Apoptosis-inducing antifungal peptides and proteins

Katrijn De Brucker, Bruno P.A. Cammue and Karin Thevissen[1]
CMPG (Centre of Microbial and Plant Genetics), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium

Abstract

Despite the availability of various classes of antimycotics, the treatment of patients with systemic fungal infections is challenging. Therefore the development of new antifungals is urgently required. Promising new antifungal candidates are antimicrobial peptides. In the present review, we provide an overview of antifungal peptides isolated from plants, insects, amphibians and mammals that induce apoptosis. Their antifungal spectrum, mode of action and toxicity are discussed in more detail.

Introduction

In the U.S.A., the number of patients with sepsis caused by fungi has increased by 207% between 1979 and 2000, resulting in approximately 204 cases per 100 000 persons [1]. *Candida* species are the fourth most common cause of bloodstream infections in the U.S.A. [2] and *Candida albicans* is the most common species of these infections. There are also other fungi, including the filamentous fungi *Aspergillus fumigatus* and *Aspergillus flavus*, that can cause invasive mycoses [3]. The number of antimycotics available for treating invasive mycoses is limited. On the basis of their mode of action, currently available antimycotics can be divided into three major classes: azoles, polyenes and echinocandins. Azoles, such as fluconazole, inhibit 14α lanosterol demethylase, a protein involved in ergosterol synthesis, thereby causing the disruption of cell membrane function and structure, resulting in growth inhibition [4]. Polyenes, on the other hand, interact with ergosterol itself, resulting in cell leakage and cell death. Echinocandins such as caspofungin inhibit the synthesis of the fungal cell wall component β-1,3-glucan by binding to the Fsk1 subunit of β-1,3-glucan synthase [5]. Despite these antifungals, treatment of immunosuppressed patients with systemic fungal infections is challenging owing to different reasons, including development of resistance, toxicity, limited activity spectrum and lack of rapid, microbe-specific diagnosis. To cope with these challenges, the development of new antifungals that target unique structures or functions of fungi, without causing severe side effects in the host, is imperative.

Key words: antifungal agent, antimicrobial peptide, apoptosis, *Candida*, plant defensin.
Abbreviations used: AMP, antimicrobial peptide; AIF1, apoptosis-inducing factor 1; DsS3, dermaseptin S3; GlcCer, glucosylceramide; MAPK, mitogen-activated protein kinase; PAF, *Penicillium chrysogenum* antifungal protein; Pir, protein with internal repeats; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species.
[1]To whom correspondence should be addressed (email karin.thevissen@biw.kuleuven.be).

AMPs (antimicrobial peptides) and proteins are promising new antifungal candidates. These peptides and proteins are produced by a wide range of organisms, from bacteria to plants and humans, and protect the host against pathogens. On the basis of their structure and topology, AMPs can be divided into five classes: (i) AMPs with an α-helical structure; (ii) AMPs with a β-sheet structure; (iii) AMPs with a mixture of α-helical/β-sheet structure; (iv) AMPs with an irregular structure; and (v) macrocyclic AMPs. AMPs form amphipathic structures and are often cationic. Whereas it is generally believed that AMPs act by simply disrupting the target cell membranes, recent research indicates that their mode of action is more complex. For example, several defensins, which have a β-sheet structure and are characterized by the presence of disulfide bridges, induce apoptosis in *C. albicans* [6,7]. Apoptosis is an evolutionarily conserved cell suicide programme that can be used by an organism to eliminate dangerous, superfluous or damaged cells [8]. Whereas apoptosis in multicellular organisms has an obvious advantage as cells die for the benefit of the whole organism, the advantages for yeast to undergo apoptosis are less clear. However, the death of a single cell can be beneficial to the whole population under certain conditions [8]. For example, apoptosis in yeast cells is induced when mating is not successful [9]. Also older and damaged cells undergo apoptosis, resulting in the release of nutrients which stimulates survival of younger and fitter cells [10]. In contrast with apoptosis, necrosis results in disruption of the cell membrane and release of factors involved in immune response stimulation [11]. As such, antifungals that induce apoptosis instead of necrosis are preferred. Assays for apoptotic cell death in yeast are available to investigate clonogenic determination of viability, accumulation of ROS (reactive oxygen species), DNA fragmentation and externalization of phosphatidylserine [12]. A combination of these assays allows precise determination of apoptosis in yeast.

