Intracellular Targeting Mechanisms by Antimicrobial Peptides

Cheng-Foh Le,^{a,b} Chee-Mun Fang,^a Shamala Devi Sekaran^c

School of Pharmacy, Faculty of Science, The University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia^a; School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia^b; Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia^c

ABSTRACT Antimicrobial peptides (AMPs) are expressed in various living organisms as first-line host defenses against potential harmful encounters in their surroundings. AMPs are short polycationic peptides exhibiting various antimicrobial activities. The principal antibacterial activity is attributed to the membrane-lytic mechanism which directly interferes with the integrity of the bacterial cell membrane and cell wall. In addition, a number of AMPs form a transmembrane channel in the membrane by self-aggregation or polymerization, leading to cytoplasm leakage and cell death. However, an increasing body of evidence has demonstrated that AMPs are able to exert intracellular inhibitory activities as the primary or supportive mechanisms to achieve efficient killing. In this review, we focus on the major intracellular targeting activities reported in AMPs, which include nucleic acids and protein biosynthesis and protein-folding, protease, cell division, cell wall biosynthesis, and lipopolysaccharide inhibition. These multifunctional AMPs could serve as the potential lead peptides for the future development of novel antibacterial agents with improved therapeutic profiles.

KEYWORDS antimicrobial peptides, intracellular targeting, membrane lysis, antibiotic resistance, novel antibiotics

Living in a complex environment, various life forms from single-cell to multicellular higher organisms are constantly interacting with each other. Such interactions include challenges that are harmful to the host. One of the important defensive strategies is the expression of antimicrobial peptides (AMPs). AMPs are short peptides (8 to 50 amino acids) of low molecular weight, the majority of which are cationic, contain multiple hydrophobic residues, are amphiphatic, and exhibit broad-spectrum antimicrobial activity (1–5). Different classes of AMPs have been discovered in various organisms from mammals, arachnids, amphibians, plants, fungi, and even single-cell microorganisms (6, 7). In humans, AMPs can be supplemented by immune cells (e.g., mast cells, macrophage, and neutrophils) at injury sites during the acute inflammatory response (8–10). Also, secretory AMPs are constitutively expressed and released into the oral cavity, gastrointestinal tract, and respiratory tract as part of mucosal innate immunity (11, 12). In the event of acute infection or the presence of infection stimuli, the expression of AMPs can be dramatically induced (13).

AMPs are commonly known for their non-receptor-mediated membrane-lytic bactericidal activity (14, 15). Three membrane disruption models by AMPs had been proposed (1): toroidal-pore, barrel-stave, and carpet models (Fig. 1). In the toroidal-pore model, initial binding of peptide to the membrane is followed by cascade aggregation of incoming monomer units, causing the lipid moieties of outer and inner membranes to fold inward, forming a continuous channel lined by multiple peptide units (16). The lipid head groups of membrane phospholipids are thus tightly associated with the peptides. Examples include melittin (17), magainin 2 (18), and lactacin Q (19). The

Accepted manuscript posted online 6 February 2017

Citation Le C-F, Fang C-M, Sekaran SD. 2017. Intracellular targeting mechanisms by antimicrobial peptides. *Antimicrob Agents Chemother* 61:e02340-16. <https://doi.org/10.1128/AAC.02340-16>.

Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Cheng-Foh Le, chengfoh.le@nottingham.edu.my.

FIG 1 The proposed models of membrane-lytic mechanisms by AMPs. (A) Toroidal pore model: cascade aggregation of peptide monomers in the membrane causes the lipid moieties to fold inward, forming a continuous channel. (B) Barrel-stave model: the peptide monomers are arranged parallel to the phospholipids of the membrane, forming a hydrophilic transmembrane channel. (C) Carpet model: accumulation of peptides on the surface induces local weaknesses and breaks down the bilayers into small areas lined by the peptide units, giving a carpet-like appearance.

barrel-stave model differs from the toroidal-pore model in that the peptide monomers inserted into the membrane are arranged parallel to the phospholipid molecules of the membrane. The lumen of the transmembrane channel will thus be lined by the hydrophilic side of the peptides, while the hydrophobic side will be facing outward in association with the lipid core of the bilayers (16). An example of an AMP of this type is alamethicin (20). For the carpet model, the cumulative effect of peptides interacting with the outer membrane would induce local weaknesses disintegrating the membrane structure, giving a carpet-like appearance (16). An example of a carpet model AMP is cecropin P1 (21).

In recent years, there have been increasing documentations of intracellular targeting AMPs (1, 16, 22). These AMPs are able to traverse the cell barriers to reach the target components in the cytoplasm of the cells. The uptake process of AMPs generally occurs via direct penetration or endocytosis (23). Endocytosis follows either micropinocytosis or receptor-mediated pathways (24). In micropinocytosis, dynamin facilitates the formation of AMP-carrying macropinosomes derived from the cell membrane (23). In a receptor-mediated pathway, formation of small vesicles in the cytoplasm is preceded by pit formation mediated by clathrin or caveolin membrane proteins (24, 25).

A number of AMPs are known to serve two or more inhibitory functions. A peptide that exerts a bactericidal effect via membrane disruption might in turn kill other bacteria using nonmembrane-lytic pathways, and vice versa (16). The activities of AMPs are dependent upon various factors, including temporal separation (simultaneous or sequential), concentration of AMPs, bacterial growth phase, species, and Gram stain, in a cooperative manner to ensure efficient and rapid killing (22, 26, 27). Conversely, AMPs with widely dissimilar primary sequence and solution structure may inhibit common pathways, as seen with histatin 5 and human neutrophil defensin-1 (HNP-1) (28). Such indispensable roles in the host throughout evolution clearly imply that the membrane and nonmembrane mechanisms of AMPs serve equally important functions.

Unfortunately, the general environment in the field in past decades has focused much on the discovery of natural AMPs and design of AMP analogues. Investigating the alternative mechanisms of AMPs has gained interest only in the past 10 years. With the enormous pool of AMP leads available in the literature, the previously overlooked aspects of AMPs should be discovered and rediscovered. From a technical perspective, the commonly performed methods (electron microscopic imaging, dye leakage, and gel retardation assays) do describe the killing mechanisms of AMPs but are otherwise insufficient to pinpoint the actual interacting components or binding sites. Microscopy

imaging using fluorescent probe/radiolabel localization in the treated cells, pulldown assay, proteomic array, whole-genome sequencing, and other newer platforms would be valuable to determine the interactions.

In this review, AMPs exhibiting nonmembrane-lytic antibacterial activities are discussed to provide a greater understanding of the mechanisms of actions of multifunctional AMPs as the potential next-generation antibiotics.

NUCLEIC ACID BIOSYNTHESIS AND METABOLISM INHIBITORS

Buforins are a class of DNA inhibitor first isolated from the stomach tissues of the Asian toad (*Bufo bufo gargarizans*) (29). Buforin II, a 21-amino-acid fragment peptide derived from endoproteinase Lys-C enzymatic cleavage of buforin I, was able to induce membrane permeation in *Escherichia coli* (30). The presence of a proline hinge region in buforin II is critical for its transmembrane activity (30, 31). Interestingly, membrane leakage was not observed even at a concentration 5-fold above the MIC (31, 32). Results from a gel-retardation experiment showed that buforin II exhibited high nucleic acid binding affinity. This strongly implies that buforin II inhibits cellular processes by interrupting DNA and RNA metabolisms (32). Later, it was determined that the parent peptide buforin I possessed high sequence homology (94.49%) to the N-terminal fragment of the DNA-binding protein histone H2A, leading to speculation that AMPs with structures resembling histone H2A (e.g., parasin I and hipposin, derived from skin mucus of catfish [33]) would exert antimicrobial activity in a fashion similar to that of buforin II (30, 32). Two designed analogues of buforin II were shown to inhibit nucleic acid metabolisms with 4-fold greater DNA-binding affinity in BF2-C than BF2-A (34). In contrast, BF2-A bound more tightly to the single-strand RNA. Based on sequence analysis, it appeared that the C-terminal regular α -helical motif RLLR contributed toward the stronger double-helix DNA-binding affinity in BF2-C. We have summarized a list of AMPs with reported intracellular targeting activities discussed in this review in Table 1. An illustration indicating the intracellular targets sites of AMPs is shown in Fig. 2.

