pheromone-response MAPK cascade (Lee and Kronstad, 2002). Likewise, expression of a dominant active *ras2* allele of *M. grisea* stimulated appressorium formation on non-inductive surfaces in the wild-type strain, but not in the *pmk1* mutant, suggesting that Ras2 functions upstream of the Mst11–Mst7–Pmk1 cascade (Park et al., 2006). A similar signalling role for Ras2 was proposed in *F. graminearum* (Bluhm et al., 2007). In this study, Ras2 orthologues containing predicted palmitoyl and farnesyl groups at the C-terminus for membrane localization were identified in all fungal species studied.

In *S. cerevisiae,* two nuclear protein substrates of Kss1, Dig1 and Dig2, negatively regulate the invasive growth pathway by repressing Ste12 action (Cook et al., 1996). We failed to detect Dig1 and Dig2 orthologues in any of the species analyzed except for the close relative *A. gossypii*, suggesting that a regulatory mechanism other than that mediated by Dig1 and Dig2 must be operating in filamentous fungi.

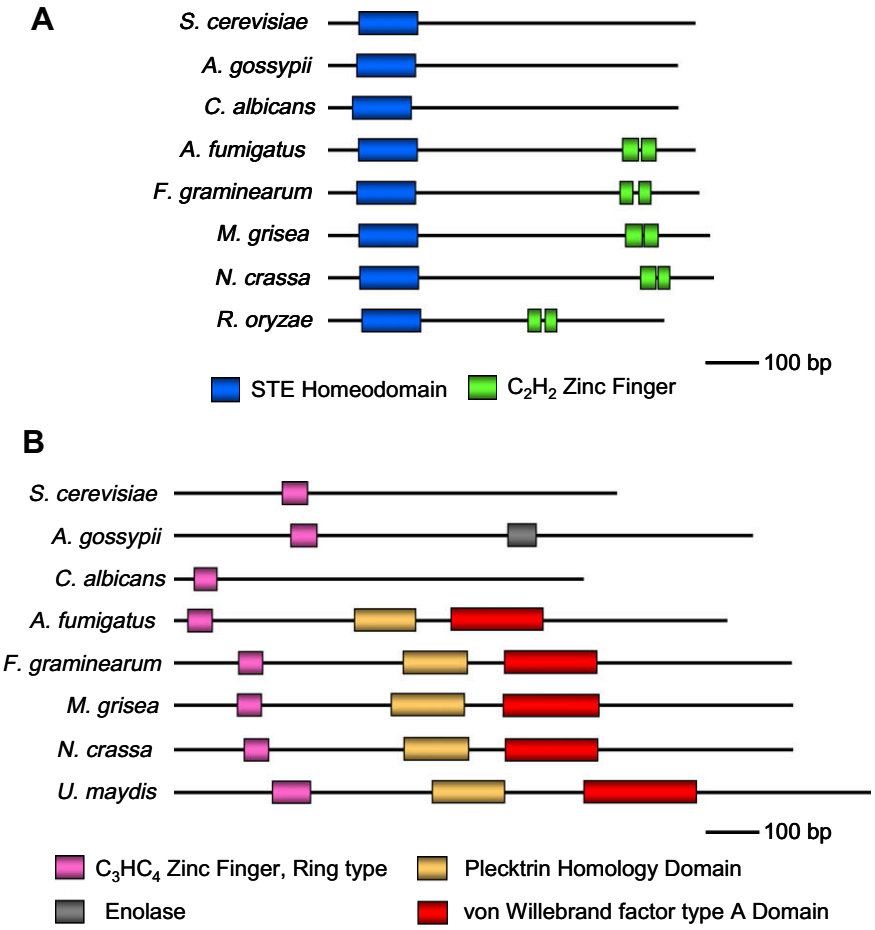

Fig. 2. Scaled cartoon of the domain structure of Ste12 and Far1 orthologues from different fungal species. (A) Ste12 orthologues all contain a characteristic STE homeodomain in the N-terminal region. In addition, filamentous ascomycetes and the zygomycete *R. oryzae* have a C_2H_2 zinc finger domain in the C-terminal region. (B) Far1 orthologues all contain a C_3HC_4 ring type zinc finger domain in the N-terminal region. In addition, filamentous ascomycetes and the basidiomycete *U. maydis* have a pleckstrin homology domain and a von Willebrand factor type A domain, whereas *A. gossypii* has an enolase domain.