In the present review, an overview of antifungal peptides and proteins that induce apoptosis in susceptible fungi is presented and summarized in Table 1.

Table 1 | **Summary of current knowledge of apoptosis-inducing antifungal peptides and proteins**

Abbreviations: n.d., not determined; HE, human erythrocytes.

	Peptide/ protein	Source	Antifungal spectrum	Interaction partners on fungal envelope	Mode of action	Interacting signalling cascades	Toxicity to mammalian cells
Micro-organism	WH1	*B. amyloliquefaciens*	*R. solani*; *C. albicans*; *F. oxysporium*	n.d.	Inhibition of glucan synthesis; apoptosis; caspase-like activity; cytochrome *c* release	n.d.	n.d.
	PAF	*P. chrysogenum*	*A. nidulans*; *A. fumigatus*; *A. niger*; *B. cinerea*	n.d.	Hyperpolarization of plasma membrane; activation of ion channels; ROS; apoptosis	cAMP/PKA pathway; PKC/MAPK pathway	Not toxic
Plant	RsAFP2	Radish seed (*R. sativus*)	*C. albicans*; *C. krusei*; *A. flavus*; *F. solani*	Sphingolipid; GlcCer	Hyperpolarization of membrane potential; membrane permeabilization; ion fluxes; ROS; apoptosis; independent of Mca1	MAPK signalling pathways	Not toxic
	HsAFP1	Coral bells (*H. sanguinea*)	*C. albicans*; *C. krusei*; *A. flavus*; *B. cinerea*; *F. culmorum*	n.d.	Membrane permeabilization; ROS; apoptosis	MAPK signalling pathways	n.d.
	Osmotin	Tobacco (*N. tabacum*)	*F. oxysporium*; *P. infestans*; *N. crassa*; *C. albicans*	Pho36	Alters membrane potential; membrane permeabilization	MAPK signalling pathways; Ras2/cAMP pathway	Not toxic
Insect	Psacotheacin	Yellow spotted long-horned beetle (*Ps. hilaris*)	*C. albicans*; *C. parapsilosis*	n.d.	Cell membrane damage; membrane polarization; pore formation; membrane permeabilization; apoptosis; increased metacaspase activity	n.d.	No haemolytic activity towards HE
	Melittin	European honeybee (*A. mellifera*)	*C. albicans*; *C. parapsilosis*	n.d.	Formation of voltage-dependent ion channels across the membrane; membrane permeabilization; apoptosis	n.d.	Haemolytic activity towards HE; toxic
Amphibian	DsS3/DsS3-(1–16)	Tree frog (*P. sauvagii*)		Might bind manno-sylphosphate from cell wall proteins	DsS3: membrane permeabilization; DsS3-(1–6): ROS; apoptosis; independent of metacaspase Yca1, requires Aif1	n.d.	No haemolytic activity towards HE
Mammal	Lactoferrin	Human/bovine	*C. albicans*; *C. tropicalis*; *C. krusei*	n.d.	Alters membrane potential; membrane permeabilization; iron sequestration; cytosolic acidification; apoptosis	n.d.	Not toxic

AMPs isolated from micro-organisms

WH1 fungin

Bacillus amyloliquefaciens WH1 produces the surfactin WH1 fungin (<2.5 kDa). This lipopeptide shows antifungal activity against several fungi including *C. albicans* and plant pathogens *Rhizoctonia solani* and *Fusarium oxysporium*. In the human pathogen *C. albicans*, WH1 fungin inhibits glucan synthesis, resulting in reduced callose levels in the fungal cell wall, and binds to ATPase on the mitochondrial membrane, causing decreased ATPase activity in the fungal cells. Whereas high concentrations of WH1 fungin induce the formation of pores, lower concentrations of WH1 fungin result in apoptosis [13]. Qi et al. [13] also showed increased caspase-like activities against substrates specific for caspases 3, 8 and 9 and cytochrome *c* release from the mitochondria after exposure of the fungal cells to WH1 fungin.