The 13-amino-acid indolicidin is a C-terminally amidated polycationic tryptophan-rich AMP derived from the cytoplasmic granules of bovine neutrophils. Indolicidin belongs to the cathelicidin family of AMPs (35). Membrane permeation induced by indolicidin at high concentration does not lead to cell lysis (36–39); rather, the increased permeabilization facilitates continuous peptide entry into the cytosol to inhibit DNA biosynthesis exclusively, but not RNA (40). The peptide specifically targets the abasic site of DNA to cross-link with single- or double-stranded DNA (41). A detailed study by Hsu et al. showed that indolicidin possessed high binding affinity toward double-stranded (ds) [AT], ds[CG], and ds[AG] but lower affinity toward ds[GT] (42). Furthermore, binding affinity was greater in complex with duplex DNA than single-stranded DNA, except in the [GT] sequence. Indolicidin binding to duplex DNA is stabilized by the central PWWP motif, thus inhibiting DNA replication and transcription (43).

Besides direct DNA binding, indolicidin prevents DNA relaxation by inactivation of DNA topoisomerase I (41). Interestingly, this step occurs without unwinding of the DNA helix, further suggesting the multiple inhibitory roles of indolicidin against various DNA-associated enzymes. The presence of homologous sequence in other DNA-binding proteins (e.g., DNA methyltransferase) indicates that the N-terminal fragment of indolicidin, rather than the PWWP motif, is responsible for its DNA-binding activity (41). Indolicidin has also been demonstrated to bind calmodulin at high affinity in a Ca^{2+} -dependent fashion (44).

The ostrich-derived β -defensins ostricacin-1 (Osp-1) and ostricacin-2 (Osp-2) exhibited antibacterial activity against Gram-negative bacteria by binding to and inhibiting DNA (45). These AMPs have been shown to interfere with protein biosynthesis and enzymatic activities in the target bacterial cells. On the other hand, Oabac5mini is an AMP isolated from ovine neutrophils displaying strong DNA-binding affinity in bacterial cells (45, 46). Tachyplesin, isolated from hemocytes of horseshoe crab (*Tachyplesus*

TABLE 1 Peptide sequence information and mode of action of intracellular targeting AMPs

AMP ^a	Residues	Sequence	Source	Mode(s) of actions
Buforin II	21	TRSSRAGLQFPVGRVHLLRK	Enzymatic cleavage of buforin I (from Asian toad <i>Bufo bufo gargarizans</i>)	Inhibits DNA, inhibits RNA
BF2-A	17	RAGLQFPVGRVHLLRK	Buforin II analogues	Inhibits DNA
BF2-C	21	RAGLQPLGLRLLRLLRLLR		Inhibits RNA
Indolicidin	13	ILPWKWPWWPWRR	Bovine neutrophil cytoplasmic granules	Direct DNA binding, inhibits DNA topoisomerase I, inhibits septation, binds calmodulin, inhibits HIV-1 integrase
Ostricacin-1	36	LFCKGTCHFGCPAHLVKVGSFCFRACKWPWDV	Ostrich β -defensins	Inhibits DNA
Ostricacin-2	41	APGNKAECEREKGYCGFLKCSFPVVGKCSRRFFCCKNW	Ovine neutrophils (truncated version of Oabac5)	Inhibits DNA
Oabac5mini	17	RFRRPPIRRPPRPFRPPFRPPVR	Horseshoe crab hemocytes	Inhibits DNA
Tachyplesin	43	KWCFRVCYRGICYRRCR	Produced by <i>E. coli</i> harboring the plasmid-borne MccB17 operon	Binds DNA minor groove
Microcin B17	43	VIGGGGGGGGGGGGGGGGGGGGSGSHI		Inhibits DNA topoisomerase II gyrase
Microcin J25	21	VGIGTPIFSYGGGAGHVEYF	<i>E. coli</i> AY25	Inhibits RNA polymerase, inhibits cytochrome c reductase, inhibits septation
Bac7 1–35	35	RRIRPPRLPRPPRP LPFPPGPRPRLPFP	35-residue N-terminal fragment of Bac7	Inhibits protein biosynthesis by targeting ribosomes, inhibits DnaK
Plaucidicin	25	GWGSFFKAAHVKHVGKAAALTHYL	Winter flounder skin secretions	Inhibits protein biosynthesis
CPT0A	13	ILAWKWAWWAWRR	Synthetic variant of indolicidin	Inhibits DNA biosynthesis, inhibits RNA biosynthesis, inhibits protein biosynthesis
DM3	13	GLFDIWKWWRWR	Hybrid AMP of CP10A and the bacterial membrane anchor analog aurein 1	Altered expression of genes associated with DNA replication and transcription, affects ribosomal rRNA subunits, affects RpoD and RNA polymerase sigma factor, affects amino acid biosynthesis pathway, downregulates expression of capsular polysaccharide subunits, downregulates cationic AMP resistance pathway
PR-39	39	RRRRPPYLP RRRPPPPFPPLPRIPRPPGPPRRPP	Porcine small intestine; porcine and human neutrophil; wound fluids	Inhibits DNA biosynthesis, affects nucleotide transport and metabolism, inhibits protein biosynthesis, inhibits translation, inhibits septation, affects coenzyme transport and metabolism
Apidaecin Hb1a	18	GNRPVYPPRPHPRL	Honeybee	Inhibits protein biosynthesis by targeting ribosomes, inhibits DnaK and GroEL, inhibits ABC transport system, binds LPS
Lactoferricin B	25	FKRRWQWRMKLGLAPSITCVRRAF	Cleaved from gastric pepsin digestion of bovine lactoferrin; human gastrointestinal tract	Inhibits phosphorylation of two-component system
HNP-1	30	ACYCRIPACIAGERYGTICYGRLWAFCC	Human neutrophil azurophilic granules	Inhibits DNA biosynthesis, inhibits RNA biosynthesis, inhibits protein biosynthesis, inhibits lipid II in peptidoglycan biosynthesis, activation of autolytic enzyme
Pyrrhocoricin	20	VDKGSYLPRPTPRPIYNRN	Fire bug <i>Pyrrhocoris apterus</i>	Inhibits DnaK and GroEL, binds LPS
Drosocin	19	GKPRPYSPRPTSHPRIRV	<i>Drosophila</i>	Inhibits DnaK and GroEL, binds LPS
Abacacin	39	FVPYNRPYQSKPFPSPGHGPNKQWPYLPNPGH	Bumblebee	Inhibits DnaK
Oncocin	19	VDKPYLP RPPRPPIYNR	Oncopeltus antibacterial peptide 4	Inhibits DnaK
Histatin-5	24	DSHAKRHGGYKRFHEKHSHRGY	Human saliva	Inhibits proteases, inhibits MMP-2 and MMP-9, inhibits generation of ROS ^c
eNAP-2	46	EVERKHPILGSGSRGRCPTVPPTGTGHACALCTGDASEPKQKCCSN	Equine leukocytes	Inhibits microbial serine proteases subtilisin A and proteinase K
Ixodidin	65	ERGSRGQRCPGGEVFNQCGSACPRVCGRPPAQAACTLQCVCSCFCR RGYIRTQGGCIPEROCHOR	Hemocytes of cattle tick <i>Boophilus microplus</i>	Inhibits DnaK, inhibits elastase and chymotrypsin
Diptericin	83	DDMTMKPTPPQYPLNLGGGGGGGDDGFGFAVGHQKQWTS DNGRRHEIGLNGYGOHLGPGYGNSEPSWKVGSTYTRFPNF	Hemolymph of injured <i>Sarcophaga peregrina</i> larvae	Inhibits septation
HD5 (oxidized)	32	ATCYCRIGCATRESLSGVCEISGRLYRLCCR	Human small intestine	Inhibits cell division
PR-26	26	RRRRPPYLP RPPPPFPFPPLPPI	Truncated fragment of PR-39	Inhibits septation
Mersacidin	20	CTFLPGGGGVCTLTSEIC	<i>Bacillus</i> sp. strain HIL-Y85/54728	Inhibits lipid II in peptidoglycan biosynthesis
HB03	45	GIINTLQKYCRVRGRCVLSCLPKKEEQIGKCTSRGKCCRKK	Human (various sites)	Inhibits lipid II in peptidoglycan biosynthesis
Nisin ^b	34	IOAUIAAPAGAKAGLMANWKAANASIHVUK	<i>Lactococcus lactis</i>	Inhibits lipid II in peptidoglycan biosynthesis
Copsin	57	QNCPTRLGVLTSGLTACRNHCRSHRGDVGVCVRCNAOCTG FLGTTCTCINPCRC	Basidiomycete <i>Coprinopsis cinerea</i>	Inhibits lipid II in peptidoglycan biosynthesis
Plectasin	40	GFGCGPWDEDDMOCHNHCHSKIGYKGYCAKGFGYCKY	Ascomycete <i>Pseudopeziza nigrella</i>	Inhibits lipid II in peptidoglycan biosynthesis
Pep5	34	TAGPAIRASVKQCKTLKATLRFVTSCKGNCK	<i>Staphylococcus epidermidis</i>	Activation of cell wall-associated autolytic enzyme amidases
tPMP-1	72	DDPKESGDLHCVCVKTSLVRPHRHTNLLEIKAGGHCPTAQLIA TLKNRKLCLDPQAAALYKVKVLLKE	Platelet granules	Activation of autolytic enzyme
MBI-27	26	KWKLKKGIGAVLKLTLTGLPALIS	Derived from part of silk moth cecropin and bee melittin peptides	Inhibits LPS
MBI-28	28	KWKLKKGIGAVLKLTLTGLPALIKLTK	Derived from part of silk moth cecropin and bee melittin peptides	Inhibits LPS
Magainin 1	23	GIGKFLHSAGKFGKAFVGEIMKS	African clawed frog <i>Xenopus laevis</i>	Inhibits energy metabolism proteins, inhibits amino acid metabolism

