

Chapter 2

Antifungal Host Defense Peptides

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Abstract Fungi infect billions of people every year, yet their contribution to the global burden of disease is largely unrecognized and the repertoire of antifungal agents is rather limited. Thus, treatment of life-threatening invasive fungal infections is still based on drugs discovered several decades ago. In addition, recent data on resistance emergence of fungi emphasize the urgent need for novel antifungal treatments. One alternative strategy is based on host defense peptides. Among the large number of antimicrobial peptides, a group of peptides show primarily antifungal activity by interfering with enzymes of cell wall biosynthesis or specific membrane lipids such as ergosterol. Both are promising targets for antifungal peptides, as they are absent in mammalian cells and hence low toxicity of peptides can be expected. However, most of the antimicrobial peptides exhibit a broad spectrum activity including antifungal activity. These peptides act on the cell membrane level and although their structures vary largely, they share a positive net charge, which facilitates electrostatic interactions with negatively charged lipids of the target cell, and an amphipathic structure, which facilitates incorporation into the cell membrane and in turn membrane disruption. Thereby, membrane lipids differing between mammals and fungi play a central role concerning specificity and efficacy of these peptides. Hence, understanding their molecular mechanism(s) of action will aid in the design of novel antifungal agents. Finally, some of these peptides were shown to act synergistically with conventional drugs, which would further widen the armory to treat especially life-threatening invasive fungal infections.

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2.1 Introduction

Of the 1.5 million fungal species, around 300 are reported to be pathogenic in humans (Taylor et al. 2001). Superficial and mucosal fungal infections are extremely common, but life-threatening invasive fungal infections have increased in importance (Polvi et al. 2015; Warnock 2007). In a very recent review about yeast pathogens, *Cryptococcus neoformans* was described as the leading cause of deaths due to fungal infections, with a global burden of nearly 1 million cases annually, and more than 620,000 deaths worldwide (Polvi et al. 2015; Park et al. 2009). Further, cryptococcal meningitis contribute up to 20 % of AIDS-related mortality in low-income and middle-income countries every year (Loyse et al. 2013). *Candida albicans*, another important fungal pathogen, causes more than 400,000 deaths per year due to invasive candidiasis (Horn et al. 2009). Risk factors for invasive candidiasis include surgery (especially abdominal surgery), burns, long-term stay in an intensive care unit, and previous administration of broad spectrum antibiotics and immunosuppressive agents (Kontoyiannis et al. 2003; Zaoutis et al. 2005; Sydnor and Perl 2011; Pfaller and Diekema 2004; Spampinato and Leonardi 2013). The Centers for Disease Control and Prevention, Atlanta, reported that roughly one third of patients, who suffer from bloodstream infections caused by drug-resistant *Candida* spp., die during their hospitalization in the US (<http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf>). Finally, patients with impaired immune function are also often infected by *Aspergillus* species (Polvi et al. 2015; Warnock 2007).

Despite the profound impact of fungal pathogens on human health worldwide, treatment can be hampered by toxicity, poor tolerability, or a narrow activity spectrum of antifungal drugs. Nevertheless, invasive fungal infections remain understudied and underdiagnosed as compared to other infectious diseases (Brown et al. 2012). Further, the repertoire of antifungal agents is rather limited and therefore treatment of life-threatening invasive fungal infections is still mainly based on drugs discovered several decades ago (Butts and Krysan 2012). Polyenes, azoles, allylamines, and echinocandins represent the most common classes of antifungals currently used in the clinics. These agents demonstrate high levels of antifungal activity, although resistance is reported for all classes including echinocandins, which represent the first and so far only class of licensed antifungal peptides (Polvi et al. 2015; Drgona et al. 2014; Spampinato and Leonardi 2013; Chen and Sorrell 2007; Perlin 2015). The fungal-derived echinocandins are cyclic hexapeptides with N-linked acyl lipid side chains, which inhibit cell wall biosynthesis at the level of (1,3)- β -D-glucan synthase (Boucher et al. 2004). Whereas native echinocandins were hemolytic and had poor solubility in water, chemical modifications resulted in molecules with improved properties (Luca and Walsh 2000; Denning 2002). The first licensed echinocandin product was caspofungin acetate (Cancidas[®]; Merck) (Denning 2002). Currently, also micafungin (Mycymine[®]; Astella Pharma) and anidulafungin (Ecalta[®], Pfizer) are available for treatment of invasive fungal infections. The inhibitory spectrum of these

Table 2.1 Secondary structure and physico-chemical properties of antifungal peptides comprised of ≤50 amino acids^a

Net charge ^b	Secondary structure	Hydrophobic residues (%)										Number of peptides			
		≤10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90					
Positive	α-helix	3	1	8	31	38	31	13	3	0		855			
	β-sheet	0	0	3	16	9	13	1	0	0		128			
	α + β (combined)	0	0	3	19	6	1	0	0	0		42			
	Neither α nor β	0	0	0	1	1	2	0	0	0		29			
	Disulfide	0	0	11	65	22	15	2	0	0		115			
	Rich in unusual aa	8	1	1	3	1	0	0	0	0		14			
Zero	Unknown	0	2	22	68	159	116	91	4	1		463			
		11	4	48	203	236	178	107	7	1		795			
	α-helix	0	0	0	0	0	0	0	0	0		0			
	β-sheet	0	0	0	0	0	0	0	0	0		0			
	α + β (combined)	0	0	0	0	0	0	0	0	0		0			
	Neither α nor β	0	0	0	0	0	0	0	0	0		0			
	Disulfide	0	0	0	2	0	0	0	0	0		2			
	Rich in unusual aa	4	2	0	0	0	0	0	0	0		6			
	Unknown	1	1	3	9	6	4	3	1	0		28			
		5	3	3	11	6	4	3	1	0		36			
Negative	α-helix	0	0	0	1	2	0	1	0	0		4			
	β-sheet	0	0	0	1	0	0	0	0	0		1			
	α + β (combined)	0	0	0	1	0	0	0	0	0		1			
	Neither α nor β	0	0	0	0	0	0	0	0	0		0			
	Disulfide	0	0	1	0	0	0	0	0	0		1			
	Rich in unusual aa	1	0	0	0	0	0	0	0	0		1			
	Unknown	1	3	1	5	2	1	0	3	0		16			
		2	3	2	8	4	1	1	3	0		24			

^aData from AP Database (Wang and Wang 2004; Wang et al. 2009)

^bAt physiological pH

synthetically modified lipopeptides, however, does not include the leading fungal pathogen *Cryptococcus neoformans* since this pathogen has little or no (1,3)- β -D-glucan synthase enzyme (Denning 1997; Hector 1993).

Currently, there is considerable interest in antifungal properties of antimicrobial peptides (AMPs) and research on this topic has strongly expanded during the past decade. Antimicrobial peptides are produced by diverse life forms including mammals, plants, amphibians, insects, fungi, and bacteria. More than 2500 natural or synthetic AMPs are listed in the Antimicrobial Peptide Database (APD, <http://aps.unmc.edu/AP>) of which around 900 have antifungal activity (Wang and Wang 2004; Wang et al. 2009) including some proteins such as ribonucleases and proteases. The vast majority of AMPs with antifungal activity is positively charged and for almost two third of these peptides no secondary structure is determined so far (Table 2.1). About 15 % of the peptides exhibit either an α -helix or structures stabilized by disulfide bonds, while peptides that adopt a β -sheet make up to only ~ 5 %. A similar amount of peptides have a combined α -helical/ β -sheet structure and a very minor fraction (2 %) is rich in unusual amino acids. It is of interest to note that peptides containing unusual amino acids have a very low content of hydrophobic residues (mostly ≤ 10 %), while the percentage of hydrophobic residues of the majority of peptides with antifungal activity is around 50 % (Table 2.1). Excellent reviews about antifungal peptides originating from insects and plants have very recently been published (Faruck et al. 2015; Lacerda et al. 2014; Vriens et al. 2014; Silva et al. 2014; Nawrot et al. 2014). Furthermore, Matejuk et al. described peptide-based strategies for antifungal therapies against emerging infections emphasizing that these peptides may have specific targets showing selective toxicity or may be multifunctional in their mode of action (Matejuk et al. 2010). The number of peptides exhibiting primarily antifungal activity such as echinocandins is much lower than peptides exhibiting a broad antimicrobial activity supposedly resulting in lysis of the cytoplasmic membrane. This review will focus on the different fungal targets of peptides that have shown selective toxicity against fungal pathogens in vitro or in vivo. Further, we will briefly discuss mechanisms of membrane lysis and describe co-applications of standard drugs and antifungal peptides.

2.2 Targets for Antifungal Therapy

In terms of numbers of classes of agents that can be used to treat life-threatening mycoses, the targets of antifungal agents are heavily focused, directly or indirectly, on the cell envelope (wall and plasma membrane), and particularly on the fungal membrane sterol, ergosterol, and its biosynthesis (Odds et al. 2003) (Table 2.2, Fig. 2.1). From the 1950s until the discovery of azoles, polyene antifungal agents such as amphotericin B, which are known to cause significant nephrotoxicity, represented the standard of therapy for systemic fungal infections (Ghannoum and Rice 1999). Amphotericin B (AmpB) has been proposed to interact with plasma

Table 2.2 Proposed targets of selected antifungal peptides

Target	Peptide	Sequence/Net charge	Reference(s)
Cell wall	Glucan synthase inhibitors	Cyclic hexapeptides	Boucher et al. (2004)
	Chitin synthase inhibitor	Peptidyl nucleoside antibiotics	Nix et al. (2009)
	Chitin binding	VPGGEGCGRFGGAGGQC ⁺ SRFGFCGSGPKYCAH net charge: +2	Rogozhin et al. (2015) (APD ID: AP02585) ^a
	Chitin binding	Cyclic thiopeptide	Mizuhara et al. (2011)
	Chitin binding	HSSGYTRPLRKPSRPIRPIGDCVYGIPSSSTARLCCFRYGDCCHL net charge: +5	(Cuthbertson et al. 2002; Destoumieux et al. 2000) (APD ID: AP00420)
	Ergosterols facilitating pore formation of SE	cyclic lipodepsi-nonapeptide	(Lucca et al. 1999; Feigin et al. 1997)
Plasma membrane ^b	High ergosterol affinity, (mitochondria?)	ACYCRIPACIAGERRYGTCTYQGRLWAFCC net charge: +3	(Selsted et al. 1985; Gonçalves et al. 2012a; Leher et al. 1988) (APD ID: AP00176)
	Interaction with sphingolipids, nucleus (cell cycle control)	KTCEHLADTYRGVCFNTASDDHCKNKAHLISGTCHNWKCFCTQNC net charge: +1	(Almeida et al. 2000; Medeiros et al. 2010; Lobo et al. 2007) (APD ID: AP00483)
	Interaction with sphingolipids	ELCEKASKTWSGNCGNTGHCDNQCKSWEGA ⁺ AHGACHVRNGKHMCFCYFNC net charge: +1	(François et al. 2002; Thevissen et al. 2000) (APD ID: AP00918)
	Interaction with sphingolipids	Synthetic peptidomimetic (arginine-tertbutyl tryptophan-arginine-phenylethanol) net charge: +2	(Isaksson et al. 2011; Bojsen et al. 2013)
	Interaction with sphingolipids	QKLCQRPSGTWSGVCNNACKNQICIRLEKARHGSCNYYFPAHKCIYFPC net charge: +6	(Terras et al. 1993; Thevissen et al. 2004) (APD ID: AP00287)
	Interaction with sphingolipids	DKLIGSCVWGA ⁺ NYTSDCNCEKRRRGYKGGHCGSFANVNCWCE ⁺ net charge: +1	(Lamberty et al. 2001; Thevissen et al. 2004) (APD ID: AP00031)
			(continued)

Table 2.2 (continued)