PAF (*Penicillium chrysogenum* antifungal protein)

PAF (55 amino acid residues) is a cysteine- and lysine-rich, low-molecular-mass and cationic antifungal protein. PAF inhibits growth of different fungi including *A. fumigatus*, *Aspergillus niger* and *Botrytis cinerea*. PAF shows, however, no activity against prokaryotes or yeasts [14], and is not cytotoxic to human endothelial cells [15]. Internalization of PAF in sensitive fungi requires ATP, an active metabolism and intact actin microfilaments, suggesting an endocytotic mechanism [16]. PAF hyperpolarizes the plasma membrane and activates ion channels. This antifungal protein also increases the level of ROS in the cells and induces apoptosis. An *Aspergillus nidulans* strain carrying a dominant interfering mutation in the α subunit of the heterotrimeric G-protein ($fadA^{G203R}$), controlling the balance between cell growth and sporulation, displays a reduced sensitivity to PAF. This indicates that G-protein signalling is involved in PAF-mediated toxicity [17]. Moreover, recently it was shown that PAF activates the cAMP/PKA (protein kinase A) pathway, possibly via heterotrimeric G-protein signalling, and interferes with the PKC (protein kinase C)/MAPK (mitogen-activated protein kinase) pathway [18].

AMPs isolated from plants

Plant defensins are small, basic peptides that have disulfide-linked cysteine bridges and possess antimicrobial activity. These defensins are produced by plants to defend against fungal pathogens but are non-toxic to either mammalian or plant cells. In the present paper, the plant defensins RsAFP2 from *Raphanus sativus* (radish) and HsAFP1 from *Heuchera sanguinea* are discussed, as well as the antifungal protein osmotin from tobacco.

RsAFP2

The plant defensin RsAFP2 (51 amino acid residues), isolated from radish seed (*R. sativus*), inhibits the growth of different fungi including the human pathogens *C. albicans* and *Candida krusei* and the crop pathogen *Fusarium solani* [19]. RsAFP2 is not toxic to mammalian cells and is prophylactically effective against murine candidiasis [20]. RsAFP2 interacts with GlcCer (glucosylceramide) in the plasma membrane of susceptible fungi. In agreement with these results, *Saccharomyces cerevisiae* and *C. glabrata*, both lacking GlcCer, are resistant to RsAFP2 [21,22]. Also, *C. albicans gcs1*Δ, impaired in GlcCer biosynthesis, is resistant to RsAFP2 [21]. Interaction of RsAFP2 with GlcCer results in permeabilization of the cell and cell growth arrest [23]. Furthermore, treatment of *C. albicans* cells with RsAFP2 induces ROS and apoptosis [6]. This RsAFP2-induced killing of *C. albicans* cells requires caspase or caspase-like proteases but is independent of metacaspase 1 [6]. In *Fusarium graminearum* MAPK pathways are involved in RsAFP2 tolerance [24].

HsAFP1

The antifungal peptide HsAFP1 (54 amino acid residues), isolated from coral bells (*H. sanguinea*), exerts strong antimicrobial activity against several fungi, including *C. albicans*, *C. krusei*, *Aspergillus flavus*, *B. cinerea* and *Fusarium culmorum* [25]. HsAFP1 permeabilizes susceptible fungal cells, resulting in the inhibition of cell growth [23]. Although the HsAFP1-binding sites remain to be identified, it is unlikely that HsAFP1 binds to GlcCer like RsAFP2, as strains defective in biosynthesis of GclCer are as sensitive to HsAFP1 as wild-type (K. Thevissen, unpublished work). Different MAPK pathways, including the cell integrity pathway and HOG (high osmolarity glycerol) pathway, are believed to be involved in yeast tolerance against HsAFP1, and a functional electron transport chain is indispensable for HsAFP1 antifungal activity. Furthermore, exposure of *C. albicans* to HsAFP1 induces ROS accumulation and apoptosis [7].