^aHNP-1, human neutrophil defensin 1; eNAP-2, equine neutrophil antimicrobial peptide 2; HB03, human β -defensin 3; tPMP-1, thrombin-activated platelet microbicidal protein 1.
^bO₂, dehydrobutyrene (Dhb); U₂, dehydroalanine (Dha); Δ , α -aminobutyric acid (Abu).
^cROS, reactive oxygen species.

FIG 2 Illustration showing the major pathways targeted by AMPs in bacterial cells. A number of AMPs, such as indolicidin, boforin II, DM3, and microcin J25, are able to inhibit multiple intracellular targets to achieve high antibacterial efficiency.

tridentatus), is also a DNA-binding peptide (47). It contains a unique antiparallel beta-sheet secondary structure to bind to the minor groove of DNA duplex (48).

Microcins are a group of enterobacterial AMPs classified into one of seven major groups (A, B, C, D, E, H, and J) based on cross-immunity criteria (49–51). The 43-residue microcin B17 is the first AMP found to inhibit type II DNA topoisomerase (gyrase), reminiscent of the conventional quinolone antibiotics (52–54). Microcin B17 is produced by *E. coli* harboring the plasmid-borne MccB17 operon (55, 56). It blocks DNA replication by trapping the gyrase-DNA complex, halting subsequent processes (52, 57). B17 specifically targets the C-terminal domain of *gyrB* of the gyrase tetramer, which consists of *gyrA* and *gyrB* subunits. This inhibits the ATP hydrolysis activity responsible for the proper negative supercoiling of DNA. *OmpF* is important for the translocation of microcin B17 across the outer membrane and *SbmA* across the inner membrane into the bacterial cells (58).

Another type of microcin 25 (mcc25 or J25) isolated from the *E. coli* fecal strain AY25 is a potent antibacterial peptide, particularly against Gram-negative bacteria, including the closely related *Shigella* and *Salmonella* genera (59, 60). Entry of J25 into the cell is facilitated by the outer membrane receptor FhuA (61). After passing the first barrier, ExbD, ExbB, and *SbmA* are responsible for peptide transport across the inner membrane into the cytoplasm (62). Microcin J25 disrupts transcription by binding to beta', which represents the largest bacterial RNA polymerase subunit encoded by *rpoC* (63). Binding of J25 blocks substrate access to the catalytic center of RNA polymerase (64). This stops transcript elongation temporarily but does not alter the elongation velocity between pauses (65). Adelman and colleagues showed that J25 can be cross-linked to

the 3' end of the nascent RNA of the elongation complexes to inhibit the GreA/GreB-dependent transcript cleavage pathway and cease backtracked complex formations (65). It was found that in J25-resistant *E. coli* strains, a mutation in *rpoC* leads to the alteration of amino acids in the conserved G, G', and F segments within the secondary channel (63).

PROTEIN BIOSYNTHESIS AND METABOLISM INHIBITORS

The concerted events of protein synthesis begin from DNA transcription to mRNA, followed by translation of mRNA via the 70S bacterial ribosomal machinery to polypeptides before undergoing chaperone-assisted folding and assembly into functional proteins. Interference with any of the associated enzymes or effector molecules could result in protein biosynthesis arrest. There are AMPs shown to inhibit these targets to achieve bacterial cell killing.

Bac7 is a considerably large 60-residue AMP isolated from the large granules of bovine neutrophils. The N-terminal 35-residue fragment (Bac7 1–35) appears to be responsible for biological activity of the intact Bac7 (66). Interestingly, Bac7 1–35 exerts intracellular activity when present at near-MIC level, following stereospecific-dependent uptake that would become membranolytic at a concentration 64-fold greater than the MIC, independent of stereospecificity (67). Internalization of Bac7 1–35 is mediated by the inner membrane protein SbmA. *E. coli* mutants with an *sbmA*-null or *sbmA* point mutation have been shown to develop resistance against Bac7 1–35 (68). Bac7 1–35 inhibits protein translation by targeting the ribosomes (69). Proteome analysis found that Bac7-treated *E. coli* bacteria showed functional enrichment in a number of pathways involving nucleic acid metabolism, including nucleotide transport and metabolism, nucleobase, nucleoside, and nucleotide interconversion (70). A previous study reported that Bac7 1–35 conferred protection against endotoxin-induced septic shock in an experimental rat model (71).

Protein synthesis arrest was also observed in *E. coli* treated with sublethal dose of pleurocidin (72). When given at the lethal dose, pleurocidin became both bactericidal and inhibited protein, DNA, and RNA synthesis (73). This was evident through observations of a reduction in the incorporation of histidine, uridine, and thymidine in pleurocidin-treated cells (73).

The 13-residue CP10A is a synthetic variant of indolicidin (74) belonging to the cathelicidin family of polypeptides (75). Previous studies documented that indolicidin and its synthetic analogues exhibited strong inhibitory effects against bacteria, fungi, and viruses (35, 76–78). In addition to membrane lysis, CP10A exerted diverse intracellular inhibitory activities against DNA, RNA, and protein synthesis (74). By using *Staphylococcus aureus* as the model organism, cells treated with CP10A had reduced incorporation of histidine, uridine, and thymidine (74).