Target	Peptide	Sequence/Net charge	Reference(s)
Intracellular	Interaction with sphingolipids	HTPTPTPICKSRSHYKGRCIQDMDCNAACVKESYSYTGFCNGRPPEKQCFCTKPKCKRERAAATLRWPGLn net charge: +6	(De-Paula et al. 2008) (APD ID: AP02018)
	Interaction with sphingolipids	DCLSGRYKGPACAVWDNETCRRVCKEEGRSSGHCSPLKWCWEGC net charge: +1	(Fehlbaum et al. 1994; Gao and Zhu 2008) (APD ID: AP00672)
	Interaction with sphingolipids, blocks mammalian L-type Ca^{2+} channel	RTCENLADKYRGPFCFSGCDTHCTTKENAVSGRCRDFRCWC ^a TKRC net charge: +3	(Ramamoorthy et al. 2007; Spelbrink et al. 2004) (APD ID: AP00978)
	Mitochondria	DSHAKRRHHGYKRRKFHEKHHSRGRY net charge: +5	(Oppenheim et al. 1988; Helmerhorst et al. 1999) (APD ID: AP00505)
	Nucleus	Pentapeptide (dovaline-valine-dolaisoleuine-dolaproine-phenylalanine-methyl-ester)	Woyke et al. (2001, 2002)
Intracellular (DNA damage)	Auristatin PHE (synthetic product of marine dolastatin 10)	ALWKNMLKGIGKLAGKAALGAVKKLVGAES net charge: +5	(Mor et al. 1994; Morton et al. 2007) (APD ID: AP00159)
	intracellular (DNA damage)	GIGKFLHSAKKFGKAFVGEIMNS net charge: +3	(Zaslouff 1987; Morton et al. 2007) (APD ID: AP00144)
	Acts on vacuolar proton pump, seems to act via Ca^{2+} influx through L-type Ca^{2+} channels	ASCNQVCSPFEMP ^b PGTGSACRCIPVGLVIGYCRNPSG net charge: +1	(Chouabe et al. 2011; Gressent et al. 2011)

^aID number of peptides in the AP Database (Wang and Wang 2004; Wang et al. 2009)^bMembrane-active peptides with broad spectrum activity are not listed. Examples can be deduced from the AP Database and from Table 2.2 (Matejuk et al. 2010)

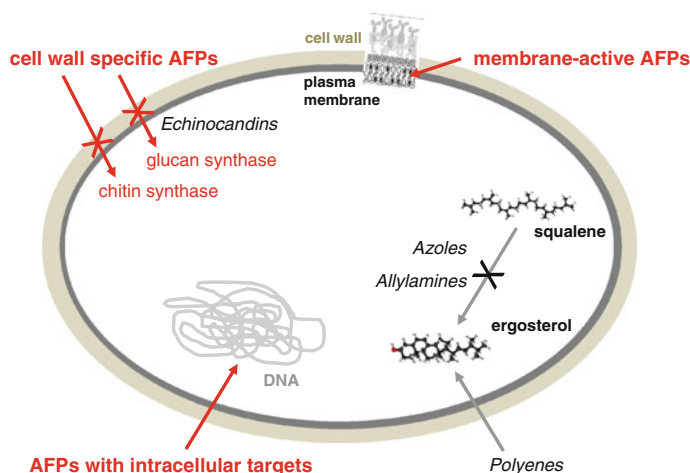


Fig. 2.1 Potential targets of antifungal peptides and conventional antifungal drugs. Latter interfere mainly with the biosynthesis of ergosterol and its physiological function, while the former predominantly interfere with cell wall biosynthesis and cell membrane integrity

membrane ergosterol resulting in the formation of ion channel aggregates that are inserted into lipid bilayers and thereby permeabilize and kill yeast (Kruijff and Demel 1974; Holz 1974). Anderson et al., however, reported that AmpB exists primarily in the form of large, extra-membranous aggregates that kill yeast by extracting ergosterol from lipid bilayers (Anderson et al. 2014; Lohner 2014). The clinical efficacy and safety of azoles, in particular fluconazole, has led to their extensive use. The primary target of azoles is a heme protein, which catalyzes cytochrome P-450-dependent 14- α -demethylation of lanosterol (Hitchcock et al. 1990). Accumulation of zymosterol and squalene was observed, when *C. albicans* cells were treated with voriconazole (Sanati et al. 1997). Mammalian cholesterol synthesis is also blocked by azoles at the stage of 14- α -demethylation, however, the dose required to effect the same degree of inhibition is much higher than that required for fungi (Hitchcock et al. 1990; van den Bossche et al. 1978; Ghannoum and Rice 1999). Allylamines, such as terbinafine and naftifine, have primarily fungicidal action against many fungi as a result of its specific inhibition of squalene epoxidase (Ryder 1992). Treated fungi accumulate squalene, while becoming deficient in ergosterol, which leads to inhibition of growth. Terbinafine has no effect on cholesterol biosynthesis in vivo (Ryder 1992). Regarding antifungal proteins and peptides, potential targets of fungal cells including several intracellular targets were described earlier (Theis and Stahl 2004; Matejuk et al. 2010). Novel antifungal drugs need to act on targets that are absent or different in mammalian cells.

2.3 Cell Wall-Specific Antifungal Peptides

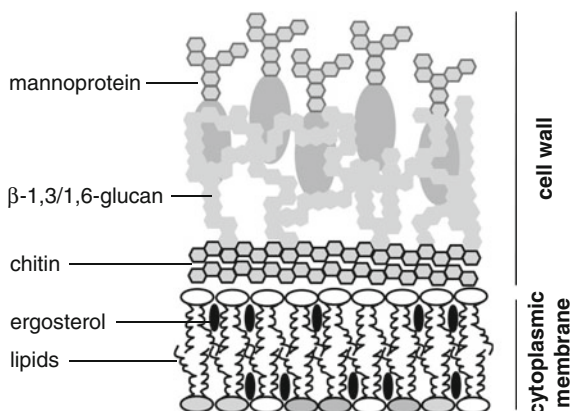
For pathogenic fungi, the cell wall (Fig. 2.2) is critical for invading the host and resisting against host defense mechanisms (Latgé and Beauvais 2014). It provides the cell with sufficient mechanical strength to withstand changes in osmotic pressure imposed by the environment. The fungal cell wall is a complex structure composed typically of chitin, 1,3- β - and 1,6- β -glucan, mannan and proteins, although cell wall composition frequently varies markedly between species of fungi (Adams 2004). Enzymes catalyzing the synthesis of cell wall components are promising targets for antifungal peptides as they are absent in mammalian cells and hence low toxicity of peptides can be expected. Disruptions of cell wall structure have a profound effect on the growth and morphology of the fungal cell, often rendering it susceptible to lysis and death (Bowman and Free 2006).

2.3.1 Inhibitors of Glucan Synthase

Glucan is the major structural polysaccharide of the fungal cell wall, constituting approximately 50–60 % of the wall by dry weight (Fleet 1985; Kapteyn et al. 1999). The 1,3- β -glucan serves as the main structural constituent to which other cell wall components are covalently attached. As a result, the synthesis of 1,3- β -glucan is required for proper cell wall formation and the normal development of fungi (Bowman and Free 2006).

Echinocandins (caspofungin, micafungin, and anidulafungin) are now the preferred first line therapy for patients with invasive candidiasis (Spampinato and Leonardi 2013). These semi-synthetic lipopeptides are non-competitive inhibitors of (1,3)- β -D-glucan synthase, an enzyme complex that forms glucan polymers in fungal cell walls (Denning 1997). This leads to the formation of fungal cell walls

Fig. 2.2 Schematic representation of the fungal cell envelope highlighting the most important components of the cell wall and cytoplasmic membrane. Membrane proteins were omitted for clarity



with impaired structural integrity, which in turn results in cell vulnerability to osmotic lysis (Grover 2010). Their low toxicity may reflect the fact that their target, (1,3)- β -D-glucan synthase, is not found in humans (Perlin 2015). Echinocandin drugs are potentially fungicidal against most clinically important *Candida* spp. but, are considered fungistatic against *Aspergillus* (Barchiesi et al. 2005; Ernst et al. 1999; Bowman et al. 2002; 2006; Pfaller et al. 2003). Although these types of drugs were licensed first in 2001, reports on *Candida* spp. isolates resistant to echinocandins are increasingly reported (Perlin 2015). Resistance is attributed to point mutations in the *FKSI* gene, which encodes the major subunit of the glucan synthase complex (Perlin 2007).

2.3.2 Inhibitors of Chitin Synthase

Chitin, a long linear homo-polymer of β -1,4-linked N-acetylglucosamine, is a structurally important component of the fungal cell wall. Chitin accounts for only 1–2 % of the yeast cell wall by dry weight (Klis 1994; Klis et al. 2002), whereas the cell walls of filamentous fungi, such as *Neurospora* and *Aspergillus*, are reported to contain 10–20 % chitin (Nobel et al. 2000; Bartnicki-Garcia 1968; Bowman et al. 2006). Disruption of chitin synthesis leads to disordered cell walls and the fungal cell becomes malformed and osmotically unstable (Bago et al. 1996; Specht et al. 1996).

Nikkomycins are a group of peptidyl nucleoside antibiotics produced by *Streptomyces ansiochromogenes* (Chen et al. 2000) and *Streptomyces tendae* (Brillinger 1979). Acting as competitive inhibitors of chitin synthase, nikkomycins inhibit the growth of filamentous fungi and yeasts (Dähn et al. 1976; Feng et al. 2014). Compared to conventional antifungal agents, including fluconazole and amphotericin B, nikkomycin Z resulted in greater killing of *Coccidioides* spp. and was able to sterilize lung lesions in seven of eight mice dosed with 50 mg/kg/day for 6 days, while the conventional agents tested did not sterilize lung lesions in any case (Hector et al. 1990). Nikkomycin Z has been used in Phase I clinical trials for the treatment of coccidioidomycosis (Nix et al. 2009). However, the peptidyl nucleoside was degraded in rat, mouse and rabbit plasma much faster than in pH 7.5 buffer (Tokumura and Horie 1997). Recently, two novel nikkomycin analogs (nikkomycin Px and Pz) were generated by mutasynthesis showing similar antifungal activities to those of natural nikkomycins, but with improved stabilities under different pHs and temperatures (Feng et al. 2014). Polyoxins, which were isolated from the culture broth of *Streptomyces cacaoi*, are closely related to nikkomycins and also act as specific inhibitors of chitin synthase (Hector 1993; Isono et al. 1969). Polyoxins, which contained hydrophobic amino acids, retained strong chitin synthase inhibitory activity and were resistant to cellular hydrolysis of *C. albicans* (Smith et al. 1986).

2.3.3 Chitin Binding Peptides

Members of the family of hevein-like antimicrobial peptides carry a conserved chitin binding site. The hevein-like peptides belong to a unique class of plant antimicrobials that show resemblance to hevein, the antimicrobial peptide (AMP) from latex of *Hevea brasiliensis* (van Parijs et al. 1991; Rogozhin et al. 2015). Their antifungal activity is supposed to be associated with their chitin binding activity. Binding to chitin is believed to interfere with hyphal growth resulting in abnormal branching, retardation of elongation and swelling. Hevein-like peptides are rarely found outside the plant kingdom. Novel hevein-like peptide precursors were identified by similarity search methods, including one from a fungal source (Porto et al. 2012). SmAMP3, a new member of the hevein-like family peptides was isolated recently from leaves of a weed species *S. media* (Rogozhin et al. 2015). It is basic and cysteine-rich, with six cysteines linked to form three disulfide bridges. SmAMP3 demonstrated significant inhibition of spore germination of fungi with highest activity against *B. cinerea* (Rogozhin et al. 2015).

Cyclothiazomycin B1 (CTB1) is an antifungal cyclic thiopeptide isolated from the culture broth of *Streptomyces* sp. HA 125-40. CTB1 inhibited the growth of several filamentous fungi including plant pathogens along with swelling of hyphae and spores, which indicates serious effects on cell wall rigidity. CTB1 does not inhibit chitin synthase activity, but it induces cell wall fragility by binding to chitin (Mizuhara et al. 2011). Also the antifungal activity of penaeidins, a family of antimicrobial peptides characterized in the shrimp *Penaeus vannamei*, can be related to their chitin binding ability (Destoumieux et al. 2000).

2.4 Membrane-Active Antifungal Peptides

As mentioned in the introduction and described above host defense peptides with primarily antifungal activity are much less abundant than peptides with broad antimicrobial activity. This is most likely due to evolution creating molecules that can protect the host from a variety of invaders. Therefore, the predominant fraction of these peptides shows a broad spectrum activity against bacteria, fungi and even viruses (Cole and Ganz 2000). Within this plethora of peptides, which predominantly act on the plasma membrane level, there are some, which interact with specific membrane lipid components such as ergosterol and sphingolipids, described in Sects. 2.4.2 and 2.4.3. However, most of them are supposed to induce lysis of the cell membrane. The molecular mechanism(s) of membrane rupture mutually depends on the nature of the peptide and membrane lipid composition (Lohner and Blondelle 2005; Lohner 2009). Thus, in terms of antifungal drug design it is crucial that antifungal peptides can discriminate between target and host membrane (Lohner 2001).