Osmotin

Originally, osmotin was identified as a major protein accumulating in tobacco cells (*Nicotiana tabacum*) grown under osmotic stress [26]. Osmotin is a member of the PR-5 (pathogen related-5) family and has *in vitro* antifungal activity against a broad range of fungi, including *Fusarium oxysporum*, *Phytophtora infestans*, *Neurospora crassa* and *C. albicans* [27,28]; however, it is not toxic to human cells [26]. Osmotin causes permeabilization of the membrane and dissipation of the membrane potential. Resistance of *S. cerevisiae* to osmotin is mediated by Pirs (proteins with internal repeats), which are cell-wall-located stress proteins induced by heat and nitrogen limitation [29] and by Ssd1, a protein involved in cell wall biogenesis and composition, including sorting of Pirs [30]. Osmotin toxicity also depends on the presence of fungal cell wall phosphomannans. These phosphomannans might serve as docking sites, facilitating diffusion of the protein across the cell wall and increasing toxicity [31]. Moreover, this plant defence protein also uses an MAPK pathway to weaken defensive cell wall barriers, resulting in increased toxicity of osmotin [32]. In addition, osmotin was shown

to suppress the Ras2/cAMP stress response pathway and to induce apoptosis in *S. cerevisiae* [33]. Pho36, a homologue of the mammalian adiponectin receptor that regulates lipid and phosphate metabolism in *S. cerevisiae*, functions upstream of Ras2 in this osmotin-induced apoptosis pathway [34].

AMPs isolated from insects

Melittin

Melittin, the main toxic component of the venom of the European honeybee *Apis mellifera*, is a cationic amphipathic peptide and has strong antimicrobial activity against different micro-organisms, including *Escherichia coli*, *Staphylococcus aureus* [35], *C. albicans* and *Candida parapsilosis* [36,37]. This peptide (26 amino acid residues) has a large hydrophobic region and a stretch of mostly hydrophilic amino acids at the C-terminal. Melittin forms voltage-dependent ion channels across the lipid bilayer. At low peptide-to-lipid ratios, melittin binds to membrane surfaces, whereas at high peptide concentrations, melittin permeabilizes membranes, resulting in micelle formation [38]. Recently, it was demonstrated that *C. albicans* cells undergo apoptosis when exposed to subinhibitory concentrations of melittin, while cells exposed to the MIC (minimal inhibitory concentration) display necrotic effects [37]. Major disadvantages of melittin are its haemolytic activity and its toxicity to mammalian cells [39].

Psacotheacin

Recently, the novel knottin-type AMP psacotheacin was isolated from the larvae of the yellow-spotted long-horned beetle *Psacothea hilaris* [36]. This 34-mer mature peptide exerts potent antimicrobial activity against different Gram-positive and Gram-negative bacteria and fungal pathogens, including *C. albicans* and *C. parapsilosis* [36]. Psacotheacin damages the cell membrane, initiates membrane polarization and pore formation, increases membrane permeability [36] and induces apoptosis in *C. albicans* cells. Moreover, metacaspase activity also increases, suggesting that metacaspase activation is required for psacotheasin-induced apoptosis [40]. In contrast with melittin, psacotheacin has no haemolytic activity towards human erythrocytes [36].

AMPs isolated from amphibians

DsS3 (dermaseptin S3)

The non-haemolytic DsS3 (29 amino acid residues) is a lysine-rich amphipathic α-helical AMP that belongs to the family of dermaseptins and is isolated from the skin of the tree-frog *Phyllomedusa sauvagii*. DsS3 binds and permeabilizes negatively charged microbial membranes [41]. DsS3-(1–16) is a truncated derivative of DsS3 with full activity. The loss of mannosylphosphate from cell wall proteins of *C. albicans* results in increased resistance to DsS3-(1–16) owing to reduced binding of DsS3-(1–16) to the cell surface. *S. cerevisiae* cells exposed to DsS3-(1–16) show apoptotic characteristics. Deletion of the metacaspase-encoding gene *YCA1* does not increase resistance to DsS3-(1–16) and no Yca1 activity was triggered on exposure to this peptide, suggesting that DsS3-(1–16)-induced apoptosis is metacaspase independent. However, deletion of *AIF1* (apoptosis-inducing factor 1) rescues yeast cells from DsS3-(1–16)-induced apoptosis, indicating that this process requires AIF1 [42].