In our previous study, the hybrid peptide DM3 exhibited broad-spectrum antibacterial activity with rapid bactericidal potency (79). In particular, standalone DM3 treatment significantly enhanced the survival rate of mice lethally infected with pneumococcal systemic infection compared to the untreated control group (80). Whole-genome transcriptomic analysis found that a number of important intracellular pathways were significantly affected. These include the expression of genes associated with DNA replication and transcription, ribosomal rRNA subunits, amino acid biosynthesis pathway, and downregulation of RpoD and RNA polymerase sigma factor (81). Downregulation of the cationic AMP resistance pathway indicated that DM3 treatment could autoenhance pneumococcal susceptibility to DM3 and other AMPs, thus reducing the chance of AMP resistance developing in the treated cells.

PR-39 is a 39-residue proline-rich (49%) and arginine-rich (24%) AMP first isolated from porcine small intestine (82) and subsequently from porcine and human neutrophils (83). Internalization of PR-39 is also mediated by the membrane receptor SbmA (68, 84). The primary mechanism of action of PR-39 is attributable to its rapid proteolytic activity causing the degradation of some DNA replication-associated proteins, leading to secondary inhibition of DNA synthesis events (85). Recent data obtained using a

proteome microarray have confirmed the inhibitory effects of PR-39 in a number of metabolic pathways, including those for translation, nucleotide transport and metabolism, coenzyme transport and metabolism, and others (70).

Apidaecin-type peptides are a group of 18- to 20-amino-acid proline- and arginine-rich AMPs isolated from honeybees, hornets, and wasps (86). Apidaecin Hb1a displays selective antibacterial activity, mainly against Gram-negative bacteria (87–89). Significant loss of antimicrobial activity with the all-*D*-enantiomer analogues showed that the biological functionality of apidaecin required high stereospecificity (90). Even though most AMPs do exhibit certain levels of membrane-lytic activity, apidaecin Hb1a is a rare exception, possessing no pore-forming or membrane disruption activity (90). Entry of apidaecin Hb1a into *E. coli* begins with the initial nonspecific contact with the outer membrane component to enter the periplasmic space, followed by irreversible binding of peptide to the inner membrane or otherwise-bound receptors. The ABC transporter system was suggested to be the potential candidate responsible for apidaecin Hb1a uptake into the cells (88). Subsequently, the peptide will be translocated intracellularly to inhibit the protein synthesis pathway by targeting the ribosomes (88).

Lactoferricin B was the first AMP shown to interfere with phosphorylation of the two-component system to suppress bacterial growth (91). It inhibits phosphorylation of the two response regulators by their cognate sensor kinases BasS and CreC. Pyruvate accumulation has also been reported in bacterial cells treated with lactoferricin B (92).

HNP-1 belongs to one of four α -defensin variants (HNP-1 to HNP-4) in human neutrophil azurophilic granules (93). Interestingly, HNP-1 induced sequential permeabilization of both the outer and inner membranes of *E. coli*, accompanied by DNA, RNA, and protein synthesis failures (94). For HNP-1, loss of inner membrane integrity appeared to be the key event associated with lethality. Besides that, lactoferricin B, PR-39, P-Der, and Bac7 were found to inhibit the arginine decarboxylase pathway and 30 other common proteins in *E. coli*-treated cells (70). Interference with arginine decarboxylase activity could reduce survival fitness of the host cells, especially under highly acidic conditions, thus reducing the pathogenicity of the bacteria. Other AMPs, such as defensins and dermaseptin, also possessed protein synthesis inhibitory activity (95).

PROTEIN-FOLDING INHIBITORS

Chaperones are proteins crucial for the proper folding and assembly of newly synthesized proteins (96). DnaK is the major bacterial heat shock protein 70 (Hsp70) and represents an important component in *E. coli* chaperone pathways (97). A group of short (18- to 20-amino acid) proline-rich insect-derived AMPs are able to inhibit bacterial growth by interrupting the protein-folding pathway. This includes pyrrhocoricin, isolated from the hemipteran insect fire bug *Pyrrhocoris apterus* (98), apidaecin, and *Drosophila* drosocin, originally discovered in the bean bug (*Riptortus clavatus*) (99). In its lipoform form, pyrrhocoricin, apidaecin, and drosocin interact strongly with bacterial lipopolysaccharides (LPS), suggesting that the multiple inhibitory events essentially begin with the initial contact against a bacterial cell wall component (100). Both pyrrhocoricin and apidaecin inhibit DnaK and GroEL as well as disrupt the ATPase activity of DnaK (101, 102). In contrast, drosocin inactivates DnaK and GroEF (100). Both pyrrhocoricin and drosocin prevent DnaK from refolding misfolded proteins by inducing permanent closure of the DnaK peptide-binding cavity (102). Binding of these three AMPs to DnaK is essentially stereospecific, which is advantageous in term of cell selectivity to prevent toxicity, as they do not target the human equivalent chaperone Hsp70 (100).

DnaK-binding activity has similarly been described for abaecin, an AMP isolated from the bumblebee *Bombus pascuorum*. Experimental results recorded a 74% quenching effect by abaecin, with a disassociation efficiency comparable to that of other proline-rich DnaK-targeting peptides, such as oncocin and pyrrhocoricin (103). Unlike the proline-rich AMPs (onc72, api88, drosocin, and pyrrhocoricin), which possess the DnaK-binding YL/IPRP motif, the WPYPLPN fragment was predicted to be the primary binding region in abaecin. Oncocin, a 19-amino-acid proline-rich AMP derived from

Oncopeltus antibacterial peptide 4, exhibits potent antibacterial activity against Gram-negative bacteria (104). The peptide penetrates the bacterial membrane freely, without causing lytic effect to the cells (104). Following that, it was demonstrated that oncocin formed a complex with DnaK, indicating the protein-folding inhibitory activity of this peptide (105). Apart from targeting the protein biosynthesis mechanisms, Bac7 1–35 also displayed high binding affinity to DnaK and inhibited the protein-refolding function of the DnaK-DnaJ-GrpE-ATP molecular chaperone system in a concentration-dependent manner (106).

PROTEASE INHIBITORS

Host matrix metalloproteases (MMPs) serve to function as the principal enzymes in the normal turnover of extracellular matrix. Dysregulation of MMPs and bacterial proteases are associated with oral diseases, arthritis, periodontitis, and tumor metastasis (107).

Histatin 5 is a member of the histatins, a group of histidine-rich cationic peptides present in the salivary secretions of human submandibular and parotid glands (108, 109). Histatin 5 is a potent antifungal peptide acting upon multiple intracellular components to induce oxidative stress and mitochondrial malfunction in *Candida albicans* (110). However, its direct antibacterial activity has only been described for *Streptococcus mutans* (111), one of the major oral bacteria implicated in dental caries (112). What is interesting about histatin 5 is its strong inhibitory activity against the host- and bacterium-secreted proteases. In a study by Gusman et al., the 50% inhibitory concentration (IC₅₀) levels of histatin 5 against human MMP-2 and MMP-9 were recorded at 0.57 and 0.25 μ M, respectively (113). In a separate study, they observed higher binding affinity of histatin 5 than for leupeptin (114), a competitive inhibitor of the cysteine proteinase clostripain produced by the anaerobic bacterium *Clostridium histolyticum* that causes gas gangrene (115). Overproduction of cysteine proteinases and collagenases could lead to extensive necrotizing muscle tissue breakdown. Besides, histatin 5 specifically inhibits a trypsin-like protease but not other proteases produced by the oral bacterial pathogen *Porphyromonas gingivalis* (116). The peptide also displays competitive inhibition against Arg-gingipain, while both competitive and non-competitive mechanisms are involved upon complex formation with Lys-gingipain (113). Based on the findings, histatin 5 could serve as a potential therapeutic agent to reduce extracellular matrix degradation caused by host- and bacterium-secreted proteases in periodontal diseases.