For activation of genes involved in filamentous and invasive growth, Ste12 forms a heterodimer with the TEA/ATTS family transcription factor Tec1 to bind filamentation response elements (FREs) (Madhani and Fink, 1997). It has been suggested that Tec1 orthologues of pathogenic fungi could be of interest due to their possible implication in virulence (Madhani and Fink, 1998). Indeed, the Tec1 orthologue of *C. albicans* regulates hyphal development and virulence (Schweizer et al., 2000). However, in our survey we failed to detect clear Tec1 orthologues in *S. pombe* and the filamentous ascomycetes *F. graminearum, M. grisea* and *N. crassa*. Interestingly, the human pathogen *A. fumigatus* contains a Tec1 orthologue that is highly similar to *A. nidulans* AbaA, a transcription factor with an ATTS DNA-binding motif required for conidiophore development (Andrianopoulos and Timberlake, 1994). The role of Tec1 in virulence of *A. fumigatus* has not been explored so far.

In summary, most components of the Fus3 and Kss1 MAPK cascades are well conserved among the fungal species studied, including basidiomycetes and zygomycetes. Exceptions are the pheromone-response scaffold protein Ste5 and the two Ste12 regulators Dig1 and Dig2. A noteworthy finding is the multiplicity of heterotrimeric G protein subunits in the zygomycete *R. oryzae*.

2.3. The Hog1 MAPK pathway

The high osmolarity glycerol (HOG) pathway mediates responses to hyperosmotic shock and to other stresses (Hohmann et al., 2007). In *S. cerevisiae*, the Hog1 pathway has two upstream branches that converge on the MAPKK Pbs2 (see Fig. 1). One branch consists of a phosphorelay system composed of the sensor histidine kinase Sln1, the phosphotransfer protein Ypd1 and the response regulator Ssk1. Hyperosmotic shock deactivates Sln1, leading to enhanced levels of dephospho-Ssk1 and sequential phosphorylation of the MAPKKKs Ssk2 and Ssk22, the MAPKK Pbs2 and the MAPK Hog1 (Posas et al., 1996). Whereas Sln1 is the only histidine kinase present in *S. cerevisiae*, other fungi contain multiple histidine kinases which can be classified into different groups according to their topology (Catlett et al., 2003). Members of group VI, which includes Sln1, contain two transmembrane domains in addition to the characteristic phosphoacceptor, ATP-binding and response regulator receiver domains. The *C. albicans* orthologue *CaSLN1* was shown to be involved in hyphal formation and virulence (Nagahashi et al., 1998), whereas deletion of the *A. fumigatus* orthologue TcsB produced no clear phenotype (Du et al., 2006). Orthologues, in which the critical Sln1 domains are conserved, were identified in most species studied. However, *S. pombe, U. maydis* and *R. oryzae* genomes do not contain any member of group VI. By contrast, a single orthologue of the phosphorelay protein Ypd1 and of the cytoplasmic response regulator Ssk1 was identified in all species. *C. albicans* mutants lacking Ssk1 are avirulent in an invasive murine model and fail to adhere to human cells (Calera et al., 2000). The orthologous RRG-1 response regulator from *N. crassa* was recently shown to function upstream of the osmoresponse MAPK pathway, and to regulate asexual develop-

ment, female fertility, osmotic stress and fungicide resistance (Jones et al., 2007).

The MAPKKKs Ssk2 and Ssk22 function downstream of the Sln1 branch to activate the MAPKK Pbs2 and the MAPK Hog1. All fungi surveyed including, *A. gossypii*, contain a single orthologue of Ssk2/Ssk22, except for *S. pombe* which has two paralogues clustering in a separate branch with the basidiomycetes and zygomycetes.