Peptides isolated from mammals

Lactoferrin

The innate human protein lactoferrin is an iron-binding glycoprotein (approximately 700 amino acid residues) and is found on mucosal surfaces and in biological fluids, including milk and saliva. Lactoferrin has a broad antimicrobial spectrum including bacteria, viruses, fungi and parasites [43]. Bovine lactoferrin has fungicidal activity against different *Candida* species such as *C. albicans*, *C. tropicalis* and *C. krusei* [44]. The ability of lactoferrin to sequester iron at sites of infection contributes to the antifungal activity as it deprives the micro-organism of iron [45]. In addition, it was shown that lactoferricin B, a peptide derived from bovine lactoferrin by enzymatic cleavage, alters the permeability of the cell surface of *C. albicans* cells [46]. Exposure of *C. albicans* cells to lactoferrin results in cytosolic acidification, changes in the cytoplasmic membrane potential and apoptosis [47,48]. ROS and K^+ channel-mediated K^+ efflux were shown to be essential for lactoferrin-induced cell death [48]. Both oral and intragastric administration of bovine lactoferrin to mice with oral candidiasis reduced the number of *C. albicans* cells in the oral cavity significantly, suggesting that bovine lactoferrin can be used to support antifungal drug treatment [49].

Concluding remarks

Whereas the AMPs discussed above are all apoptosis-inducing AMPs, this is not a general characteristic of AMPs. For example, human salivary histatin 5, which has antifungal activity against different *Candida* species and *Cryptococcus neoformans*, induces a non-lytic form of cell death; however, it does not initiate apoptosis [50]. Also DmAMP1, an antifungal plant defensin isolated from seeds of dahlia (*Dahlia merckii*) [25], does not induce apoptosis in *S. cerevisiae* or *C. albicans* (K. Thevissen, unpublished work).

Apart from their antimicrobial activities, AMPs can have other activities as well. Lactoferrin, for instance, also modulates inflammation and protects against tumorigenesis and metastasis [43]. Whereas melittin lyses both cancer and normal, healthy cells, murine L1210 leukaemia cells are significantly more sensitive to melittin-mediated cytotoxicity than normal mouse splenocytes or bone marrow cells [51]. Dermaseptin B2 and B3, isolated from the South American tree frog *Phyllomedusa bicolor*, which have antimicrobial activity, also inhibit the proliferation of cancer cells [52].

In the future, AMP will probably give rise to a class of very promising novel antimycotics as they combine potent

antimicrobial activity with a low risk of developing antibiotic-resistant pathogens [53] and are known to act synergistically with existing antibiotics.

Funding

K.T. and B.P.A.C. acknowledge support from KULeuven through a research manager fellowship (to K.T.) and the Research Foundation Flanders [grant number G.0440.07].