2.4.1 Broad Spectrum Antimicrobial Peptides

Mammalian and fungal membranes are composed of proteins and three main lipids: phospholipids, sphingolipids, and sterols (Zinser et al. 1993; Löffler et al. 2000; van Meer and de Kroon 2011). The phospholipid classes of eukaryotic plasma membranes are asymmetrically distributed as they actively sequester phosphatidylcholine (PC) and sphingomyelin (SM) within the outer monolayer of the membrane (van Meer et al. 2008; Devaux and Morris 2004). PC accounts for >50 % of the phospholipids in most eukaryotic membranes. It self-organizes spontaneously as a planar bilayer, in which PC has a nearly cylindrical molecular geometry (Fig. 2.3). Most PC molecules have one *cis*-unsaturated fatty acyl chain, which renders the membrane fluid at room temperature (van Meer et al. 2008; van Meer and de Kroon 2011). Sphingolipids usually contain a long to very long saturated fatty acid (C16–C32) with an amide linkage to the sphingoid base. They generally adopt a solid gel phase, but are fluidized by sterols, which supposedly preferentially interact with them in the membrane (van Meer and de Kroon 2011). Phosphatidylethanolamine (PE) as well as the negatively charged lipids phosphatidylserine (PS) and phosphatidylinositol (PI) are found almost exclusively in the inner leaflet of the bilayer

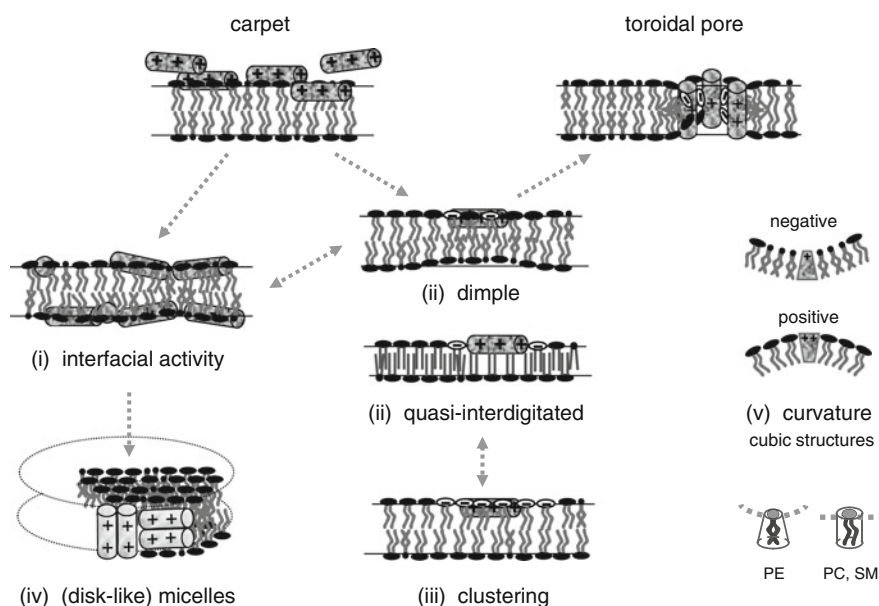


Fig. 2.3 Schematic representation of various modes of action of membrane-active peptides. Arrows indicate some possible mutual reactions, e.g., after peptide binding to and accumulation at the membrane surface followed by insertion into the membrane interface (*carpet model*) various molecular mechanisms may occur, which strongly depend on the nature of both peptide and lipids. At high peptide concentrations micellization may occur. In the *right hand lower corner* the molecular shape of representative lipids are indicated

(van Meer et al. 2008; Devaux and Morris 2004). The non-bilayer propensity of PE is essential for the functional embedding of membrane proteins and for processes such as membrane fusion and fission (Kruijff 1997; Lohner 1996). PE assumes a conical molecular geometry because of the relatively small size of its polar head-group (Fig. 2.3) (Seddon and Templer 1995). Membrane asymmetry is known to affect various bilayer properties, including membrane potential, surface charge, permeability, shape as well as stability (Devaux 1991; Cheng et al. 2009; Marquardt et al. 2015). Owing to this asymmetric phospholipid distribution mainly uncharged, zwitterionic phospholipids are exposed to the outside of the cell membrane of eukaryotes (Lohner 2001).

Studies on the surface potential and the translocation of anionic phospholipids in *Saccharomyces carlsbergensis* unveiled that about 5 % of anionic phospholipids are in the exofacial side of the plasma membrane (Cerbón and Calderón 1994). When cations were added to the culture medium this value increased slightly but significantly to 7 %. On the other hand, most of the membrane-active antimicrobial peptides exhibit a positive net charge under physiological conditions, which facilitates electrostatic interactions with negatively charged lipids of the target cell, while their amphipathic structure facilitates incorporation into membrane layers (Tossi et al. 2000; Lohner and Blondelle 2005). Therefore, owing to the comparatively low content of anionic lipids at the surface of fungi as compared to bacteria membrane-destabilization of antifungal peptides was suggested not to be facilitated by strong electrostatic interactions but rather by cell leakage due to pore formation, which is supposed to appear far below micromolar concentrations (Matsuzaki et al. 1995; Matsuzaki 1998). In the toroidal pore model (Fig. 2.3) peptides together with lipids form transmembrane pores, with the hydrophilic residues facing the lumen of the pore (Matsuzaki et al. 1996; Huang 2006). However, similar amounts of anionic lipids, i.e., PS, were found to be exposed on the outer membrane leaflet of cancer cells (Riedl et al. 2011a) shown to be sufficient to render them as target for cationic antimicrobial peptides without affecting significantly membranes of normal cells (Riedl et al. 2011b; Riedl et al. 2015; Hoskin and Ramamoorthy 2008). Thus, membrane permeabilization of fungal membranes may also occur by other modes of action than pore formation. In this respect, the most frequently discussed mechanism is the carpet model (Fig. 2.3), where AMPs accumulate at the cell membrane being aligned parallel to the bilayer surface and insert into the membrane above a certain threshold concentration resulting in membrane permeabilization and eventually disruption (Shai 2002). At the molecular level different processes may apply that can lead to loss of membrane integrity briefly listed here and schematically shown in Fig. 2.3:

- (i) interfacial activity model, defined as the propensity of amphipathic peptides to partition into the membrane interface in a way to disrupt the normally strict segregation of polar and non-polar groups of the lipids (Wimley 2010),
- (ii) free volume model, interfacial alignment parallel to the membrane plane creating “voids” in the hydrophobic core of the membrane, which leads to a quasi-interdigitated structure in the gel phase and membrane thinning/dimple formation in the fluid phase (Lohner 2009; Sevcsik et al. 2007; Huang 2000),

- (iii) phase separation, creating domains with different physico-chemical properties between lipid bulk and peptide-enriched domains (Arouri et al. 2009; Epand et al. 2010; Epand and Epand 2009, 2011; Lohner 2009),
- (iv) disruption of the membrane similar to detergents occurring particularly at high peptide concentration (Bechinger and Lohner 2006),
- (v) modifying membrane curvature strain (Koller and Lohner 2014; Lohner and Blondelle 2005).

These models may be considered as special cases within the complex interaction of amphipathic peptides and membrane lipids, which besides of their nature also depend on a number of factors including environmental factors such as pH, ionic strength or temperature. Taking this into consideration and the fact that both molecules are highly dynamic, the SMART (soft membranes adapt and respond) model was introduced to account for the full range of possibilities (Bechinger 2015). Notably, the fast killing rate within minutes (Boman 2003) as well as the nature of the target (lipids of the plasma membrane) makes the occurrence of resistance less likely, since substantial modification of the lipid composition would affect fungal cell viability.

Although mammalian and fungal plasma membranes are similar in structure and composition, differences may arise when fungal species that infect humans switch from yeast cells to mycelium, which is considered to be an important factor in pathogenesis and in turn may facilitate the design of novel antifungal peptides. The primary function of hypha formation is to invade the substrate they are adhered to (Brand 2012). The levels of total lipids, sterols and phospholipids were found to be different in the mycelial form (log phase) of *Candida albicans* and in its yeast form (Mishra and Prasad 1990; Goyal and Khuller 1994). The contents of PC, PS, and PI in the mycelial form are higher than in the yeast form, whereas the opposite is true for PE (Mishra and Prasad 1990; Goyal and Khuller 1994). Analyses of the fatty acid composition showed that mycelial apolar and polar lipid fractions contained higher levels of polyunsaturated fatty acids (C18:2 and C18:3) as well as C16:0, C16:1 and C18:0, but lower levels of oleic acid (C18:1) than the corresponding yeast fractions (Ghannoum et al. 1986). The differences in the fatty acid pattern resulted in alterations in the thermotropic phase behavior and thus physico-chemical properties of *C. albicans* membrane lipids corresponding to its morphological form (Goyal and Khuller 1994; Ghannoum et al. 1986). The fatty acid pattern of mycelial lipids from *A. niger* were also different from its yeast form lipids (Chattopadhyay et al. 1985). An unusual lipid species, pyrophosphatidic acid (pyro-PA), was identified in *Cryptococcus neoformans* (Itoh and Kaneko 1977). Pyro-PA may have a potential role in signaling and stress response in *C. neoformans* and it is important for the mammalian immune response (Shea et al. 2006; Balboa et al. 1999). Unlike other fungi, membranes of clinical isolates of the pathogenic yeast *Cryptococcus neoformans* contain obtusifoliol as major sterol, followed by ergosterol (Ghannoum et al. 1994). Obtusifoliol is an important intermediate in the synthesis of sterols and has been observed in several fungal species following treatment with azoles (Vanden Bossche et al. 1990; Ghannoum et al. 1994). As with the total sterol

content, there was considerable variation in the types and quantities of sterols present in isolates from individual patients (Ghannoum et al. 1994). In contrast to *C. neoformans*, *C. albicans* does not show significant strain-to-strain variation in sterol patterns. Moreover, ergosterol is the predominant sterol in *C. albicans* (Ghannoum et al. 1994). In this respect, it is highly interesting to note that minor structural differences of sterols as deduced from NMR experiments can account for differential binding of amphotericin B to ergosterol (strong), cholesterol (weak) and lanosterol (no binding) (Anderson et al. 2014). It was suggested that this has also important implications for the design of novel antifungal compounds that distinguish between ergosterol of fungal and cholesterol of mammalian cell membranes thereby reducing unwanted side effects (Lohner 2014).

2.4.2 Antifungal Peptides and Ergosterol

Fungal membranes differ from those of higher eukaryotes concerning sterols, which regulate membrane fluidity. Ergosterol is the major sterol in the membranes of lower eukaryotes like yeast and fungi, whereas cholesterol predominates in the plasma membrane of mammalian cells (Henriksen et al. 2006). Antifungal substances like polyenes, azoles, and allylamines act on ergosterol or its synthesis (Ryder 1992; Sabatelli et al. 2006; Anderson et al. 2014). Cholesterol and ergosterol are similar molecules, but there are slight structural differences: ergosterol has two additional double bonds as well as a methyl group on the side chain (Hsueh et al. 2007). These small differences in sterol structure, however, result in stronger conformational ordering of lipid acyl chains in case of cholesterol and weaker effects on membrane packing for ergosterol (Hsueh et al. 2007; Urbina et al. 1995).

The small cyclic lipodepsipeptide syringomycin E from *Pseudomonas syringae* is a potent antifungal peptide (Segre et al. 1989; Lucca et al. 1999). Syringomycin E acts on the fungal plasma membrane and alters several of its functions, including ion transport, protein phosphorylation, and H⁺-ATPase activity (Zhang and Takemoto 1986; Suzuki et al. 1992; Reidl et al. 1989; Feigin et al. 1997). The antifungal activity of syringomycin E is dependent on the presence of sterols in the plasma membrane of the fungal cells (Takemoto et al. 1993). Furthermore, the pore-forming activity of syringomycin E can be modulated by the type of sterol. The energy barrier for the channel formation in membrane bilayers was highest in presence of cholesterol, while ergosterol was promoting pore-forming activity of this lipopeptide (Feigin et al. 1997; Blasko et al. 1998). Although syringomycins are fungicidal against important human pathogenic yeasts, they caused lysis of sheep erythrocytes (Sorensen et al. 1996).