Equine leukocytes contain eNAP-2, an AMP exhibiting antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus zooepidemicus* (117). This peptide selectively forms a noncovalent complex with the microbial serine proteases subtilisin A and proteinase K but not the mammalian serine proteases (118). Selective inactivation of microbial serine proteases by eNAP-2 represents an important host defense mechanism against microbial infection in horses. Ixodidin is a considerably large 65-residue cysteine-rich AMP isolated from tick (*Boophilus microplus*) hemocytes. This AMP inhibits proteases, such as elastase and chymotrypsin, to induce cellular metabolism cessation (119).

CELL DIVISION INHIBITORS

There are AMPs demonstrated to have significant inhibitory activity on bacterial cell processes. Cell filamentation might occur as a result of inhibition of DNA replication, SOS induction, chromosome segregation, or failure of septation process (120). As shown by Salomón and Fariás, *E. coli* treated with microcin J25 became significantly elongated, resulting in a filamentous morphology, but septation was otherwise not initiated, thus prohibiting the cell division processes (59). It was found that microcin J25 acted on a non-SOS-dependent mechanism, and this confirmed its bacteriostatic mode of action (59). Similar to microcin J25, *E. coli* treated with dipterocin had a significantly elongated morphology, suggesting that dipterocin may act upon cellular targets in-

volved in cell division to induce cell death (121). Notably, dipteridin was effective against actively growing *E. coli* but not cells at its resting state (121).

The human α -defensin 5 (HD5) is able to traverse the outer membrane and impairs the inner membrane of *E. coli* (122, 123). Peptide entering the cytosol will then induce multiple cellular damages, as observed with blebbing and clumping of cells. Extensive elongation of cells was also noted, indicating disruption to cell division events (124). Such treatment outcomes were observed in *P. aeruginosa*, *K. pneumoniae*, and *Acinetobacter baumannii*, suggesting that HD5 produced a common inhibitory activity against Gram-negative bacteria. AMP-induced filamentation had also been described with indolicidin (40), PR39, and the truncated variant PR26 (83).

CELL WALL BIOSYNTHESIS INHIBITORS

Cell wall compositional differences and the presence of capsular polysaccharide in certain bacteria (e.g., *Streptococcus pneumoniae*) are important factors influencing the potency and antibacterial selectivity of AMPs. The bacterial cell wall consists of alternating amino sugars in linear form that cross-link via peptide bridges to form the peptidoglycan layer (125). Lipid II is an important component in peptidoglycan synthesis. It resides at the cytoplasmic face of the cell membrane to transport cell wall subunits across the membrane to be polymerized and inserted into the existing cell wall (126). The bacterial peptidoglycan layer is crucial for the integrity and survival of bacteria and represents the prominent target for a number of antibiotics (127).

Mersacidin is a globular lantibiotic formed by the presence of four intramolecular thioether bridges (128). The peptide targets the peptidoglycan precursor lipid II to form a complex with the sugar phosphate head group (129). Binding of mersacidin to lipid II blocks the transglycosylation step in cell wall biosynthesis (130). Accumulation of the peptidoglycan precursor complex UDP-MurNAc-pentapeptide has been observed in the cytoplasm of mersacidin-treated bacterial cells (130). Since mersacidin targets the peptidoglycan biosynthesis pathway, Gram-positive bacteria, such as methicillin-resistant *S. aureus* (MRSA), appear to be more susceptible to the peptide than Gram-negative bacteria and fungi (131, 132). An *in vivo* experiment using a murine rhinitis model reported that mersacidin was able to eradicate MRSA colonization without stimulating inflammatory responses (133). Moreover, newer evidence showed that human β -defensin 3 (HBD3) and HNP-1 were able to bind to lipid II to inhibit cell wall biosynthesis (134, 135).

Nisin represents the most characterized lantibiotic that acts by membrane pore formation to induce cell lysis (136). The pore complex is formed between the lanthionine ring A and B complex of nisin with the pyrophosphate group of lipid II (137). Hasper et al. showed that lantibiotics with an N-terminal pyrophosphate group cage displayed an alternative bactericidal mechanism which was lipid II dependent by sequestering lipid II from its intracellular functional site to inhibit cell wall biosynthesis (138). The finding supported the hypothesis that other lantibiotics possessing similar lanthionine A/B ring structures may exhibit similar mechanisms of action (139–141). This might help explain the potent non-pore-forming antibacterial activity of staphylococcin T, gallidermin, and epidermin (142, 143).

Copsin is extremely resistant to proteolysis and wide range of pH and temperature due to the presence of the unusually high number of (six) disulfide bonds, compared to only three or four in other cysteine-stabilized α/β defensins (144). It is able to bind to the precursor lipid II, particularly at the third position of the pentapeptide, leading to inhibition of cell wall biosynthesis (144). In addition, the fungal AMP plectasin produced by the saprophytic ascomycete *Pseudoplectania nigrella* effectively inhibits lipid II for its intracellular activity (145). Plectasin also displayed high therapeutic efficacy in mouse pneumococcal peritonitis and pneumonia models, with minimal toxicity (146).

Staphylococcus epidermitis produces the lantibiotic Pep5, which is characterized by the presence of three intramolecular rings (147). The peptide is involved indirectly in cell wall lysis through the activation of cell wall-associated lytic enzymes (148). Pep5

exhibits high binding affinity to teichoic and teichuronic acids of the Gram-positive bacterial cell wall, which competitively displaces the cell wall-associated amidases (148). This leads to premature release of the autolytic enzymes, resulting in cell lysis. Apart from that, transcriptomic analysis revealed that our designed peptide, DM3, downregulated the capsular polysaccharide subunits of penicillin-resistant pneumococci (81). This implies that DM3 could compromise cell wall biosynthesis activity to weaken and exert bactericidal activity.

In mammalian blood cell granules, defensins expressed in the neutrophil azurophilic granules and the small endogenous cationic peptide platelet microbicidal proteins (PMPs) present within the platelet α -granules are two well-characterized AMPs exhibiting very similar antimicrobial activities against *C. albicans* and *S. aureus*, *E. coli*, and other bacteria (94, 149–152). Their potent membrane-lytic activities, particularly against *S. aureus*, have been described in the literature (153–155). Previous studies reported that pretreatment of bacteria with the cell wall-targeting antibiotics penicillin and vancomycin would facilitate uptake of the thrombin-activated PMP-1 (tPMP-1) and HNP-1 into the cytoplasm (156). The observed microbicidal activity by tPMP-1 and HNP-1 might be assisted by the direct activation of autolytic enzyme in the bacterial cells or indirectly via penicillin- and vancomycin-enhanced cell killing. However, these mechanisms are likely the supportive but not primary antibacterial mode of action (156). Of note, HNPs have also been shown to enhance the susceptibility of bacteria against rifampin, and this indicates that AMPs causing outer membrane permeabilization might act synergistically with conventional antibiotics to improve therapeutic outcome (157).

LIPOPOLYSACCHARIDE-BINDING PEPTIDES

The LPS layer constitutes the outer leaflet component of the outer membrane of Gram-negative bacteria. The hydrophobic anchor lipid A (LA) molecule (commonly referred to as LPS) of LPS monomer is generally known as the bacterial endotoxin largely responsible for the toxicity and clinical manifestations associated with Gram-negative bacterial infections (158, 159). LPS can be shed from the cell during the process of infection or the course of antimicrobial chemotherapy treatment (160). However, in the case of endotoxemia, where endotoxin is being released into the blood, overproduction of inflammatory stimulatory molecules by the immune cells could lead to septic shock (161–163). Endotoxin-targeting AMPs have great potential to be developed as antiendotoxin or endotoxin-neutralizing agents in patients with endotoxic shock during or after treatment.