References

1 Martin, G.S., Mannino, D.M., Eaton, S. and Moss, M. (2003) The epidemiology of sepsis in the United States from 1979 through 2000. N. Engl. J. Med. **348**, 1546–1554
2 Gudlaugsson, O., Gillespie, S., Lee, K., Vande, B.J., Hu, J., Messer, S., Herwaldt, L., Pfaller, M. and Diekema, D. (2003) Attributable mortality of nosocomial candidemia, revisited. Clin. Infect. Dis. **37**, 1172–1177
3 Vonberg, R.P. and Gastmeier, P. (2006) Nosocomial aspergillosis in outbreak settings. J. Hosp. Infect. **63**, 246–254
4 Como, J.A. and Dismukes, W.E. (1994) Oral azole drugs as systemic antifungal therapy. N. Engl. J. Med. **330**, 263–272
5 Mathew, B.P. and Nath, M. (2009) Recent approaches to antifungal therapy for invasive mycoses. Chem. Med. Chem. **4**, 310–323
6 Aerts, A.M., Carmona-Gutierrez, D., Lefevre, S., Govaert, G., Francois, I.E., Madeo, F., Santos, R., Cammue, B.P. and Thevissen, K. (2009) The antifungal plant defensin RsAFP2 from radish induces apoptosis in a metacaspase independent way in *Candida albicans*. FEBS Lett. **583**, 2513–2516
7 Aerts, A.M., Bammens, L., Govaert, G., Carmona-Gutierrez, D., Madeo, F., Cammue, B.P.A. and Thevissen, K. (2011) The antifungal plant defensin HsAFP1 from *Heuchera sanguinea* induces apoptosis in *Candida albicans*. Front. Microbiol. **2**, 47
8 Buttner, S., Eisenberg, T., Herker, E., Carmona-Gutierrez, D., Kroemer, G. and Madeo, F. (2006) Why yeast cells can undergo apoptosis: death in times of peace, love, and war? J. Cell Biol. **175**, 521–525
9 Severin, F.F. and Hyman, A.A. (2002) Pheromone induces programmed cell death in *S. cerevisiae*. Curr. Biol. **12**, R233–R235
10 Váchová, L. and Palková, Z. (2005) Physiological regulation of yeast cell death in multicellular colonies is triggered by ammonia. J. Cell Biol. **169**, 711–717
11 Eisenberg, T., Carmona-Gutierrez, D., Buttner, S., Tavernarakis, N. and Madeo, F. (2010) Necrosis in yeast. Apoptosis **15**, 257–268
12 Carmona-Gutierrez, D., Eisenberg, T., Buttner, S., Meisinger, C., Kroemer, G. and Madeo, F. (2010) Apoptosis in yeast: triggers, pathways, subroutines. Cell Death Differ. **17**, 763–773
13 Qi, G., Zhu, F., Du, P., Yang, X., Qiu, D., Yu, Z., Chen, J. and Zhao, X. (2010) Lipopeptide induces apoptosis in fungal cells by a mitochondria-dependent pathway. Peptides **31**, 1978–1986
14 Marx, F., Binder, U., Leiter, E. and Pocsi, I. (2008) The *Penicillium chrysogenum* antifungal protein PAF, a promising tool for the development of new antifungal therapies and fungal cell biology studies. Cell. Mol. Life Sci. **65**, 445–454
15 Szappanos, H., Szigeti, G.P., Pal, B., Rusznak, Z., Szucs, G., Rajnavolgyi, E., Balla, J., Balla, G., Nagy, E., Leiter, E. et al. (2005) The *Penicillium chrysogenum*-derived antifungal peptide shows no toxic effects on mammalian cells in the intended therapeutic concentration. Naunyn-Schmiedeberg's Arch. Pharmacol. **371**, 122–132
16 Oberparleiter, C., Kaiserer, L., Haas, H., Ladurner, P., Andratsch, M. and Marx, F. (2003) Active internalization of the *Penicillium chrysogenum* antifungal protein PAF in sensitive aspergilli. Antimicrob. Agents Chemother. **47**, 3598–3601
17 Leiter, E., Szappanos, H., Oberparleiter, C., Kaiserer, L., Csernoch, L., Pusztahelyi, T., Emri, T., Pocsi, I., Salvenmoser, W. and Marx, F. (2005) Antifungal protein PAF severely affects the integrity of the plasma membrane of *Aspergillus nidulans* and induces an apoptosis-like phenotype. Antimicrob. Agents Chemother. **49**, 2445–2453
18 Binder, U., Oberparleiter, C., Meyer, V. and Marx, F. (2010) The antifungal protein PAF interferes with PKC/MPK and cAMP/PKA signalling of *Aspergillus nidulans*. Mol. Microbiol. **75**, 294–307
19 Thevissen, K., Kristensen, H.H., Thomma, B.P., Cammue, B.P. and Francois, I.E. (2007) Therapeutic potential of antifungal plant and insect defensins. Drug Discovery Today **12**, 966–971