In the second osmosensing branch, the plasma membrane protein Sho1 recruits the MAPKKK Ste11 and the MAPKK Pbs2 to the cell surface. Orthologues of Sho1 displaying conserved structural features were detected in all species studied, except *S. pombe* and *R. oryzae*. Sho1 orthologues were found to link oxidative stress to morphogenesis and cell wall biosynthesis in *C. albicans* (Bermejo et al., 2008; Roman et al., 2005) and to regulate hyphal growth, morphology and oxidant adaptation in *A. fumigatus* (Ma et al., 2008), but were dispensable for virulence in both human pathogens. The role of Sho1 in fungal pathogenicity on plants has not been determined yet.

Similar to the Fus3 and Kss1 pathway, activation of Ste11 by the Sho1 branch of the osmoresponse pathway requires the small G protein Cdc42, the adaptor protein Ste50 and the PAK kinase Ste20 (Raitt et al., 2000). A second PAK kinase, Cla4, functions in parallel with Ste20 (Tatebayashi et al., 2006). In this study we detected a single Cla4 orthologue in all the fungal species studied.

The MAPKK Pbs2 serves as a scaffold for several components of the HOG pathway and integrates the two upper branches of the pathway. Phosphorylation of Pbs2 via Ssk2 and Ssk22 occurs under severe osmotic stress (Posas et al., 1996), whereas its activation by Ste11 takes place under less severe hyperosmotic conditions, whereby Pbs2 acts as a scaffold for Sho1, Ste11 and Hog1 (Posas and Saito, 1997). Single Pbs2 orthologues showing a conserved domain composition were detected in all the species studied.

All fungi surveyed contain a single orthologue of the osmoresponse MAPK Hog1, except for *A. fumigatus* which has two orthologues, similar to other *Aspergilli* (Miskei et al., 2009). The role of Hog1 orthologues has been studied in different fungal pathogens. *C. albicans hog1* mutants are de-repressed in serum-induced hyphal formation and show reduced virulence (Alonso-Monge et al., 1999). In *A. fumigatus,* two Hog1 orthologues, SakA and MpkC play distinct roles in the response to oxidative and nutritional stresses but are not required for virulence (Reyes et al., 2006; Xue et al., 2004). Likewise, *M. grisea* mutants lacking the Hog1 orthologue Osm1 were sensitive to osmotic stress, but formed functional appressoria and were fully virulent on rice plants (Dixon et al., 1999). In *F. graminearum,* deletion mutants of MAPKKK FgOs4, MAPKK FgOs5 and MAPK FgOs2 showed markedly enhanced pigmentation and failed to produce trichothecenes in aerial hyphae, although their virulence phenotype has yet to be determined (Ochiai et al., 2007).

Downstream targets of Hog1 in *S. cerevisiae* include the MAPK-dependent protein kinases Rck1 and Rck2 (Bilsland et al., 2004), as well as the transcription factors Sko1 (Rep et al., 2001), Msn2 and Msn4 (Martinez-Pastor et al., 1996), Hot1 (Rep et al., 2000), Smp1 and Rlm1 (de Nadal et al., 2003), and Mcm1 (Yu et al., 1995). Our study indicates that all these downstream components are well conserved across the fungal phyla, except for Hot1 whose presence is limited to the *Saccharomycotina*. In most cases, single orthologues of each component were detected, although *R. oryzae* has two orthologues for the MADS-box transcription factors Smp1, Rlm1 and Mcm1. In summary, the components of the Hog MAPK pathway are very well conserved throughout the fungal kingdoms, with the exception of the transcription factor Hot1 which is specific for the *Saccharomycotina*.