Psd1, a defensin isolated from seeds of the pea *Pisum sativum* with a compact cysteine-stabilized α/β motif, showed high partitioning into ergosterol-containing membranes (as fungal membranes), whereas partitioning of Psd1 into cholesterol-containing membranes was undetectable (Gonçalves et al. 2012b). This suggests low toxicity of Psd1 to mammalian (cholesterol-rich) membranes. The

cationic Psd1 has also increased affinity for membranes containing glucosylceramide, which is the most common fungal glycosphingolipid (Gonçalves et al. 2012b; Vriens et al. 2014; Wilmes et al. 2011). Upon interaction with their target membrane, plant defensins are either internalized by the fungal cell and interact with internal targets, or they stay at the cell surface and induce cell death through induction of a signaling cascade (Vriens et al. 2014).

The human neutrophil peptide 1 (HNP1) showed low interaction with glucosylceramide rich membranes, but high sterol selectivity for ergosterol-containing membranes in vitro (Gonçalves et al. 2012a). The histidine-rich glycoprotein (HRG) as well as the epithelium-produced growth factor midkine preferentially lysed ergosterol-containing liposomes over cholesterol-containing ones, indicating a specificity for fungal versus mammalian membranes (Rydengård et al. 2008; Nordin et al. 2012). Although these peptides show selectivity for fungal membranes in vitro, their therapeutic application would be accompanied by dose-limited toxicities towards human cells.

2.4.3 Antifungal Peptides and Sphingolipids

Sphingolipids are potentially specific targets for antifungal molecules due to structural differences between fungal and mammalian sphingolipids such as 9-methyl group branching of the sphingoid base and different degrees of unsaturation in fungal sphingolipids (Thevissen et al. 2005). Sphingolipids and their biosynthesis have been investigated intensively for the yeast *S. cerevisiae*. The three types of sphingolipids (IPC, MIPC, and M(IP)2C) are located primarily in the plasma membrane (Patton and Lester 1991; Hechtberger et al. 1994). Disruption of the biosynthetic pathway for the sphingolipid mannosyl di-(inositol phosphoryl) ceramide (M(IP)2C) in *S. cerevisiae* resulted in resistance to the plant defensin DmAMP1 and the synthetic amphipathic peptide mimetic LTX109 indicating that M(IP)2C is essential for their antifungal action (Thevissen et al. 2000; Bojsen et al. 2013). DmAMP1 was shown to bind to purified M(IP)2C and this binding was enhanced in the presence of ergosterol (Thevissen et al. 2003).

Another plant defensin, RsAFP2, as well as the insect defensin-like heliomicin, selectively binds to glucosylceramide from fungi like *P. pastoris* and *C. albicans*, but not to glucosylceramide from human source (Thevissen et al. 2004). *S. cerevisiae* that do not contain this sphingolipid is resistant to RsAFP2-induced permeabilization and growth inhibition. In contrast to DmAMP1, the interaction of RsAFP2 with glucosylceramide was not increased in the presence of ergosterol (Thevissen et al. 2004).

Other plant and insect defensins interacting specifically with sphingolipids are Psd1 isolated from pea seeds (Medeiros et al. 2010; Wilmes et al. 2011); Sd5 isolated from *Saccharum officinarum* (De-Paula et al. 2008); MsDef1 from *Medicago sativa* (Ramamoorthy et al. 2007) and Drosomycin, an inducible insect defensin isolated from *Drosophila* (Gao and Zhu 2008).

2.5 Intracellular Targets

Some antifungal peptides enter the fungal cell and interact with intracellular targets after crossing the plasma membrane. Nevertheless, membrane lipids play a role concerning specificity and efficacy of these antifungal peptides.

Histatin 5 (Hst5), a human basic salivary peptide with strong fungicidal properties *in vitro*, becomes internalized and targets to energized mitochondria (Helmerhorst et al. 1999). The killing of *C. albicans* by Hst5 is accomplished by an increase in membrane potential and permeability and the subsequent release of intracellular ATP (Koshlukova et al. 1999, 2000; Bobek and Situ 2003). However, non-respiring yeast cells were protected against histatin 5 killing activity (Helmerhorst et al. 1999). The importance of metabolic activity in the susceptibility of *C. albicans* cells to basic proteins, like protamine or HNP-1, was already reported by Olson et al. (1977) and Lehrer et al. (1988). Interestingly, the amino acid sequence of histatin 5 resembles the mitochondrial targeting sequence characteristic for mitochondrial proteins that target proteins from cytosol to mitochondria (Nicolay et al. 1994; Helmerhorst et al. 1999). Perturbation of mitochondrial membranes by antifungal peptides may be facilitated by the divalent negative phospholipid cardiolipin, which is highly enriched in the inner mitochondrial membrane (Daum 1985).

Antifungal peptides may also cause inhibition of nuclear migration and nuclear division as shown for the penta-peptide auristatin PHE (Woyke et al. 2002), which has fungicidal activity against *C. neoformans*. This peptide caused complete disruption of both spindle and cytoplasmic microtubules in *C. neoformans*. As a consequence cell cycle arrest was leading to uninucleate, large-budded cells. The nucleus itself is the intracellular target of the plant defensin PsD1 (Lobo et al. 2007). PsD1 was shown to interact with the cell cycle control protein cyclin F from *N. crassa* cells and thereby impaired the progression of the cell cycle (Lobo et al. 2007).

Dermaseptin S3(1-16) and magainin 2 are two unrelated, amphibian-derived cationic peptides that interacted with DNA *in vitro*. Both peptides also interfered with DNA integrity of *S. cerevisiae* *in vivo* (Morton et al. 2007). This implies that both peptides are able to pass through the cytoplasmic membrane of yeast cells and damage DNA.

PA1b (pea albumin 1 subunit b) is a plant peptide of 37 amino acids purified from *Pisum sativum* and acts as an insecticide. The toxicity of PA1b is due to a specific and direct interaction with the V0 complex of the vacuolar proton pump (Chouabe et al. 2011). PA1b adopts a typical knottin fold with a triple-stranded antiparallel β -sheet and three buried interlocked disulfide bonds (Jouvensal et al. 2003). Antifungal activity has been reported for the knottin-type peptides Mj-AMP1 and PAFP-S (Cammue et al. 1992; Gao et al. 2001; van der Weerden et al. 2013).

2.6 Synergism with Conventional Antifungal Drugs

An attractive therapeutic option might be a combination of antifungal peptides with conventional antifungal drugs like amphotericin B and azoles. In fact, a substantial cooperative effect of lactoferrin with amphotericin B, fluconazole, and 5-fluorocytosine was observed against *Candida* species (Kuipers et al. 1999). The combination of lactoferrin and fluconazole appeared to be the most successful combination. Wakabayashi et al. reported on cooperative effects of lactoferrin with clomitrazone agents against *Candida* growth (Wakabayashi et al. 1996). Lactoferrin is an innate host defense protein, which exerts a candidacidal effect in a cation concentration-dependent manner (Viejo-Díaz et al. 2004). Peptide 2, a short and potent lactoferrin derivative, suppressed the growth of *Candida* cells additively by a combination of peptide 2 with amphotericin B or miconazole (Ueta et al. 2001). Furthermore, in pilot experiments the effect on the minimal inhibitory concentration of amphotericin B, fluconazole, and 5-fluorocytosine upon addition of sub-inhibitory concentrations of the frog skin antimicrobial peptide, PGLa, as well as of Hst5 and designed analogs was tested (van't Hof et al. 2000). Thereby, addition of the peptides to amphotericin B resulted in a synergistic effect against several *Aspergillus*, *Candida* and *Cryptococcus* strains, while no enhanced activity was found in combination with fluconazole or 5-fluorocytosine. Tanida et al. also reported that Hst5 and the human neutrophil peptide, HNP1, acted synergistically with amphotericin B and itraconazole to suppress *Candida* colony formation (Tanida et al. 2006). The synergism between HNP1 and itraconazole was weak compared to combinations with other peptides. Inhibition of sterol synthesis by itraconazole might reduce membrane affinity of HNP1 as this peptide was shown to have high sterol selectivity for ergosterol-containing membranes in vitro (Gonçalves et al. 2012a). A number of studies concerning synergism between antifungal peptides of the echinocandin family and amphotericin B or azoles were performed. Disturbing the integrity of fungal cell walls by echinocandins may facilitate access of polyenes and triazoles to the cell membrane. Synergy between cilofungin and amphotericin B, a polyene derivative, was first reported for a murine model of candidiasis in 1991 (Hanson et al. 1991). Anidulafungin increased the antimycotic efficacy of amphotericin B and fluconazole against *Candida* spp. (Rosato et al. 2012) and pneumocandin L-743,872 enhanced the efficacy of fluconazole and amphotericin B in vitro against *C. neoformans* (Franzot and Casadevall 1997). Caspofungin and amphotericin B were synergistic or synergistic to additive for a number of clinical isolates of *Aspergillus* and *Fusarium* spp. (Arikan et al. 2002). A successful combined antifungal treatment of a life-threatening systemic fungal infection by *Aspergillus flavus* was reported by Krivan et al. (2006). The infection which developed in a central venous catheter tunnel progressed rapidly in spite of conventional and subsequent liposomal amphotericin B therapy. However, the deep fungal infection resolved after 30 days of dual therapy with liposomal amphotericin B and caspofungin. Therapy with co-administration of two or three antifungals has been applied by clinicians in

difficult-to-treat infection. However, there is still no support from randomized, controlled clinical trials (Hatipoglu and Hatipoglu 2013). Nevertheless, in summary these studies indicate that the growth inhibitory activity of conventional antifungal drugs can be enhanced by sub-inhibitory concentrations of antimicrobial peptides without affecting the cytotoxic activity against mammalian cells, suggesting that combination therapy can be a promising strategy for treatment of fungal infections.

2.7 Concluding Remarks

A global rise in incidences of invasive fungal infections has been reported, although true mortality rates are unknown because of a lack of good epidemiological data. This development has been largely related to modern medical interventions and immunosuppressive diseases (Brown et al. 2012). For example, in her annual report of 2011 the UK Chief Medical Officer Dame Sally C. Davies summed up: “Thus we are now seeing the paradoxical emergence of new infectious disease threats, and the re-emergence of infections that had previously been thought to be a problem of the past, as a direct consequence of the success of modern medicine. Examples include the increased risk of infection in general, but also of unusual infections such as invasive fungal disease, in patients being treated for non-infectious diseases, such as patients on immunosuppressive treatments for cancer or inflammatory disease.” Further, demographic changes resulting in an ever elderly population favors such disease pattern and demand to manage also infectious complications common in patients undergoing dialysis for renal failure, and surgery, especially organ transplantation. Unfortunately, clinically available drugs have had only modest success in reducing the high mortality rates of invasive fungal infections such as candidiasis and cryptococcosis, their treatment relying on a limited number of antifungal drugs. In terms of such life-threatening systemic infections amphotericin B, which was brought onto the market in the 1950s, still remains the first line treatment and is considered as the gold standard despite its low therapeutic index, which may cause severe side effects. Furthermore, recent data indicate the emergence of drug-resistant fungi within hospitals and possibly the larger environment (Mesa-Arango et al. 2012). Therefore, as a consequence of the current situation Brown et al. proposed to tackle human fungal infections by (i) raising the general awareness of the problem, (ii) developing rapid, simple, and cheap diagnostics as well as (iii) safer and more effective antifungal drugs (Brown et al. 2012).

In this contribution, we focused on one alternative strategy for the development of novel specific antifungal drugs, which is based on host defense peptides. Among these peptides a minor group shows primarily antifungal activity, while the majority of peptides exhibit broad antimicrobial activity. Both classes have targets, which are absent in mammalian cells and therefore will have strongly reduced or no side effects. Peptides belonging to the former group of peptides bind to (i) enzymes, which are essential for the biosynthesis of the cell wall, (ii) ergosterol and (iii) sphingolipids, both being essential for plasma membrane function. The latter

group of peptides interacts with the cytoplasmic membrane inducing membrane permeabilization and cell lysis. Biophysical studies on membrane mimetic systems demonstrated that these membrane-active peptides have no specific receptor and thus they should be less prone to resistance development. The molecular mechanism(s) of killing depends on both the physico-chemical properties of the peptides and the membrane lipid composition. A detailed mechanistic understanding of antifungal activity will be important to understand the molecular basis for selective targeting of fungal cells. This in turn is essential for the rational development of novel antifungal agents that lead to more specific and hence safer therapeutics. Finally, these peptides may also be used synergistically in combination with conventional antifungal drugs, which would further widen the armory to treat especially life-threatening invasive fungal infections.