20 Tavares, P.M., Thevissen, K., Cammue, B.P., Francois, I.E., Barreto-Bergter, E., Taborda, C.P., Marques, A.F., Rodrigues, M.L. and Nimrichter, L. (2008) *In vitro* activity of the antifungal plant defensin RsAFP2 against *Candida* isolates and its *in vivo* efficacy in prophylactic murine models of candidiasis. Antimicrob. Agents Chemother. **52**, 4522–4525
21 Thevissen, K., Warnecke, D.C., Francois, I.E., Leipelt, M., Heinz, E., Ott, C., Zahringer, U., Thomma, B.P., Ferket, K.K. and Cammue, B.P. (2004) Defensins from insects and plants interact with fungal glucosylceramides. J. Biol. Chem. **279**, 3900–3905
22 Saito, K., Takakuwa, N., Ohnishi, M. and Oda, Y. (2006) Presence of glucosylceramide in yeast and its relation to alkali tolerance of yeast. Appl. Microbiol. Biotechnol. **71**, 515–521
23 Thevissen, K., Terras, F.R. and Broekaert, W.F. (1999) Permeabilization of fungal membranes by plant defensins inhibits fungal growth. Appl. Environ. Microbiol. **65**, 5451–5458
24 Ramamoorthy, V., Zhao, X., Snyder, A.K., Xu, J.R. and Shah, D.M. (2007) Two mitogen-activated protein kinase signalling cascades mediate basal resistance to antifungal plant defensins in *Fusarium graminearum*. Cell. Microbiol. **9**, 1491–1506
25 Osborn, R.W., De Samblanx, G.W., Thevissen, K., Goderis, I., Torrekens, S., Van, L.F., Attenborough, S., Rees, S.B. and Broekaert, W.F. (1995) Isolation and characterisation of plant defensins from seeds of Asteraceae, Fabaceae, Hippocastanaceae and Saxifragaceae. FEBS Lett. **368**, 257–262
26 Singh, N.K., Bracker, C.A., Hasegawa, P.M., Handa, A.K., Buckel, S., Hermodson, M.A., Pfankoch, E., Regnier, F.E. and Bressan, R.A. (1987) Characterization of osmotin: a thaumatin-like protein associated with osmotic adaptation in plant cells. Plant Physiol. **85**, 529–536
27 Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., van den Elzen, P.J. and Cornelissen, B.J. (1991) Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell **3**, 619–628
28 Narasimhan, M.L., Lee, H., Damsz, B., Singh, N.K., Ibeas, J.I., Matsumoto, T.K., Woloshuk, C.P. and Bressan, R.A. (2003) Overexpression of a cell wall glycoprotein in *Fusarium oxysporum* increases virulence and resistance to a plant PR-5 protein. Plant J. **36**, 390–400
29 Yun, D.J., Zhao, Y., Pardo, J.M., Narasimhan, M.L., Damsz, B., Lee, H., Abad, L.R., D'Urzo, M.P., Hasegawa, P.M. and Bressan, R.A. (1997) Stress proteins on the yeast cell surface determine resistance to osmotin, a plant antifungal protein. Proc. Natl. Acad. Sci. U.S.A. **94**, 7082–7087
30 Ibeas, J.I., Yun, D.J., Damsz, B., Narasimhan, M.L., Uesono, Y., Ribas, J.C., Lee, H., Hasegawa, P.M., Bressan, R.A. and Pardo, J.M. (2001) Resistance to the plant PR-5 protein osmotin in the model fungus *Saccharomyces cerevisiae* is mediated by the regulatory effects of SSD1 on cell wall composition. Plant J. **25**, 271–280
31 Ibeas, J.I., Lee, H., Damsz, B., Prasad, D.T., Pardo, J.M., Hasegawa, P.M., Bressan, R.A. and Narasimhan, M.L. (2000) Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein. Plant J. **23**, 375–383
32 Yun, D.J., Ibeas, J.I., Lee, H., Coca, M.A., Narasimhan, M.L., Uesono, Y., Hasegawa, P.M., Pardo, J.M. and Bressan, R.A. (1998) Osmotin, a plant antifungal protein, subverts signal transduction to enhance fungal cell susceptibility. Mol. Cell **1**, 807–817
33 Narasimhan, M.L., Damsz, B., Coca, M.A., Ibeas, J.I., Yun, D.J., Pardo, J.M., Hasegawa, P.M. and Bressan, R.A. (2001) A plant defense response effector induces microbial apoptosis. Mol. Cell **8**, 921–930
34 Narasimhan, M.L., Coca, M.A., Jin, J., Yamauchi, T., Ito, Y., Kadowaki, T., Kim, K.K., Pardo, J.M., Damsz, B., Hasegawa, P.M. et al. (2005) Osmotin is a homolog of mammalian adiponectin and controls apoptosis in yeast through a homolog of mammalian adiponectin receptor. Mol. Cell **17**, 171–180
35 Han, S., Yeo, J., Baek, H., Lin, S.M., Meyer, S. and Molan, P. (2009) Postantibiotic effect of purified melittin from honeybee (*Apis mellifera*) venom against *Escherichia coli* and *Staphylococcus aureus*. J. Asian Nat. Prod. Res. **11**, 796–804