2.4. The Mpk1 cell integrity pathway

The Mpk1 cell integrity cascade is responsible for orchestrating changes in the cell wall through the cell cycle and in response to various forms of stress (Levin, 2005). This pathway is activated by the integrin-like proteins Wsc1,2,3 which share a conserved extracellular motif of eight cysteines (Verna et al., 1997). A second activator of the Mpk1 pathway is Mid2, an O-glycosylated plasma membrane protein that interacts with Rom2, the guanine nucleotide exchange factor for Rho1, and with the cell integrity pathway protein Zeo1 (Philip and Levin, 2001). Orthologues of the Wsc1 and Wsc2 and 3 proteins are present in most ascomycetes, whereas Mid2 and Zeo1 are restricted to the *Saccharomycotina*. Wsc1 and Mid2 are linked to the guanine nucleotide exchange factors (GEFs) Rom1 and 2 which activate the GTPase Rho1 (Ozaki et al., 1996). Similar to *S. cerevisiae*, Rho1 is required for cell viability in *C. albicans* (Smith et al., 2002). In contrast, *rho1* knockout mutants of the soilborne pathogen *Fusarium oxysporum* were viable and showed drastically reduced virulence on plants, but retained full virulence on immunodepressed mice (Martinez-Rocha et al., 2008). Both Rom1 and 2, as well as Rho1, are widely conserved in fungi, with a single orthologue of the two GEFs present in almost all species studied. In addition, Rho1 activity in *S. cerevisiae* is regulated by the GEF Tus1 and the GTPase activating proteins Sac7 and Bem2 (Levin, 2005). Both Tus1 and Sac7 are widely conserved among the fungal species studied. By contrast, Bem2 was detected in the *Saccharomycotina*, *U. maydis* and *R. oryzae*, but not in the other ascomycetes. The evolutionary and functional implications of this interesting differential distribution are currently unknown.

Rho1 activates protein kinase C (PKC) 1 which, in turn, activates a three-tiered kinase module composed of the MAPKKK Bck1, the MAPKKs Mkk1 and Mkk2 and the MAPK Mpk1 (Levin, 2005). In contrast to most fungi surveyed in this study, *R. oryzae* has two Pkc1 orthologues, as previously described for *S. pombe* (Kobori et al., 1994). Single orthologues were detected for each of the three components of the Mpk1 MAPK module. The role of Mpk1 orthologues has been determined in a number of fungal pathogens. Mps1 is essential for conidiation, appressorial penetration, and plant infection in *M. grisea* (Xu et al., 1998). In *F. graminearum*, Mgv1 is required for hyphal fusion and heterokaryon formation (Hou et al., 2002). In *C. albicans*, Mkc1 regulates cell wall integrity, growth at high temperatures, morphological transition and pathogenesis (Diez-Orejas et al., 1997). Recently, it was shown that the *A. fumigatus* orthologue MpkA controls cell wall signalling and oxidative stress response, but is dispensable for virulence (Valiante et al., 2008).

Mpk1 regulates multiple nuclear targets, including the SBF complex which is formed by DNA-binding component Swi4, Mbp1 and co-factor Swi6 and acts as a transcriptional activator of cell cycle-dependent genes (Nasmyth and Dirick, 1991). Two Swi4 and Mbp1 orthologues were found in *A. gossypii, S. pombe* and *R. oryzae*, opposed to only one in the other fungi. By contrast, all species studied have a single Swi6 orthologue.

A second nuclear target of Mpk1 is the MADS-box transcription factor Rlm1 which regulates the expression of at least 25 genes in *S. cerevisiae*, most of which have been implicated in cell wall biogenesis and function (Jung et al., 2002). These include the glycosylphosphatidylinositol (GPI)-anchored protein Pst1, the O-glycosylated protein Pir3 (Jung and Levin, 1999) and the glucan synthase catalytic subunit Fks2. Conserved orthologues were detected for Rlm1 itself, Pst1 (except in *U. maydis*) and Fks2 (including four orthologues in *S. pombe*). By contrast, no Pir3 orthologues were found outside of the *Saccharomycotina*.

The serine/threonine protein phosphatases Ppz1 and Ppz2 are key regulators of K^+ and pH homeostasis, thus determining salt tolerance, cell wall integrity and cell cycle progression (Yenush et al., 2002). In contrast to *S. cerevisiae*, all fungal species studied with the exception of *R. oryzae* contain only a single Ppz1 and 2 orthologue.

In summary, a high conservation of the Mpk1 MAPK cascade components was detected throughout the species studied, except for certain plasma membrane sensors such as Mid2, and for the downstream effector protein Pir3.