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References

- Adams DJ (2004) Fungal cell wall chitinases and glucanases. *Microbiology* (Reading, Engl) 150 (Pt 7):2029–2035. doi:[10.1099/mic.0.26980-0](https://doi.org/10.1099/mic.0.26980-0)
- Almeida MS, Cabral KM, Zingali RB, Kurtenbach E (2000) Characterization of two novel defense peptides from pea (*Pisum sativum*) seeds. *Arch Biochem Biophys* 378(2):278–286. doi:[10.1006/abbi.2000.1824](https://doi.org/10.1006/abbi.2000.1824)
- Anderson TM, Clay MC, Cioffi AG, Diaz KA, Hisao GS, Tuttle MD, Nieuwkoop AJ, Comellas G, Maryum N, Wang S, Uno BE, Wildeman EL, Gonen T, Rienstra CM, Burke MD (2014) Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nat Chem Biol* 10 (5):400–406. doi:[10.1038/nchembio.1496](https://doi.org/10.1038/nchembio.1496)
- Arikan S, Lozano-Chiu M, Paetznick V, Rex JH (2002) In vitro synergy of caspofungin and amphotericin B against *Aspergillus* and *Fusarium* spp. *Antimicrob Agents Chemother* 46 (1):245–247
- Arouri A, Dathe M, Blume A (2009) Peptide induced demixing in PG/PE lipid mixtures: a mechanism for the specificity of antimicrobial peptides towards bacterial membranes? *Biochim Biophys Acta* 1788(3):650–659. doi:[10.1016/j.bbamem.2008.11.022](https://doi.org/10.1016/j.bbamem.2008.11.022)
- Bago B, Chamberland H, Goulet A, Vierheilig H, Lafontaine J, Piché Y (1996) Effect of Nikkomycin Z, a chitin-synthase inhibitor, on hyphal growth and cell wall structure of two arbuscular-mycorrhizal fungi. *Protoplasma* 192(1–2):80–92. doi:[10.1007/BF01273247](https://doi.org/10.1007/BF01273247)
- Balboa MA, Balsinde J, Dillon DA, Carman GM, Dennis EA (1999) Proinflammatory macrophage-activating properties of the novel phospholipid diacylglycerol pyrophosphate. *J Biol Chem* 274(1):522–526
- Barchiesi F, Spreghini E, Tomassetti S, Arzeni D, Giannini D, Scalise G (2005) Comparison of the fungicidal activities of caspofungin and amphotericin B against *Candida glabrata*. *Antimicrob Agents Chemother* 49(12):4989–4992. doi:[10.1128/AAC.49.12.4989-4992.2005](https://doi.org/10.1128/AAC.49.12.4989-4992.2005)
- Bartnicki-Garcia S (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annu Rev Microbiol* 22:87–108. doi:[10.1146/annurev.mi.22.100168.000511](https://doi.org/10.1146/annurev.mi.22.100168.000511)
- Bechinger B (2015) The SMART model: soft membranes adapt and respond, also transiently, in the presence of antimicrobial peptides. *J Pept Sci* 21(5):346–355. doi:[10.1002/psc.2729](https://doi.org/10.1002/psc.2729)
- Bechinger B, Lohner K (2006) Detergent-like actions of linear amphipathic cationic antimicrobial peptides. *Biochim Biophys Acta* 1758(9):1529–1539. doi:[10.1016/j.bbamem.2006.07.001](https://doi.org/10.1016/j.bbamem.2006.07.001)

- Blasko K, Schagina LV, Agner G, Kaulin YA, Takemoto JY (1998) Membrane sterol composition modulates the pore forming activity of syringomycin E in human red blood cells. *Biochim Biophys Acta* 1373(1):163–169
- Bobek LA, Situ H (2003) MUC7 20-Mer: investigation of antimicrobial activity, secondary structure, and possible mechanism of antifungal action. *Antimicrob Agents Chemother* 47(2):643–652. doi:[10.1128/AAC.47.2.643-652.2003](https://doi.org/10.1128/AAC.47.2.643-652.2003)
- Bojsen R, Torbensen R, Larsen CE, Folkesson A, Regenberg B (2013) The synthetic amphipathic peptidomimetic LTX109 is a potent fungicide that disturbs plasma membrane integrity in a sphingolipid dependent manner. *PLoS ONE* 8(7):e69483. doi:[10.1371/journal.pone.0069483](https://doi.org/10.1371/journal.pone.0069483)
- Boman HG (2003) Antibacterial peptides: basic facts and emerging concepts. *J Intern Med* 254(3):197–215
- Boucher HW, Groll AH, Chiou CC, Walsh TJ (2004) Newer systemic antifungal agents. *Drugs* 64(18):1997–2020. doi:[10.2165/00003495-200464180-00001](https://doi.org/10.2165/00003495-200464180-00001)
- Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. *BioEssays* 28(8):799–808. doi:[10.1002/bies.20441](https://doi.org/10.1002/bies.20441)
- Bowman JC, Hicks PS, Kurtz MB, Rosen H, Schmatz DM, Liberator PA, Douglas CM (2002) The antifungal echinocandin caspofungin acetate kills growing cells of *Aspergillus fumigatus* in vitro. *Antimicrob Agents Chemother* 46(9):3001–3012
- Bowman JC, Abruzzo GK, Flattery AM, Gill CJ, Hickey EJ, Hsu MJ, Kahn JN, Liberator PA, Misura AS, Pelak BA, Wang TC, Douglas CM (2006) Efficacy of caspofungin against *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus nidulans*. *Antimicrob Agents Chemother* 50(12):4202–4205. doi:[10.1128/AAC.00485-06](https://doi.org/10.1128/AAC.00485-06)
- Brand A (2012) Hyphal growth in human fungal pathogens and its role in virulence. *Int J Microbiol* 2012:517529. doi:[10.1155/2012/517529](https://doi.org/10.1155/2012/517529)
- Brillinger GU (1979) Metabolic products of microorganisms. 181. Chitin synthase from fungi, a test model for substances with insecticidal properties. *Arch Microbiol* 121(1):71–74
- Brown GD, Denning DW, Levitz SM (2012) Tackling human fungal infections. *Science* 336(6082):647. doi:[10.1126/science.1222236](https://doi.org/10.1126/science.1222236)
- Butts A, Krysan DJ (2012) Antifungal drug discovery: something old and something new. *PLoS Pathog* 8(9):e1002870. doi:[10.1371/journal.ppat.1002870](https://doi.org/10.1371/journal.ppat.1002870)
- Cammue BP, de Bolle MF, Terras FR, Proost P, van Damme J, Rees SB, Vanderleyden J, Broekaert WF (1992) Isolation and characterization of a novel class of plant antimicrobial peptides from *Mirabilis jalapa* L. seeds. *J Biol Chem* 267(4):2228–2233
- Cerbón J, Calderón V (1994) Surface potential regulation of phospholipid composition and in-out translocation in yeast. *Eur J Biochem* 219(1–2):195–200
- Chattopadhyay P, Banerjee SK, Sen K, Chakrabarti P (1985) Lipid profiles of *Aspergillus niger* and its unsaturated fatty acid auxotroph, UFA2. *Can J Microbiol* 31(4):352–355
- Chen SCA, Sorrell TC (2007) Antifungal agents. *Med J Aust* 187(7):404–409
- Chen W, Zeng H, Tan H (2000) Cloning, sequencing, and function of *sanF*: a gene involved in nikkomycin biosynthesis of *Streptomyces ansochromogenes*. *Curr Microbiol* 41(5):312–316
- Cheng H, Megha London E (2009) Preparation and properties of asymmetric vesicles that mimic cell membranes: effect upon lipid raft formation and transmembrane helix orientation. *J Biol Chem* 284(10):6079–6092. doi:[10.1074/jbc.M806077200](https://doi.org/10.1074/jbc.M806077200)
- Chouabe C, Eyraud V, Da Silva P, Rahioui I, Royer C, Soulage C, Bonvallet R, Huss M, Gressent F (2011) New mode of action for a knottin protein bioinsecticide: pea albumin 1 subunit b (PA1b) is the first peptidic inhibitor of V-ATPase. *J Biol Chem* 286(42):36291–36296. doi:[10.1074/jbc.M111.281055](https://doi.org/10.1074/jbc.M111.281055)
- Cole AM, Ganz T (2000) Human antimicrobial peptides: analysis and application. *BioTech* 29(4):822–826, 828, 830–831
- Cuthbertson BJ, Shepard EF, Chapman RW, Gross PS (2002) Diversity of the penaeidin antimicrobial peptides in two shrimp species. *Immunogenetics* 54(6):442–445. doi:[10.1007/s00251-002-0487-z](https://doi.org/10.1007/s00251-002-0487-z)

- Dähn U, Hagenmaier H, Höhne H, König WA, Wolf G, Zähler H (1976) Stoffwechselprodukte von mikroorganismen. 154. Mitteilung. Nikkomycin, ein neuer hemmstoff der chitinsynthese bei pilzen. Arch Microbiol 107(2):143–160
- Daum G (1985) Lipids of mitochondria. Biochim Biophys Acta 822(1):1–42
- de Kruijff B (1997) Biomembranes. Lipids beyond the bilayer. Nature 386(6621):129–130. doi:[10.1038/386129a0](https://doi.org/10.1038/386129a0)
- de Kruijff B, Demel RA (1974) Polyene antibiotic-sterol interactions in membranes of *Acholeplasma laidlawii* cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic-cholesterol complexes. Biochimica et Biophysica Acta (BBA) Biomembranes 339 (1):57–70. doi:[10.1016/0005-2736\(74\)90332-0](https://doi.org/10.1016/0005-2736(74)90332-0)
- de Luca AJ, Walsh TJ (2000) Antifungal peptides: origin, activity, and therapeutic potential. Rev Iberoam Micol 17(4):116–120
- de Lucca AJ, Jacks TJ, Takemoto J, Vinyard B, Peter J, Navarro E, Walsh TJ (1999) Fungal lethality, binding, and cytotoxicity of syringomycin-E. Antimicrob Agents Chemother 43 (2):371–373
- de Medeiros LN, Angeli R, Sarzedas CG, Barreto-Bergter E, Valente AP, Kurtenbach E, Almeida FCL (2010) Backbone dynamics of the antifungal Psd1 pea defensin and its correlation with membrane interaction by NMR spectroscopy. Biochim Biophys Acta 1798 (2):105–113. doi:[10.1016/j.bbamem.2009.07.013](https://doi.org/10.1016/j.bbamem.2009.07.013)
- de Nobel H, van Den Ende H, Klis FM (2000) Cell wall maintenance in fungi. Trends Microbiol 8 (8):344–345
- Denning DW (1997) Echinocandins and pneumocandins—a new antifungal class with a novel mode of action. J Antimicrob Chemother 40(5):611–614
- Denning DW (2002) Echinocandins: a new class of antifungal. J Antimicrob Chemother 49 (6):889–891
- De-Paula VS, Razzera G, Medeiros L, Miyamoto CA, Almeida MS, Kurtenbach E, Almeida FCL, Valente AP (2008) Evolutionary relationship between defensins in the Poaceae family strengthened by the characterization of new sugarcane defensins. Plant Mol Biol 68(4–5):321–335. doi:[10.1007/s11103-008-9372-y](https://doi.org/10.1007/s11103-008-9372-y)
- Destoumieux D, Muñoz M, Cosseau C, Rodriguez J, Bulet P, Comps M, Bachère E (2000) Penaeidins, antimicrobial peptides with chitin-binding activity, are produced and stored in shrimp granulocytes and released after microbial challenge. J Cell Sci 113(Pt 3):461–469
- Devaux PF (1991) Static and dynamic lipid asymmetry in cell membranes. Biochemistry 30 (5):1163–1173
- Devaux PF, Morris R (2004) Transmembrane asymmetry and lateral domains in biological membranes. Traffic 5(4):241–246. doi:[10.1111/j.1600-0854.2004.0170.x](https://doi.org/10.1111/j.1600-0854.2004.0170.x)
- Drgona L, Khachatryan A, Stephens J, Charbonneau C, Kantecki M, Haider S, Barnes R (2014) Clinical and economic burden of invasive fungal diseases in Europe: focus on pre-emptive and empirical treatment of *Aspergillus* and *Candida* species. Eur J Clin Microbiol Infect Dis 33 (1):7–21. doi:[10.1007/s10096-013-1944-3](https://doi.org/10.1007/s10096-013-1944-3)
- Epand RM, Epand RF (2009) Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochim Biophys Acta 1788(1):289–294. doi:[10.1016/j.bbamem.2008.08.023](https://doi.org/10.1016/j.bbamem.2008.08.023)
- Epand RM, Epand RF (2011) Bacterial membrane lipids in the action of antimicrobial agents. J Pept Sci 17(5):298–305. doi:[10.1002/psc.1319](https://doi.org/10.1002/psc.1319)
- Epand RF, Maloy WL, Ramamoorthy A, Epand RM (2010) Probing the “charge cluster mechanism” in amphipathic helical cationic antimicrobial peptides. Biochemistry 49 (19):4076–4084. doi:[10.1021/bi100378m](https://doi.org/10.1021/bi100378m)
- Ernst EJ, Klepser ME, Ernst ME, Messer SA, Pfaller MA (1999) In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. Diagn Microbiol Infect Dis 33(2):75–80
- Faruck MO, Yusof F, Chowdhury S (2015) An overview of antifungal peptides derived from insect. Peptides. doi:[10.1016/j.peptides.2015.06.001](https://doi.org/10.1016/j.peptides.2015.06.001)