36 Hwang, B., Hwang, J.S., Lee, J. and Lee, D.G. (2010) Antifungal properties and mode of action of psacotheasin, a novel knottin-type peptide derived from *Psacothea hilaris*. Biochem. Biophys. Res. Commun. **400**, 352–357
37 Park, C. and Lee, D.G. (2010) Melittin induces apoptotic features in *Candida albicans*. Biochem. Biophys. Res. Commun. **394**, 170–172
38 Raghuraman, H. and Chattopadhyay, A. (2007) Melittin: a membrane-active peptide with diverse functions. Biosci. Rep. **27**, 189–223
39 Asthana, N., Yadav, S.P. and Ghosh, J.K. (2004) Dissection of antibacterial and toxic activity of melittin: a leucine zipper motif plays a crucial role in determining its hemolytic activity but not antibacterial activity. J. Biol. Chem. **279**, 55042–55050
40 Hwang, B., Hwang, J.S., Lee, J. and Lee, D.G. (2011) The antimicrobial peptide, psacotheasin induces reactive oxygen species and triggers apoptosis in *Candida albicans*. Biochem. Biophys. Res. Commun. **405**, 267–271
41 Ghosh, J.K., Shaool, D., Guillaud, P., Ciceron, L., Mazier, D., Kustanovich, I., Shai, Y. and Mor, A. (1997) Selective cytotoxicity of dermaseptin S3 toward intraerythrocytic *Plasmodium falciparum* and the underlying molecular basis. J. Biol. Chem. **272**, 31609–31616
42 Morton, C.O., Dos Santos, S.C. and Coote, P. (2007) An amphibian-derived, cationic, α-helical antimicrobial peptide kills yeast by caspase-independent but AIF-dependent programmed cell death. Mol. Microbiol. **65**, 494–507
43 Legrand, D., Pierce, A., Elass, E., Carpentier, M., Mariller, C. and Mazurier, J. (2008) Lactoferrin structure and functions. Adv. Exp. Med. Biol. **606**, 163–194
44 Xu, Y.Y., Samaranayake, Y.H., Samaranayake, L.P. and Nikawa, H. (1999) *In vitro* susceptibility of *Candida* species to lactoferrin. Med. Mycol. **37**, 35–41
45 Kirkpatrick, C.H., Green, I., Rich, R.R. and Schade, A.L. (1971) Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host-defense mechanisms in chronic mucocutaneous candidiasis. J. Infect. Dis. **124**, 539–544
46 Bellamy, W., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S. and Tomita, M. (1993) Killing of *Candida albicans* by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med. Microbiol. Immunol. **182**, 97–105
47 Viejo-Diaz, M., Andres, M.T. and Fierro, J.F. (2004) Effects of human lactoferrin on the cytoplasmic membrane of *Candida albicans* cells related with its candidacidal activity. FEMS Immunol. Med. Microbiol. **42**, 181–185
48 Andrés, M.T., Viejo-Diaz, M. and Fierro, J.F. (2008) Human lactoferrin induces apoptosis-like cell death in *Candida albicans*: critical role of K^+-channel-mediated K^+ efflux. Antimicrob. Agents Chemother. **52**, 4081–4088
49 Takakura, N., Wakabayashi, H., Ishibashi, H., Teraguchi, S., Tamura, Y., Yamaguchi, H. and Abe, S. (2003) Oral lactoferrin treatment of experimental oral candidiasis in mice. Antimicrob. Agents Chemother. **47**, 2619–2623
50 Wunder, D., Dong, J., Baev, D. and Edgerton, M. (2004) Human salivary histatin 5 fungicidal action does not induce programmed cell death pathways in *Candida albican*s. Antimicrob. Agents Chemother. **48**, 110–115
51 Killion, J.J. and Dunn, J.D. (1986) Differential cytolysis of murine spleen, bone-marrow and leukemia cells by melittin reveals differences in membrane topography. Biochem. Biophys. Res. Commun. **139**, 222–227
52 van Zoggel, H., Hamma-Kourbali, Y., Galanth, C., Ladram, A., Nicolas, P., Courty, J., Amiche, M. and Delbe, J. (2010) Antitumor and angiostatic peptides from frog skin secretions. Amino Acids, doi:10.1007/s00726-010-0815-9
53 Kazemzadeh-Narbat, M., Kindrachuk, J., Duan, K., Jenssen, H., Hancock, R.E. and Wang, R. (2010) Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. Biomaterials **31**, 9519–9526

Received 10 May 2011
doi:10.1042/BST0391527