2.5. MAPK-regulatory protein phosphatases

Tyrosine, serine/threonine and dual-specificity phosphatases co-ordinately dephosphorylate and thereby inactivate different MAPKs in *S. cerevisiae* (Martin et al., 2005). The dual-specificity protein phosphatase Msg5 and the tyrosine phosphatase Ptp3 dephosphorylate Fus3, thereby regulating the adaptive response to pheromone (Zhan et al., 1997). Ptp2 and 3, as well as the type 2C protein phosphatase (PP2C) Ptc1 antagonize the osmosensing MAPK cascade by dephosphorylating Hog1 (Warmka et al., 2001; Wurgler-Murphy et al., 1997). The stress-inducible dual-specificity MAPK phosphatase Sdp1 negatively regulates the cell integrity pathway by dephosphorylating Mpk1 (Hahn and Thiele, 2002). In general, orthologues of these protein phosphatases were detected in all fungal species surveyed, suggesting that the mechanisms of regulating MAPK activity via dephosphorylation are broadly conserved in fungi. We detected mostly single orthologues, except for the *Saccharomycotina* and, in some cases, *U. maydis* and *R. oryzae*. The *M. grisea* genome database lacked an annotated Msg5 orthologue, but inspection of a genomic region from the excluded reads of annotated strain 70-15 and subsequent analysis of a genomic fragment from a field isolate (Y34) revealed the presence of a reliable Msg5 orthologue which was included in the analysis.

2.6. The calcium/calcineurin signalling pathway

Calcium signalling through the Ca^{2+}-binding protein calmodulin and the Ca^{2+}–calmodulin-dependent phosphatase calcineurin has been implicated in a multitude of processes, including stress response, mating, budding, and actin-based processes (Cyert, 2001) as well as in determining tolerance to antifungal drugs (Cruz et al., 2002; Del Poeta et al., 2000; Kontoyiannis et al., 2003; Sanglard et al., 2003; Steinbach et al., 2007; Walker et al., 2008). Cellular calcium levels in *S. cerevisiae* are regulated by multiple channels and transporters, including the voltage-gated high-affinity calcium channel Cch1 which functions together with the stretch-activated cation channel Mid1 (Locke et al., 2000). In contrast to the transmembrane protein Cch1, Mid1 is anchored to the membrane by a GPI-anchor.

A third player in calcium regulation is Fig1, an integral membrane protein required for efficient mating which may participate in the low affinity Ca^{2+} influx system, affecting intracellular signalling and cell–cell fusion (Muller et al., 2003). Cch1, Mid1 and Fig. 1 have single orthologues in all fungal species studied, except for the presence of two putative Cch1 orthologues in *R. oryzae,* and for the absence of Fig. 1 orthologues in *U. maydis* and *R. oryzae*. Both *R. oryzae* Cch1 sequences in the database contained annotation errors that were corrected manually. The role of these upstream sensors in filamentous fungi is largely unknown. Recently, a *N. crassa* mutant lacking a Mid1 orthologue was found to be affected in calcium homeostasis and vegetative growth (Lew et al., 2008). Moreover, deletion of a Cch1 orthologue affected ascospore discharge and mycelial growth in *F. graminearum* (Hallen and Trail, 2008).

In addition to plasma membrane channels, the calcium-transporting ATPases Pmr1 and Pmc1, the vacuolar ion exchanger Vcx1 and the vacuolar cation channel Yvc1 also contribute to regulation of cellular calcium levels and calcium signalling. Strikingly, while *S. cerevisiae* only has one of each of these components, filamentous fungi consistently contain two Pmr1 orthologues and most of them have between three and five orthologues of Pmc1 and Vcx1. Whether these multiple components have distinct or redundant functions is currently an open question.