Both MBI-27 (26 amino acids) and MBI-28 (28 amino acids) exhibit antibacterial activities against Gram-negative bacteria (164, 165). Strikingly, these two peptides were found to bind the purified whole-cell LPS prepared from *P. aeruginosa* with a binding affinity comparable to that of polymyxin B (164–166). MBI-28 was more potent than MBI-27, due to the presence of two additional lysine residues at its C-terminal (164). AMP binding and neutralization of endotoxin would prevent the uncontrollable activation of inflammatory responses as a result of imbalanced cytokine (e.g., interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF- α]) production, mainly by the LPS-stimulated mononuclear and macrophage cells (167–169). Amphipathicity and a high proportion of cationic residues in the AMP sequence have been suggested to be the principal binding motifs against the oppositely charged LA (170). Even for the relatively short β -sheet AMPs, a complex can still be formed between the β -sheet cationic side chains and the anionic phosphates of LA (171). Based on the structural characteristics of the LPS-binding protein family, Muhle and Tam generated two β -stranded cyclic AMPs with >200-fold greater selectivity against the Gram-negative *E. coli* than the Gram-positive *S. aureus* (172).

Magainins represent one of the most studied families of AMPs, well known for their potent membrane-lytic activity. However, the intracellular targeting effects of magainins have not received considerable attention until recently. Comparative proteomic analysis between magainin 1-resistant and -susceptible strains revealed that many proteins associated with energy metabolism (49%) were extensively altered, followed

by nitrogen metabolism (12%), amino acid metabolism (7%), stress response (7%), and others (25%) (173). This study provides strong evidence of the intracellular activities of magainin 1. Microorganisms are able to develop resistance by altering the important cellular components in response to antimicrobial selective pressure to ensure survival.

IMPORTANT REMARKS

AMPs are generally perceived as membrane-active molecules act by perturbation of the cell wall/membrane or via formation of transmembrane pores leading to cell death. Based on the findings collected in this paper and elsewhere, we should now understand that a substantial number of AMPs possess multifunctional activities at membranous and/or intracellular sites to achieve efficient killing. Furthermore, the intracellular inhibitory mechanisms of AMPs serve equally important roles, contributing to the overall antibacterial efficiency. Unfortunately, these aspects are commonly overlooked or unreported.

The factors that determine the modes of action of AMPs are the long-sought answers for researchers in the field and are still largely unclear. As reviewed in this paper, a single AMP could target multiple sites of the bacterial cells, and vice versa. These AMPs varied largely in their sequence identities, and secondary structure alone is not sufficient to explain the findings. Although higher net charge, hydrophobicity, helical propensity, and presence of a conserved sequence are able to enhance the antimicrobial activity of AMPs in general, however, these sets of rules do not apply equally to all classes of AMPs. For example, our previous studies showed that the antibacterial activity of five designed AMPs (DM1 to DM5) was correlated to net charge within specific window values (79, 174, 175). Anionic AMPs have also been documented in the literature (176, 177). Other factors, including concentration of peptide, target species, and growth phase of bacteria, have significant influences on the mode of actions of AMPs. For most AMPs, there are conserved fragments which are responsible for biological activity of the peptides. However, designing peptide analogues with improved activity based on these conserved fragments requires more in-depth considerations than cut-and-join strategies. Most importantly, determinants contributing to the antimicrobial activity of a particular class of AMPs are unlikely to be applicable to another unrelated class of AMPs. The determinants of AMPs' mechanisms of actions should involve the complex interplay between the sequence and physicochemical factors and not be treated as discrete dependents.

Given the cascade of events and time required to produce eventual metabolic inhibition in the bacterial cells in contrast to the nonspecific and rapid membrane-lytic activity, an important question may arise whether the bacteria would have better chance of developing resistance against the intracellular acting AMPs than the membrane-active AMPs? This is true, but only partially true. In fact, AMPs are unique in that these molecules exhibit multiple inhibitory actions which could act both nonselectively on cell barriers (cell wall and cell membrane) and selectively against one or more intracellular targets. Interestingly, these mechanisms do not occur independently. The same AMP could simultaneously lyse the membrane and act on intracellular targets of the same cell to exert a killing effect. Under such selective pressures, the likelihood of developing multiple resistances via simultaneous genetic mutations and altered cellular processes would be very low, as such drastic alterations would be too metabolically costly to the survival and pathogenicity of bacteria. Moreover, we postulate that there are AMPs that act via an indirect mechanism by extensive activation of resistance mechanisms, which in turn severely disrupt the normal cellular metabolisms of bacterial cells, leading to a detrimental outcome. The combination of multiple targeting and rapid bactericidal activities would essentially minimize the chance of AMP resistance development in the bacteria.

There has been increasing interest in developing AMPs as cell-penetrating peptides (CPPs) against intracellular infections. For example, LL-37 exhibited extracellular and intracellular bactericidal activities against *S. aureus* (178). Although it is still unclear if there is a correlation between antimicrobial activity and cell-penetrating capacity, we

consider AMPs with strong antimicrobial potential to be the prerequisite to act as an effective CPP, as it is meaningless to have AMP candidates penetrating the host cell at high efficiency but that are otherwise unable to kill the bacteria residing in the host cell. This is an emerging area in AMP research, and many unknowns are yet to be explored. The AMP uptake mechanisms and compositions of bacterial cell wall and cell membrane would be largely different from those in the eukaryotic cell. Additionally, the AMPs must not be cytotoxic to the host cell to be developed as antimicrobial agents.

CONCLUSION

In the face of increasing reports of multidrug-resistant and panresistant bacteria, a novel class of antimicrobials is in extreme demand. Recently, a female patient in her 70s from Nevada, USA, diagnosed with systemic inflammatory response syndrome, later developed septic shock and died in September 2016 (179). The causative pathogen, carbapenem-resistant *K. pneumoniae*, was resistant to all 26 antibiotics approved for her treatment in the United States. Antibiotic resistance is expanding and spreading worldwide at a far higher rate than that of the discovery of and eventual approval of new antimicrobials for clinical use. Ineffective and limited choices for empirical treatment would increase the rate of morbidity and mortality in patients, because simple bacterial infections are allowed more time to establish secondary invasion, which could be highly lethal. AMP-based antibiotics represent the most promising candidates to be developed as new-generation antimicrobial agents. The continuous discovery of natural AMPs from various sources will further expand the AMP database currently known to humans. These natural AMPs are valuable leads in the design of AMP variants with improved therapeutic profiles as the new-generation antibiotics.

ACKNOWLEDGMENTS

We declare no conflicts of interest.

This work was supported by the University of Nottingham Malaysia Campus Faculty of Science Pump Priming Research Grant (P044/14) and Small Grant Project (UNHP0005).