- Fehlbaum P, Bulet P, Michaut L, Lagueux M, Broekaert WF, Hetru C, Hoffmann JA (1994) Insect immunity. Septic injury of *Drosophila* induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. *J Biol Chem* 269(52):33159–33163
- Feigin AM, Schagina LV, Takemoto JY, Teeter JH, Brand JG (1997) The effect of sterols on the sensitivity of membranes to the channel-forming antifungal antibiotic, syringomycin E. *Biochim Biophys Acta* 1324(1):102–110
- Feng C, Ling H, Du D, Zhang J, Niu G, Tan H (2014) Novel nikkomycin analogues generated by mutasynthesis in *Streptomyces ansochromogenes*. *Microb Cell Fact* 13(1):59. doi:[10.1186/1475-2859-13-59](https://doi.org/10.1186/1475-2859-13-59)
- Fleet GH (1985) Composition and structure of yeast cell walls. *Curr Top Med Mycol* 1:24–56
- François Isabelle E J A, Bolle De, Miguel FC, Dwyer G, Goderis Inge J W M, Woutors PFJ, Verhaert PD, Proost P, Schaaper WMM, Cammue BPA, Broekaert WF (2002) Transgenic expression in Arabidopsis of a polyprotein construct leading to production of two different antimicrobial proteins. *Plant Physiol* 128(4):1346–1358. doi:[10.1104/pp.010794](https://doi.org/10.1104/pp.010794)
- Franzot SP, Casadevall A (1997) Pneumocandin L-743,872 enhances the activities of amphotericin B and fluconazole against *Cryptococcus neoformans* in vitro. *Antimicrob Agents Chemother* 41(2):331–336
- Gao B, Zhu S (2008) Differential potency of drosomycin to *Neurospora crassa* and its mutant: implications for evolutionary relationship between defensins from insects and plants. *Insect Mol Biol* 17(4):405–411. doi:[10.1111/j.1365-2583.2008.00810.x](https://doi.org/10.1111/j.1365-2583.2008.00810.x)
- Gao GH, Liu W, Dai JX, Wang JF, Hu Z, Zhang Y, Wang DC (2001) Solution structure of PAFP-S: a new knottin-type antifungal peptide from the seeds of *Phytolacca americana*. *Biochemistry* 40(37):10973–10978
- Ghannoum MA, Rice LB (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin Microbiol Rev* 12(4):501–517
- Ghannoum MA, Janini G, Khamis L, Radwan SS (1986) Dimorphism-associated variations in the lipid composition of *Candida albicans*. *J Gen Microbiol* 132(8):2367–2375. doi:[10.1099/00221287-132-8-2367](https://doi.org/10.1099/00221287-132-8-2367)
- Ghannoum MA, Spellberg BJ, Ibrahim AS, Ritchie JA, Currie B, Spitzer ED, Edwards JE, Casadevall A (1994) Sterol composition of *Cryptococcus neoformans* in the presence and absence of fluconazole. *Antimicrob Agents Chemother* 38(9):2029–2033
- Gonçalves S, Abade J, Teixeira A, Santos NC (2012a) Lipid composition is a determinant for human defensin HNP1 selectivity. *Biopolymers* 98(4):313–321
- Gonçalves S, Teixeira A, Abade J, de Medeiros LN, Kurtenbach E, Santos NC (2012b) Evaluation of the membrane lipid selectivity of the pea defensin Psd1. *Biochim Biophys Acta* 1818(5):1420–1426. doi:[10.1016/j.bbmem.2012.02.012](https://doi.org/10.1016/j.bbmem.2012.02.012)
- Goyal S, Khuller GK (1994) Structural and functional role of lipids in yeast and mycelial forms of *Candida albicans*. *Lipids* 29(11):793–797
- Gressent F, Da Silva P, Eyraud V, Karaki L, Royer C (2011) Pea Albumin 1 subunit b (PA1b), a promising bioinsecticide of plant origin. *Toxins (Basel)* 3(12):1502–1517. doi:[10.3390/toxins3121502](https://doi.org/10.3390/toxins3121502)
- Grover ND (2010) Echinocandins: a ray of hope in antifungal drug therapy. *Indian J Pharmacol* 42(1):9–11. doi:[10.4103/0253-7613.62396](https://doi.org/10.4103/0253-7613.62396)
- Hanson LH, Perlman AM, Clemons KV, Stevens DA (1991) Synergy between cilofungin and amphotericin B in a murine model of candidiasis. *Antimicrob Agents Chemother* 35(7):1334–1337
- Hatipoglu N, Hatipoglu H (2013) Combination antifungal therapy for invasive fungal infections in children and adults. *Expert Rev Anti Infect Ther* 11(5):523–535. doi:[10.1586/eri.13.29](https://doi.org/10.1586/eri.13.29)
- Hechtberger P, Zinser E, Saf R, Hummel K, Paltauf F, Daum G (1994) Characterization, quantification and subcellular localization of inositol-containing sphingolipids of the yeast. *Saccharomyces cerevisiae*. *Eur J Biochem* 225(2):641–649
- Hector RF (1993) Compounds active against cell walls of medically important fungi. *Clin Microbiol Rev* 6(1):1–21

- Hector RF, Zimmer BL, Pappagianis D (1990) Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. *Antimicrob Agents Chemother* 34(4):587–593
- Helmerhorst EJ, Breeuwer P, van't Hof W, Walgreen-Weterings E, Oomen LC, Veerman EC, Amerongen AV, Abee T (1999) The cellular target of histatin 5 on *Candida albicans* is the energized mitochondrion. *J Biol Chem* 274(11):7286–7291
- Henriksen J, Rowat AC, Brief E, Hsueh YW, Thewalt JL, Zuckermann MJ, Ipsen JH (2006) Universal behavior of membranes with sterols. *Biophys J* 90(5):1639–1649. doi:[10.1529/biophysj.105.067652](https://doi.org/10.1529/biophysj.105.067652)
- Hitchcock CA, Dickinson K, Brown SB, Evans EG, Adams DJ (1990) Interaction of azole antifungal antibiotics with cytochrome P-450-dependent 14 alpha-sterol demethylase purified from *Candida albicans*. *Biochem J* 266(2):475–480
- Holz RW (1974) The effects of the polyene antibiotics nystatin and amphotericin B on thin lipid membranes. *Ann N Y Acad Sci* 235:469–479
- Horn DL, Neofytos D, Anaissie EJ, Fishman JA, Steinbach WJ, Olyaei AJ, Marr KA, Pfaller MA, Chang C, Webster KM (2009) Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. *Clin Infect Dis* 48(12):1695–1703. doi:[10.1086/599039](https://doi.org/10.1086/599039)
- Hoskin DW, Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. *Biochim Biophys Acta* 1778(2):357–375. doi:[10.1016/j.bbamem.2007.11.008](https://doi.org/10.1016/j.bbamem.2007.11.008)
- Hsueh Y, Chen M, Patty PJ, Code C, Cheng J, Frisken BJ, Zuckermann M, Thewalt J (2007) Ergosterol in POPC membranes: physical properties and comparison with structurally similar sterols. *Biophys J* 92(5):1606–1615. doi:[10.1529/biophysj.106.097345](https://doi.org/10.1529/biophysj.106.097345)
- Huang HW (2000) Action of antimicrobial peptides: two-state model. *Biochemistry* 39(29):8347–8352
- Huang HW (2006) Molecular mechanism of antimicrobial peptides: the origin of cooperativity. *Biochim Biophys Acta* 1758(9):1292–1302. doi:[10.1016/j.bbamem.2006.02.001](https://doi.org/10.1016/j.bbamem.2006.02.001)
- Isaksson J, Brandsdal BO, Engqvist M, Flaten GE, Svendsen JSM, Stensen W (2011) A synthetic antimicrobial peptidomimetic (LTX 109): stereochemical impact on membrane disruption. *J Med Chem* 54(16):5786–5795. doi:[10.1021/jm200450h](https://doi.org/10.1021/jm200450h)
- Isono K, Asahi K, Suzuki S (1969) Studies on polyoxins, antifungal antibiotics. 13. The structure of polyoxins. *J Am Chem Soc* 91(26):7490–7505
- Itoh T, Kaneko H (1977) The in vivo incorporation of [32P]-labeled orthophosphate into pyrophosphatidic acid and other phospholipids of *Cryptococcus neoformans* through cell growth. *Lipids* 12(10):809–813
- Jouvansal L, Quillien L, Ferrasson E, Rahbé Y, Guéguen J, Vovelle F (2003) PA1b, an insecticidal protein extracted from pea seeds (*Pisum sativum*): 1H-2-D NMR study and molecular modeling. *Biochemistry* 42(41):11915–11923. doi:[10.1021/bi034803l](https://doi.org/10.1021/bi034803l)
- Kapteyn JC, van Den Ende H, Klis FM (1999) The contribution of cell wall proteins to the organization of the yeast cell wall. *Biochim Biophys Acta* 1426(2):373–383
- Klis FM (1994) Review: cell wall assembly in yeast. *Yeast* 10(7):851–869. doi:[10.1002/yea.320100702](https://doi.org/10.1002/yea.320100702)
- Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 26(3):239–256
- Koller D, Lohner K (2014) The role of spontaneous lipid curvature in the interaction of interfacially active peptides with membranes. *Biochim Biophys Acta* 1838(9):2250–2259. doi:[10.1016/j.bbamem.2014.05.013](https://doi.org/10.1016/j.bbamem.2014.05.013)
- Kontoyiannis DP, Mantadakis E, Samonis G (2003) Systemic mycoses in the immunocompromised host: an update in antifungal therapy. *J Hosp Infect* 53(4):243–258. doi:[10.1053/jhin.2002.1278](https://doi.org/10.1053/jhin.2002.1278)
- Koshlukova SE, Lloyd TL, Araujo MW, Edgerton M (1999) Salivary histatin 5 induces non-lytic release of ATP from *Candida albicans* leading to cell death. *J Biol Chem* 274(27):18872–18879