S. cerevisiae calmodulin is a small, essential Ca^{2+}-binding protein encoded by *CMD1*, which has both Ca^{2+}-dependent and independent targets. One of the major Ca^{2+}-dependent targets is calcineurin, a Ca^{2+} and calmodulin dependent phosphatase. *S. cerevisiae* calcineurin is composed of a heterodimer of a catalytic A subunit encoded by *CMP2* and *CNA1*, and of a regulatory B subunit encoded by *CNB1*. In the presence of stimulatory levels of Ca^{2+}, calmodulin binds to the A subunit of calcineurin, displacing an autoinhibitory domain. Calmodulin–calcineurin-activated gene expression is triggered by multiple external cues, including high temperature, high concentrations of ions, cell wall stress and exposure to mating pheromone (Cyert, 2003; Kraus and Heitman, 2003). In our survey we detected single orthologues for Cmd1, Cnb1, Cna1 and Cmp2, respectively, except for *R. oryzae* which has two Cna1 and Cmp2 orthologues. All these components showed a high degree of sequence identity (40–90%) among the fungal species studied. In the human pathogens *C. albicans*, *C. neoformans* and *A. fumigatus*, calcineurin was required for survival in serum and for virulence (Bader et al., 2003; Blankenship et al., 2003; Da Silva Ferreira et al., 2007; Fox et al., 2001; Odom et al., 1997; Steinbach et al., 2006, 2007). In the plant pathogen *Sclerotinia sclerotiorum*, the calcineurin orthologue controls sclerotial development and infection (Harel et al., 2006).

A key target of calcineurin is the zinc finger transcription factor Crz1, whose nuclear localization is positively regulated by calcineurin-mediated dephosphorylation (Cyert, 2001). Orthologues of Crz1 were recently shown to act as virulence factors in the human pathogen *A. fumigatus* (Cramer et al., 2008; Soriani et al., 2008) and the plant pathogen *Botrytis cinerea* (Schumacher et al., 2008). The fungal species surveyed in this study all contain a single orthologue of Crz1, except for *S. pombe* and *R. oryzae* which have two orthologues.

Fpr1 and Cpr1, two peptidyl–prolyl cis–trans isomerases that catalyze the cis–trans isomerization of peptide bonds N-terminal to proline residues, are the cellular targets of the drugs Cyclosporin A and FK506 and function as inhibitors of calcineurin through formation of a ternary complex (Wang and Heitman, 2005). Orthologues of these two proteins were detected in all fungal species studied, although their role in calcineurin regulation has not been determined. Similarly, orthologues of the Rcn1 protein, which is involved in regulation of calcineurin during calcium signalling (Kingsbury and Cunningham, 2000), were found in all species except *R. oryzae*.

In summary, all the components of the calcium–calcineurin pathway were highly conserved throughout the different fungal kingdoms. A noteworthy feature, whose biological significance is unclear, is the multiplicity of the calcium-transporting ATPases and the vacuolar calcium channels in filamentous fungal species.

3. Conclusions

Three conserved MAPK cascades and the calcium–calcineurin pathway play crucial roles in fungal pathogenicity. Here we have taken advantage of the availability of complete fungal genome sequences to survey the inventory of predicted MAPK and calcium–calcineurin signalling components in ten fungal species, including several plant and human pathogens, covering a wide array of taxonomical and biological diversity (Fig. 1, Table 1). While most components were found to be conserved among the model yeast *S. cerevisiae* and filamentous fungi, some components such as the scaffold protein Ste5, the regulatory proteins Dig1 and Dig2 and the transcription factor Hot1 are limited to the *Saccharomycotina*. The incorporation of new domains which are lacking in *S. cerevisi-*

ae, such as the RA domain in Ste11, the zinc finger in Ste12, or the pleckstrin homology domain and VWA domains in Far1 (Fig. 2), might reflect functional adaptations as filamentous fungi have evolved to occupy different ecological niches, including their roles as pathogenic agents. The presence of multiple paralogues of many signalling components in the zygomycete *R. oryzae* is striking, although the evolutionary and functional significance of this finding is currently unknown. Thus, while the model yeast *S. cerevisiae* has provided an excellent roadmap of the components of MAPK and calcium–calcineurin pathways, functional analysis in pathogenic species represents the only way to understand the role of these signalling cascades in fungal virulence. So far, most studies have focused on a few core pathway components. While this has provided a useful overview of the general implication of these pathways in fungal virulence, a detailed analysis of the upstream and downstream factors that interact with these core signalling cascades is now clearly necessary. Such an approach should allow a more careful and critical evaluation of the specific role of each signalling pathway in infection-associated functions, and extend our understanding regarding how these conserved signalling cascades have been recruited by fungal pathogens to infect their eukaryotic hosts.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fgb.2009.01.002.

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