REFERENCES

1. Brogden KA. 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3:238–250. <https://doi.org/10.1038/nrmicro1098>.
2. Hancock REW, Chapple DS. 1999. Peptide antibiotics. *Antimicrob Agents Chemother* 43:1317–1323.
3. Nicolas P, Mor A. 1995. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. *Annu Rev Microbiol* 49:277–304. <https://doi.org/10.1146/annurev.mi.49.100195.001425>.
4. Oren Z, Shai Y. 1998. Mode of action of linear amphipathic alpha-helical antimicrobial peptides. *Biopolymers* 47:451–463.
5. Hancock RE. 1997. Peptide antibiotics. *Lancet* 349:418–422. [https://doi.org/10.1016/S0140-6736\(97\)80051-7](https://doi.org/10.1016/S0140-6736(97)80051-7).
6. Hancock RE. 2001. Cationic peptides: effectors in innate immunity and novel antimicrobials. *Lancet Infect Dis* 1:156–164. [https://doi.org/10.1016/S1473-3099\(01\)00092-5](https://doi.org/10.1016/S1473-3099(01)00092-5).
7. Shah P, Hsiao FS, Ho YH, Chen CS. 2016. The proteome targets of intracellular targeting antimicrobial peptides. *Proteomics* 16:1225–1237. <https://doi.org/10.1002/pmic.201500380>.
8. Di Nardo A, Vitiello A, Gallo RL. 2003. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol* 170:2274–2278. <https://doi.org/10.4049/jimmunol.170.5.2274>.
9. Rosenberger CM, Gallo RL, Finlay BB. 2004. Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication. *Proc Natl Acad Sci U S A* 101:2422–2427. <https://doi.org/10.1073/pnas.0304455101>.
10. Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. 1998. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. *Antimicrob Agents Chemother* 42:2206–2214.
11. Lee HY, Andalibi A, Webster P, Moon SK, Teufert K, Kang SH, Li JD, Nagura M, Ganz T, Lim DJ. 2004. Antimicrobial activity of innate immune molecules against *Streptococcus pneumoniae*, *Moraxella catarrhalis* and nontypeable *Haemophilus influenzae*. *BMC Infect Dis* 4:12. <https://doi.org/10.1186/1471-2334-4-12>.
12. Zasloff M. 2002. Innate immunity, antimicrobial peptides, and protection of the oral cavity. *Lancet* 360:1116–1117. [https://doi.org/10.1016/S0140-6736\(02\)11239-6](https://doi.org/10.1016/S0140-6736(02)11239-6).
13. Schonwetter BS, Stolzenberg ED, Zasloff MA. 1995. Epithelial antibiotics induced at sites of inflammation. *Science* 267:1645–1648. <https://doi.org/10.1126/science.7886453>.
14. Shai Y. 2002. Mode of action of membrane active antimicrobial peptides. *Biopolymers* 66:236–248. <https://doi.org/10.1002/bip.10260>.
15. Yeaman MR, Yount NY. 2003. Mechanisms of antimicrobial peptide action and resistance. *Pharmacol Rev* 55:27–55. <https://doi.org/10.1124/pr.55.1.2>.
16. Hale JD, Hancock RE. 2007. Alternative mechanisms of action of cationic antimicrobial peptides on bacteria. *Expert Rev Anti Infect Ther* 5:951–959. <https://doi.org/10.1586/14787210.5.6.951>.
17. Sengupta D, Leontiadou H, Mark AE, Marrink SJ. 2008. Toroidal pores formed by antimicrobial peptides show significant disorder. *Biochim Biophys Acta* 1778:2308–2317. <https://doi.org/10.1016/j.bbame.2008.06.007>.
18. Yang L, Harroun TA, Heller WT, Weiss TM, Huang HW. 1998. Neutron off-plane scattering of aligned membranes. I. Method of measurement. *Biophys J* 75:641–645.
19. Yoneyama F, Imura Y, Ohno K, Zendo T, Nakayama J, Matsuzaki K, Sonomoto K. 2009. Peptide-lipid huge toroidal pore, a new antimicrobial mechanism mediated by a lactococcal bacteriocin, lactacin Q. *Antimicrob Agents Chemother* 53:3211–3217. <https://doi.org/10.1128/AAC.00209-09>.
20. Yang L, Harroun TA, Weiss TM, Ding L, Huang HW. 2001. Barrel-stave model or toroidal model? A case study on melittin pores. *Biophys J* 81:1475–1485.