- Koshlukova SE, Araujo MW, Baev D, Edgerton M (2000) Released ATP is an extracellular cytotoxic mediator in salivary histatin 5-induced killing of *Candida albicans*. *Infect Immun* 68 (12):6848–6856
- Kriván G, Sinkó J, Nagy IZ, Goda V, Reményi P, Bátaí A, Lueff S, Kapás B, Réti M, Tremmel A, Masszi T (2006) Successful combined antifungal salvage therapy with liposomal amphotericin B and caspofungin for invasive *Aspergillus flavus* infection in a child following allogeneic bone marrow transplantation. *Acta Biomed* 77(Suppl 2):17–21
- Kuipers ME, de Vries HG, Eikelboom MC, Meijer DK, Swart PJ (1999) Synergistic fungistatic effects of lactoferrin in combination with antifungal drugs against clinical *Candida* isolates. *Antimicrob Agents Chemother* 43(11):2635–2641
- Lacerda AF, Vasconcelos EAR, Pelegrini PB, de Sa Grossi, Maria F (2014) Antifungal defensins and their role in plant defense. *Front Microbiol* 5:116. doi:[10.3389/fmicb.2014.00116](https://doi.org/10.3389/fmicb.2014.00116)
- Lamberty M, Caille A, Landon C, Tassin-Moindrot S, Hetru C, Bulet P, Vovelle F (2001) Solution structures of the antifungal heliomicin and a selected variant with both antibacterial and antifungal activities. *Biochemistry* 40(40):11995–12003
- Latgé J, Beauvais A (2014) Functional duality of the cell wall. *Curr Opin Microbiol* 20:111–117. doi:[10.1016/j.mib.2014.05.009](https://doi.org/10.1016/j.mib.2014.05.009)
- Lehrer RI, Ganz T, Szklarek D, Selsted ME (1988) Modulation of the in vitro candidacidal activity of human neutrophil defensins by target cell metabolism and divalent cations. *J Clin Invest* 81 (6):1829–1835. doi:[10.1172/JCI113527](https://doi.org/10.1172/JCI113527)
- Lobo DS, Pereira IB, Fragel-Madeira L, Medeiros LN, Cabral LM, Faria J, Bellio M, Campos RC, Linden R, Kurtenbach E (2007) Antifungal *Pisum sativum* defensin 1 interacts with *Neurospora crassa* cyclin F related to the cell cycle. *Biochemistry* 46(4):987–996. doi:[10.1021/bi061441j](https://doi.org/10.1021/bi061441j)
- Löffler J, Einsele H, Hebart H, Schumacher U, Hraštík C, Daum G (2000) Phospholipid and sterol analysis of plasma membranes of azole-resistant *Candida albicans* strains. *FEMS Microbiol Lett* 185(1):59–63
- Lohner K (1996) Is the high propensity of ethanolamine plasmalogens to form non-lamellar lipid structures manifested in the properties of biomembranes? *Chem Phys Lipids* 81(2):167–184
- Lohner K (2001) The role of membrane lipid composition in cell targeting of antimicrobial peptides. In: Lohner K (ed) *Development of novel antimicrobial agents: emerging strategies*. Horizon Scientific Press, Wymondham, Norfolk, U.K., pp 149–165
- Lohner K (2009) New strategies for novel antibiotics: peptides targeting bacterial cell membranes. *gpb* 28(2):105–116. doi: [10.4149/gpb_2009_02_105](https://doi.org/10.4149/gpb_2009_02_105)
- Lohner K (2014) Antimicrobial mechanisms: a sponge against fungal infections. *Nat Chem Biol* 10(6):411–412. doi:[10.1038/nchembio.1518](https://doi.org/10.1038/nchembio.1518)
- Lohner K, Blondelle SE (2005) Molecular mechanisms of membrane perturbation by antimicrobial peptides and the use of biophysical studies in the design of novel peptide antibiotics. *Comb Chem High Throughput Screen* 8(3):241–256
- Loyse A, Thangaraj H, Easterbrook P, Ford N, Roy M, Chiller T, Govender N, Harrison TS, Bicanic T (2013) Cryptococcal meningitis: improving access to essential antifungal medicines in resource-poor countries. *Lancet Infect Dis* 13(7):629–637. doi:[10.1016/S1473-3099\(13\)70078-1](https://doi.org/10.1016/S1473-3099(13)70078-1)
- Marquardt D, Geier B, Pabst G (2015) Asymmetric lipid membranes: towards more realistic model systems. *Membranes* 5(2):180–196. doi:[10.3390/membranes5020180](https://doi.org/10.3390/membranes5020180)
- Matejuk A, Leng Q, Begum MD, Woodle MC, Scaria P, Chou S, Mixson AJ (2010) Peptide-based antifungal therapies against emerging infections. *Drugs Future* 35(3):197
- Matsuzaki K (1998) Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim Biophys Acta* 1376(3):391–400
- Matsuzaki K, Murase O, Fujii N, Miyajima K (1995) Translocation of a channel-forming antimicrobial peptide, magainin 2, across lipid bilayers by forming a pore. *Biochemistry* 34 (19):6521–6526

- Matsuzaki K, Murase O, Fujii N, Miyajima K (1996) An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. *Biochemistry* 35(35):11361–11368. doi:[10.1021/bi960016v](https://doi.org/10.1021/bi960016v)
- Mesa-Arango AC, Scorzoni L, Zaragoza O (2012) It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Front Microbiol* 3:286. doi:[10.3389/fmicb.2012.00286](https://doi.org/10.3389/fmicb.2012.00286)
- Mishra P, Prasad R (1990) An overview of lipids of *Candida albicans*. *Prog Lipid Res* 29(2):65–85
- Mizuhara N, Kuroda M, Ogita A, Tanaka T, Usuki Y, Fujita K (2011) Antifungal thiopeptide cyclothiazomycin B1 exhibits growth inhibition accompanying morphological changes via binding to fungal cell wall chitin. *Bioorg Med Chem* 19(18):5300–5310. doi:[10.1016/j.bmc.2011.08.010](https://doi.org/10.1016/j.bmc.2011.08.010)
- Mor A, Hani K, Nicolas P (1994) The vertebrate peptide antibiotics dermaseptins have overlapping structural features but target specific microorganisms. *J Biol Chem* 269(50):31635–31641
- Morton CO, Hayes A, Wilson M, Rash BM, Oliver SG, Coote P (2007) Global phenotype screening and transcript analysis outlines the inhibitory mode(s) of action of two amphibian-derived, alpha-helical, cationic peptides on *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* 51(11):3948–3959. doi:[10.1128/AAC.01007-07](https://doi.org/10.1128/AAC.01007-07)
- Nawrot R, Barylski J, Nowicki G, Broniarczyk J, Buchwald W, Goździcka-Józefiak A (2014) Plant antimicrobial peptides. *Folia Microbiol (Praha)* 59(3):181–196. doi:[10.1007/s12223-013-0280-4](https://doi.org/10.1007/s12223-013-0280-4)
- Nicolay K, Laterveer FD, van Heerde WL (1994) Effects of amphipathic peptides, including presequences, on the functional integrity of rat liver mitochondrial membranes. *J Bioenerg Biomembr* 26(3):327–334
- Nix DE, Swezey RR, Hector R, Galgiani JN (2009) Pharmacokinetics of nikkomycin Z after single rising oral doses. *Antimicrob Agents Chemother* 53(6):2517–2521. doi:[10.1128/AAC.01609-08](https://doi.org/10.1128/AAC.01609-08)
- Nordin SL, Sonesson A, Malmsten M, Mörgelin M, Egesten A (2012) The epithelium-produced growth factor midkine has fungicidal properties. *J Antimicrob Chemother* 67(8):1927–1936. doi:[10.1093/jac/dks136](https://doi.org/10.1093/jac/dks136)
- Odds FC, Brown AJ, Gow NA (2003) Antifungal agents: mechanisms of action. *Trends Microbiol* 11(6):272–279. doi:[10.1016/S0966-842X\(03\)00117-3](https://doi.org/10.1016/S0966-842X(03)00117-3)
- Olson VL, Hansing RL, McClary DO (1977) The role of metabolic energy in the lethal action of basic proteins on *Candida albicans*. *Can J Microbiol* 23(2):166–174
- Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offner GD, Troxler RF (1988) Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on *Candida albicans*. *J Biol Chem* 263(16):7472–7477
- Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM (2009) Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 23(4):525–530. doi:[10.1097/QAD.0b013e328322ffac](https://doi.org/10.1097/QAD.0b013e328322ffac)
- Patton JL, Lester RL (1991) The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane. *J Bacteriol* 173(10):3101–3108
- Perlin DS (2007) Resistance to echinocandin-class antifungal drugs. *Drug Resist Updat* 10(3):121–130. doi:[10.1016/j.drug.2007.04.002](https://doi.org/10.1016/j.drug.2007.04.002)
- Perlin DS (2015) Mechanisms of echinocandin antifungal drug resistance. *Ann N Y Acad Sci*. doi:[10.1111/nyas.12831](https://doi.org/10.1111/nyas.12831)
- Pfaller MA, Diekema DJ (2004) Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. *J Clin Microbiol* 42(10):4419–4431. doi:[10.1128/JCM.42.10.4419-4431.2004](https://doi.org/10.1128/JCM.42.10.4419-4431.2004)
- Pfaller MA, Messer SA, Boyken L, Rice C, Tendolkar S, Hollis RJ, Diekema DJ (2003) Caspofungin activity against clinical isolates of fluconazole-resistant *Candida*. *J Clin Microbiol* 41(12):5729–5731

- Polvi EJ, Li X, O'Meara TR, Leach MD, Cowen LE (2015) Opportunistic yeast pathogens: reservoirs, virulence mechanisms, and therapeutic strategies. *Cell Mol Life Sci* 72(12):2261–2287. doi:[10.1007/s00018-015-1860-z](https://doi.org/10.1007/s00018-015-1860-z)
- Porto WF, Souza VA, Nolasco DO, Franco OL (2012) In silico identification of novel hevein-like peptide precursors. *Peptides* 38(1):127–136. doi:[10.1016/j.peptides.2012.07.025](https://doi.org/10.1016/j.peptides.2012.07.025)
- Ramamoorthy V, Cahoon EB, Li J, Thokala M, Minto RE, Shah DM (2007) Glucosylceramide synthase is essential for alfalfa defensin-mediated growth inhibition but not for pathogenicity of *Fusarium graminearum*. *Mol Microbiol* 66(3):771–786. doi:[10.1111/j.1365-2958.2007.05955.x](https://doi.org/10.1111/j.1365-2958.2007.05955.x)
- Reidl HH, Grover TA, Takemoto JY (1989) 31P-NMR evidence for cytoplasmic acidification and phosphate extrusion in syringomycin-treated cells of *Rhodotorula pilimanae*. *Biochim Biophys Acta* 1010(3):325–329
- Riedl S, Rinner B, Asslaber M, Schaidler H, Walzer S, Novak A, Lohner K, Zweytick D (2011a) In search of a novel target—phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. *Biochim Biophys Acta* 1808(11):2638–2645. doi:[10.1016/j.bbamem.2011.07.026](https://doi.org/10.1016/j.bbamem.2011.07.026)
- Riedl S, Zweytick D, Lohner K (2011b) Membrane-active host defense peptides—challenges and perspectives for the development of novel anticancer drugs. *Chem Phys Lipids* 164(8):766–781. doi:[10.1016/j.chemphyslip.2011.09.004](https://doi.org/10.1016/j.chemphyslip.2011.09.004)
- Riedl S, Leber R, Rinner B, Schaidler H, Lohner K, Zweytick D (2015) Human lactoferricin derived di-peptides deploying loop structures induce apoptosis specifically in cancer cells through targeting membranous phosphatidylserine. *Biochim Biophys Acta* 1848(11 Pt A):2918–2931. doi: [10.1016/j.bbamem.2015.07.018](https://doi.org/10.1016/j.bbamem.2015.07.018)
- Rogozhin EA, Slezina MP, Slavokhotova AA, Istomina EA, Korostyleva TV, Smirnov AN, Grishin EV, Egorov TA, Odintsova TI (2015) A novel antifungal peptide from leaves of the weed *Stellaria media* L. *Biochimie* 116:125–132. doi:[10.1016/j.biochi.2015.07.014](https://doi.org/10.1016/j.biochi.2015.07.014)
- Rosato A, Piarulli M, Schiavone BIP, Montagna MT, Caggiano G, Muraglia M, Carone A, Franchini C, Corbo F (2012) In vitro synergy testing of anidulafungin with fluconazole, tioconazole, 5-flucytosine and amphotericin B against some *Candida* spp. *Med Chem* 8(4):690–698
- Rydengård V, Shannon O, Lundqvist K, Kacprzyk L, Chalupka A, Olsson A, Mörgelin M, Jähnen-Dechent W, Malmsten M, Schmidtchen A (2008) Histidine-rich glycoprotein protects from systemic *Candida* infection. *PLoS Pathog* 4(8):e1000116. doi:[10.1371/journal.ppat.1000116](https://doi.org/10.1371/journal.ppat.1000116)
- Ryder NS (1992) Terbinafine: mode of action and properties of the squalene epoxidase inhibition. *Br J Dermatol* 126(Suppl 39):2–7
- Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, Loeberberg D, Black TA, McNicholas PM (2006) In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 50(6):2009–2015. doi:[10.1128/AAC.00163-06](https://doi.org/10.1128/AAC.00163-06)
- Sanati H, Belanger P, Fratti R, Ghannoum M (1997) A new triazole, voriconazole (UK-109,496), blocks sterol biosynthesis in *Candida albicans* and *Candida krusei*. *Antimicrob Agents Chemother* 41(11):2492–2496
- Seddon JM, Templer RH (1995) Polymorphism of lipid-water systems. In: Lipowsky R, Sackmann E (eds) *Handbook of biological physics: structure and dynamics of membranes*. Elsevier SPC, Amsterdam, pp 97–160
- Segre A, Bachmann RC, Ballio A, Bossa F, Grgurina I, Iacobellis NS, Marino G, Pucci P, Simmaco M, Takemoto JY (1989) The structure of syringomycins A1, E and G. *FEBS Lett* 255(1):27–31
- Selsted ME, Harwig SS, Ganz T, Schilling JW, Lehrer RI (1985) Primary structures of three human neutrophil defensins. *J Clin Invest* 76(4):1436–1439. doi:[10.1172/JCI112121](https://doi.org/10.1172/JCI112121)
- Sevcsik E, Pabst G, Jilek A, Lohner K (2007) How lipids influence the mode of action of membrane-active peptides. *Biochim Biophys Acta* 1768(10):2586–2595. doi:[10.1016/j.bbamem.2007.06.015](https://doi.org/10.1016/j.bbamem.2007.06.015)

- Shai Y (2002) Mode of action of membrane active antimicrobial peptides. *Biopolymers* 66 (4):236–248. doi:[10.1002/bip.10260](https://doi.org/10.1002/bip.10260)
- Shea JM, Henry JL, Del Poeta M (2006) Lipid metabolism in *Cryptococcus neoformans*. *FEMS Yeast Res* 6(4):469–479. doi:[10.1111/j.1567-1364.2006.00080.x](https://doi.org/10.1111/j.1567-1364.2006.00080.x)
- Silva PM, Gonçalves S, Santos NC (2014) Defensins: antifungal lessons from eukaryotes. *Front Microbiol* 5:97. doi:[10.3389/fmicb.2014.00097](https://doi.org/10.3389/fmicb.2014.00097)
- Smith HA, Shenbagamurthi P, Naider F, Kundu B, Becker JM (1986) Hydrophobic polyoxins are resistant to intracellular degradation in *Candida albicans*. *Antimicrob Agents Chemother* 29 (1):33–39
- Sorensen KN, Kim KH, Takemoto JY (1996) In vitro antifungal and fungicidal activities and erythrocyte toxicities of cyclic lipodepsinonapeptides produced by *Pseudomonas syringae* pv. *syringae*. *Antimicrob Agents Chemother* 40(12):2710–2713
- Spampinato C, Leonardi D (2013) *Candida* infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. *Biomed Res Int* 2013:204237. doi:[10.1155/2013/204237](https://doi.org/10.1155/2013/204237)
- Specht CA, Liu Y, Robbins PW, Bulawa CE, Iartchouk N, Winter KR, Riggle PJ, Rhodes JC, Dodge CL, Culp DW, Borgia PT (1996) The *chsD* and *chsE* genes of *Aspergillus nidulans* and their roles in chitin synthesis. *Fungal Genet Biol* 20(2):153–167. doi:[10.1006/fgbi.1996.0030](https://doi.org/10.1006/fgbi.1996.0030)
- Spelbrink RG, Dilmac N, Allen A, Smith TJ, Shah DM, Hockerman GH (2004) Differential antifungal and calcium channel-blocking activity among structurally related plant defensins. *Plant Physiol* 135(4):2055–2067. doi:[10.1104/pp.104.040873](https://doi.org/10.1104/pp.104.040873)
- Suzuki YS, Wang Y, Takemoto JY (1992) Syringomycin-stimulated phosphorylation of the plasma membrane H-ATPase from red beet storage tissue. *Plant Physiol* 99(4):1314–1320
- Sydnor ERM, Perl TM (2011) Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* 24(1):141–173. doi:[10.1128/CMR.00027-10](https://doi.org/10.1128/CMR.00027-10)
- Takemoto JY, Yu Y, Stock SD, Miyakawa T (1993) Yeast genes involved in growth inhibition by *Pseudomonas syringae* pv. *syringae* syringomycin family lipodepsipeptides. *FEMS Microbiol Lett* 114(3):339–342
- Tanida T, Okamoto T, Ueta E, Yamamoto T, Osaki T (2006) Antimicrobial peptides enhance the candidacidal activity of antifungal drugs by promoting the efflux of ATP from *Candida* cells. *J Antimicrob Chemother* 57(1):94–103. doi:[10.1093/jac/dki402](https://doi.org/10.1093/jac/dki402)
- Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. *Philos Trans R Soc Lond B Biol Sci* 356(1411):983–989. doi:[10.1098/rstb.2001.0888](https://doi.org/10.1098/rstb.2001.0888)
- Terras FR, Torrekens S, van Leuven F, Osborn RW, Vanderleyden J, Cammue BP, Broekaert WF (1993) A new family of basic cysteine-rich plant antifungal proteins from *Brassicaceae* species. *FEBS Lett* 316(3):233–240
- Theis T, Stahl U (2004) Antifungal proteins: targets, mechanisms and prospective applications. *Cell Mol Life Sci* 61(4):437–455. doi:[10.1007/s00018-003-3231-4](https://doi.org/10.1007/s00018-003-3231-4)
- Thevissen K, Cammue BP, Lemaire K, Winderickx J, Dickson RC, Lester RL, Ferket KK, van Even F, Parret AH, Broekaert WF (2000) A gene encoding a sphingolipid biosynthesis enzyme determines the sensitivity of *Saccharomyces cerevisiae* to an antifungal plant defensin from dahlia (*Dahlia merckii*). *Proc Natl Acad Sci U S A* 97(17):9531–9536. doi:[10.1073/pnas.160077797](https://doi.org/10.1073/pnas.160077797)
- Thevissen K, François IEJA, Takemoto JY, Ferket KKA, Meert EMK, Cammue BPA (2003) DmAMP1, an antifungal plant defensin from dahlia (*Dahlia merckii*), interacts with sphingolipids from *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 226(1):169–173
- Thevissen K, Warnecke DC, François IEJA, Leipelt M, Heinz E, Ott C, Zähringer U, Thomma BPHJ, Ferket KKA, Cammue BPA (2004) Defensins from insects and plants interact with fungal glucosylceramides. *J Biol Chem* 279(6):3900–3905. doi:[10.1074/jbc.M311165200](https://doi.org/10.1074/jbc.M311165200)
- Thevissen K, François IEJA, Aerts AM, Cammue BPA (2005) Fungal sphingolipids as targets for the development of selective antifungal therapeutics. *Curr Drug Targets* 6(8):923–928
- Tokumura T, Horie T (1997) Kinetics of nikkomycin Z degradation in aqueous solution and in plasma. *Biol Pharm Bull* 20(5):577–580

- Tossi A, Sandri L, Giangaspero A (2000) Amphipathic, alpha-helical antimicrobial peptides. *Biopolymers* 55(1):4–30. doi:[10.1002/1097-0282\(2000\)55:1<4::AID-BIP30>3.0.CO;2-M](https://doi.org/10.1002/1097-0282(2000)55:1<4::AID-BIP30>3.0.CO;2-M)
- Ueta E, Tanida T, Osaki T (2001) A novel bovine lactoferrin peptide, FKRRWQWRM, suppresses *Candida* cell growth and activates neutrophils. *J Pept Res* 57(3):240–249
- Urbina JA, Pekerar S, Le HB, Patterson J, Montez B, Oldfield E (1995) Molecular order and dynamics of phosphatidylcholine bilayer membranes in the presence of cholesterol, ergosterol and lanosterol: a comparative study using ²H-, ¹³C- and ³¹P-NMR spectroscopy. *Biochim Biophys Acta* 1238(2):163–176
- van den Bossche H, Willemsens G, Cools W, Lauwers WF, Le Jeune L (1978) Biochemical effects of miconazole on fungi. II. Inhibition of ergosterol biosynthesis in *Candida albicans*. *Chem Biol Interact* 21(1):59–78
- van der Weerden NL, Bleackley MR, Anderson MA (2013) Properties and mechanisms of action of naturally occurring antifungal peptides. *Cell Mol Life Sci* 70(19):3545–3570. doi:[10.1007/s00018-013-1260-1](https://doi.org/10.1007/s00018-013-1260-1)
- van Meer G, de Kroon Anton I P M (2011) Lipid map of the mammalian cell. *J Cell Sci* 124(Pt 1):5–8. doi:[10.1242/jcs.071233](https://doi.org/10.1242/jcs.071233)
- van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol* 9(2):112–124. doi:[10.1038/nrm2330](https://doi.org/10.1038/nrm2330)
- van Parijs J, Broekaert WF, Goldstein IJ, Peumans WJ (1991) Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. *Planta* 183(2):258–264. doi:[10.1007/BF00197797](https://doi.org/10.1007/BF00197797)
- van't Hof W, Reijnders IM, Helmerhorst EJ, Walgreen-Weterings E, Simoons-Smit IM, Veerman EC, Amerongen AV (2000) Synergistic effects of low doses of histatin 5 and its analogues on amphotericin B anti-mycotic activity. *Antonie Van Leeuwenhoek* 78(2):163–169
- Vanden Bossche H, Marichal P, Willemsens G, Bellens D, Gorrens J, Roels I, Coene MC, Le Jeune L, Janssen PA (1990) Saperconazole: a selective inhibitor of the cytochrome P-450-dependent ergosterol synthesis in *Candida albicans*. *Aspergillus fumigatus* and *Trichophyton mentagrophytes*. *Mycoses* 33(7–8):335–352
- Viejo-Díaz M, Andrés MT, Fierro JF (2004) Effects of human lactoferrin on the cytoplasmic membrane of *Candida albicans* cells related with its candidacidal activity. *FEMS Immunol Med Microbiol* 42(2):181–185. doi:[10.1016/j.femsim.2004.04.005](https://doi.org/10.1016/j.femsim.2004.04.005)
- Vriens K, Cammue BPA, Thevissen K (2014) Antifungal plant defensins: mechanisms of action and production. *Molecules* 19(8):12280–12303. doi:[10.3390/molecules190812280](https://doi.org/10.3390/molecules190812280)
- Wakabayashi H, Abe S, Okutomi T, Tansho S, Kawase K, Yamaguchi H (1996) Cooperative anti-*Candida* effects of lactoferrin or its peptides in combination with azole antifungal agents. *Microbiol Immunol* 40(11):821–825
- Wang Z, Wang G (2004) APD: the antimicrobial peptide database. *Nucleic Acids Res* 32(Database issue):D590–2. doi:[10.1093/nar/gkh025](https://doi.org/10.1093/nar/gkh025)
- Wang G, Li X, Wang Z (2009) APD2: the updated antimicrobial peptide database and its application in peptide design. *Nucleic Acids Res* 37(Database issue):D933–7. doi:[10.1093/nar/gkn823](https://doi.org/10.1093/nar/gkn823)
- Warnock DW (2007) Trends in the epidemiology of invasive fungal infections. *Nippon Ishinkin Gakkai Zasshi* 48(1):1–12. doi:[10.3314/jjmm.48.1](https://doi.org/10.3314/jjmm.48.1)
- Wilmes M, Cammue BPA, Sahl H, Thevissen K (2011) Antibiotic activities of host defense peptides: more to it than lipid bilayer perturbation. *Nat Prod Rep* 28(8):1350–1358. doi:[10.1039/c1np00022e](https://doi.org/10.1039/c1np00022e)
- Wimley WC (2010) Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem Biol* 5(10):905–917. doi:[10.1021/cb1001558](https://doi.org/10.1021/cb1001558)
- Woyke T, Pettit GR, Winkelmann G, Pettit RK (2001) In vitro activities and postantifungal effects of the potent dolastatin 10 derivative auristatin PHE. *Antimicrob Agents Chemother* 45(12):3580–3584. doi:[10.1128/AAC.45.12.3580-3584.2001](https://doi.org/10.1128/AAC.45.12.3580-3584.2001)
- Woyke T, Roberson RW, Pettit GR, Winkelmann G, Pettit RK (2002) Effect of Auristatin PHE on microtubule integrity and nuclear localization in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 46(12):3802–3808. doi:[10.1128/AAC.46.12.3802-3808.2002](https://doi.org/10.1128/AAC.46.12.3802-3808.2002)

- Zaoutis TE, Argon J, Chu J, Berlin JA, Walsh TJ, Feudtner C (2005) The epidemiology and attributable outcomes of Candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 41(9):1232–1239. doi:[10.1086/496922](https://doi.org/10.1086/496922)
- Zasloff M (1987) Magainins, a class of antimicrobial peptides from *Xenopus* skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc Natl Acad Sci U S A* 84(15):5449–5453
- Zhang L, Takemoto JY (1986) Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin. Interaction with the plasma membrane of wild-type and respiratory-deficient strains of *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 861(1):201–204
- Zinser E, Paltauf F, Daum G (1993) Sterol composition of yeast organelle membranes and subcellular distribution of enzymes involved in sterol metabolism. *J Bacteriol* 175(10):2853–2858

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