21. Gazit E, Boman A, Boman HG, Shai Y. 1995. Interaction of the mammalian antibacterial peptide cecropin P1 with phospholipid vesicles. *Biochemistry* 34:11479–11488. <https://doi.org/10.1021/bi00036a021>.
22. Nguyen LT, Haney EF, Vogel HJ. 2011. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol* 29:464–472. <https://doi.org/10.1016/j.tibtech.2011.05.001>.
23. Madani F, Lindberg S, Langel U, Futaki S, Graslund A. 2011. Mechanisms of cellular uptake of cell-penetrating peptides. *J Biophys* 2011:414729.
24. Jones AT. 2007. Macropinocytosis: searching for an endocytic identity and role in the uptake of cell penetrating peptides. *J Cell Mol Med* 11:670–684. <https://doi.org/10.1111/j.1582-4934.2007.00062.x>.
25. Mayor S, Pagano RE. 2007. Pathways of clathrin-independent endocytosis. *Nat Rev Mol Cell Biol* 8:603–612. <https://doi.org/10.1038/nrm2216>.
26. Peschel A. 2002. How do bacteria resist human antimicrobial peptides? *Trends Microbiol* 10:179–186. [https://doi.org/10.1016/S0966-842X\(02\)02333-8](https://doi.org/10.1016/S0966-842X(02)02333-8).
27. Powers JP, Hancock RE. 2003. The relationship between peptide structure and antibacterial activity. *Peptides* 24:1681–1691. <https://doi.org/10.1016/j.peptides.2003.08.023>.
28. Edgerton M, Koshlukova SE, Araujo MW, Patel RC, Dong J, Bruenn JA. 2000. Salivary histatin 5 and human neutrophil defensin 1 kill *Candida albicans* via shared pathways. *Antimicrob Agents Chemother* 44:3310–3316. <https://doi.org/10.1128/AAC.44.12.3310-3316.2000>.
29. Park CB, Kim MS, Kim SC. 1996. A novel antimicrobial peptide from *Bufo bufo* gargarizans. *Biochem Biophys Res Commun* 218:408–413. <https://doi.org/10.1006/bbrc.1996.0071>.
30. Park CB, Yi KS, Matsuzaki K, Kim MS, Kim SC. 2000. Structure-activity analysis of buforin II, a histone H2A-derived antimicrobial peptide: the proline hinge is responsible for the cell-penetrating ability of buforin II. *Proc Natl Acad Sci U S A* 97:8245–8250. <https://doi.org/10.1073/pnas.150518097>.
31. Kobayashi S, Takeshima K, Park CB, Kim SC, Matsuzaki K. 2000. Interactions of the novel antimicrobial peptide buforin 2 with lipid bilayers: proline as a translocation promoting factor. *Biochemistry* 39:8648–8654. <https://doi.org/10.1021/bi0004549>.
32. Park CB, Kim HS, Kim SC. 1998. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem Biophys Res Commun* 244:253–257. <https://doi.org/10.1006/bbrc.1998.8159>.
33. Birkemo GA, Luders T, Andersen O, Nes IF, Nissen-Meyer J. 2003. Hippoglossin, a histone-derived antimicrobial peptide in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Biochim Biophys Acta* 1646:207–215. [https://doi.org/10.1016/S1570-9639\(03\)00018-9](https://doi.org/10.1016/S1570-9639(03)00018-9).
34. Hao G, Shi YH, Tang YL, Le GW. 2013. The intracellular mechanism of action on *Escherichia coli* of BF2-A/C, two analogues of the antimicrobial peptide Buforin 2. *J Microbiol* 51:200–206. <https://doi.org/10.1007/s12275-013-2441-1>.
35. Selsted ME, Novotny MJ, Morris WL, Tang YQ, Smith W, Cullor JS. 1992. Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. *J Biol Chem* 267:4292–4295.
36. Falla TJ, Karunaratne DN, Hancock RE. 1996. Mode of action of the antimicrobial peptide indolicidin. *J Biol Chem* 271:19298–19303. <https://doi.org/10.1074/jbc.271.32.19298>.
37. Schibli DJ, Epand RF, Vogel HJ, Epand RM. 2002. Tryptophan-rich antimicrobial peptides: comparative properties and membrane interactions. *Biochem Cell Biol* 80:667–677. <https://doi.org/10.1139/o02-147>.
38. Ladokhin AS, Selsted ME, White SH. 1997. Bilayer interactions of indolicidin, a small antimicrobial peptide rich in tryptophan, proline, and basic amino acids. *Biophys J* 72:794–805. [https://doi.org/10.1016/S0006-3495\(97\)78713-7](https://doi.org/10.1016/S0006-3495(97)78713-7).
39. Subbalakshmi C, Krishnakumari V, Nagaraj R, Sitaram N. 1996. Requirements for antibacterial and hemolytic activities in the bovine neutrophil derived 13-residue peptide indolicidin. *FEBS Lett* 395:48–52. [https://doi.org/10.1016/0014-5793\(96\)00996-9](https://doi.org/10.1016/0014-5793(96)00996-9).
40. Subbalakshmi C, Sitaram N. 1998. Mechanism of antimicrobial action of indolicidin. *FEMS Microbiol Lett* 160:91–96. <https://doi.org/10.1111/j.1574-6968.1998.tb12896.x>.
41. Marchand C, Krajewski K, Lee HF, Antony S, Johnson AA, Amin R, Roller P, Kvaratskhelia M, Pommier Y. 2006. Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites. *Nucleic Acids Res* 34:5157–5165. <https://doi.org/10.1093/nar/gkl667>.
42. Hsu CH, Chen C, Jou ML, Lee AY, Lin YC, Yu YP, Huang WT, Wu SH. 2005. Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. *Nucleic Acids Res* 33:4053–4064. <https://doi.org/10.1093/nar/gki725>.
43. Ghosh A, Kar RK, Jana J, Saha A, Jana B, Krishnamoorthy J, Kumar D, Ghosh S, Chatterjee S, Bhunia A. 2014. Indolicidin targets duplex DNA: structural and mechanistic insight through a combination of spectroscopy and microscopy. *ChemMedChem* 9:2052–2058. <https://doi.org/10.1002/cmdc.201402215>.
44. Sitaram N, Subbalakshmi C, Nagaraj R. 2003. Indolicidin, a 13-residue basic antimicrobial peptide rich in tryptophan and proline, interacts with Ca^{2+} -calmodulin. *Biochem Biophys Res Commun* 309:879–884. <https://doi.org/10.1016/j.bbrc.2003.08.095>.
45. Sugiarto H, Yu PL. 2007. Mechanisms of action of ostrich beta-defensins against *Escherichia coli*. *FEMS Microbiol Lett* 270:195–200. <https://doi.org/10.1111/j.1574-6968.2007.00642.x>.
46. Anderson RC, Yu PL. 2003. Isolation and characterisation of proline/arginine-rich cathelicidin peptides from ovine neutrophils. *Biochem Biophys Res Commun* 312:1139–1146. <https://doi.org/10.1016/j.bbrc.2003.11.045>.
47. Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S, Niwa M, Takao T, Shimonishi Y. 1988. Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachyplesus tridentatus*). Isolation and chemical structure. *J Biol Chem* 263:16709–16713.
48. Yonezawa A, Kuwahara J, Fujii N, Sugiura Y. 1992. Binding of tachyplesin I to DNA revealed by footprinting analysis: significant contribution of secondary structure to DNA binding and implication for biological action. *Biochemistry* 31:2998–3004. <https://doi.org/10.1021/bi00126a022>.
49. Baquero F, Moreno F. 1984. The microcins. *FEMS Microbiol Lett* 23:117–124. <https://doi.org/10.1111/j.1574-6968.1984.tb01046.x>.
50. Laviña M, Gaggero C, Moreno F. 1990. Microcin H47, a chromosome-encoded microcin antibiotic of *Escherichia coli*. *J Bacteriol* 172:6585–6588. <https://doi.org/10.1128/jb.172.11.6585-6588.1990>.
51. Solbiati JO, Ciccio M, Farias RN, Salomon RA. 1996. Genetic analysis of plasmid determinants for microcin J25 production and immunity. *J Bacteriol* 178:3661–3663. <https://doi.org/10.1128/jb.178.12.3661-3663.1996>.
52. Vizán JL, Hernandez-Chico C, del Castillo I, Moreno F. 1991. The peptide antibiotic microcin B17 induces double-strand cleavage of DNA mediated by *E. coli* DNA gyrase. *EMBO J* 10:467–476.
53. Davagnino J, Herrero M, Furlong D, Moreno F, Kolter R. 1986. The DNA replication inhibitor microcin B17 is a forty-three-amino-acid protein containing sixty percent glycine. *Proteins* 1:230–238. <https://doi.org/10.1002/prot.340010305>.
54. Heddle JG, Blance SJ, Zamble DB, Hollfelder F, Miller DA, Wentzell LM, Walsh CT, Maxwell A. 2001. The antibiotic microcin B17 is a DNA gyrase poison: characterisation of the mode of inhibition. *J Mol Biol* 307:1223–1234. <https://doi.org/10.1006/jmbi.2001.4562>.
55. Moreno F, San Millán JL, Hernandez-Chico C, Kolter R. 1995. Microcins. *Biotechnology* 28:307–321.
56. Roy RS, Gehring AM, Milne JC, Belshaw PJ, Walsh CT. 1999. Thiazole and oxazole peptides: biosynthesis and molecular machinery. *Nat Prod Rep* 16:249–263. <https://doi.org/10.1039/a806930a>.
57. Parks WM, Bottrill AR, Pierrat OA, Durrant MC, Maxwell A. 2007. The action of the bacterial toxin, microcin B17, on DNA gyrase. *Biochimie* 89:500–507. <https://doi.org/10.1016/j.biochi.2006.12.005>.
58. Laviña M, Pugsley AP, Moreno F. 1986. Identification, mapping, cloning and characterization of a gene (*sbmA*) required for microcin B17 action on *Escherichia coli* K12. *J Gen Microbiol* 132:1685–1693.
59. Salomón RA, Farias RN. 1992. Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. *J Bacteriol* 174:7428–7435. <https://doi.org/10.1128/jb.174.22.7428-7435.1992>.
60. Bayro MJ, Mukhopadhyay J, Swapna GV, Huang JY, Ma LC, Sineva E, Dawson PE, Montelione GT, Ebright RH. 2003. Structure of antibacterial peptide microcin J25: a 21-residue lariat protoknot. *J Am Chem Soc* 125:12382–12383. <https://doi.org/10.1021/ja036677e>.
61. Salomón RA, Farias RN. 1993. The FhuA protein is involved in microcin 25 uptake. *J Bacteriol* 175:7741–7742. <https://doi.org/10.1128/jb.175.23.7741-7742.1993>.
62. Salomón RA, Farias RN. 1995. The peptide antibiotic microcin 25 is imported through the TonB pathway and the SbmA protein. *J Bacteriol* 177:3323–3325. <https://doi.org/10.1128/jb.177.11.3323-3325.1995>.

63. Yuzenkova J, Delgado M, Nechaev S, Savalia D, Epshtein V, Artsimovitch I, Mooney RA, Landick R, Farias RN, Salomon R, Severinov K. 2002. Mutations of bacterial RNA polymerase leading to resistance to microcin J25. *J Biol Chem* 277:50867–50875. <https://doi.org/10.1074/jbc.M209425200>.
64. Mukhopadhyay J, Sineva E, Knight J, Levy RM, Ebricht RH. 2004. Antibacterial peptide microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel. *Mol Cell* 14:739–751. <https://doi.org/10.1016/j.molcel.2004.06.010>.
65. Adelman K, Yuzenkova J, La Porta A, Zenkin N, Lee J, Lis JT, Borukhov S, Wang MD, Severinov K. 2004. Molecular mechanism of transcription inhibition by peptide antibiotic microcin J25. *Mol Cell* 14:753–762. <https://doi.org/10.1016/j.molcel.2004.05.017>.
66. Schnapp D, Kemp GD, Smith VJ. 1996. Purification and characterization of a proline-rich antibacterial peptide, with sequence similarity to bactenecin-7, from the haemocytes of the shore crab, *Carcinus maenas*. *Eur J Biochem* 240:532–539. <https://doi.org/10.1111/j.1432-1033.1996.0532h.x>.
67. Podda E, Benincasa M, Pacor S, Micali F, Mattiuzzo M, Gennaro R, Scocchi M. 2006. Dual mode of action of Bac7, a proline-rich antibacterial peptide. *Biochim Biophys Acta* 1760:1732–1740. <https://doi.org/10.1016/j.bbagen.2006.09.006>.
68. Mattiuzzo M, Bandiera A, Gennaro R, Benincasa M, Pacor S, Antcheva N, Scocchi M. 2007. Role of the *Escherichia coli* SbmA in the antimicrobial activity of proline-rich peptides. *Mol Microbiol* 66:151–163. <https://doi.org/10.1111/j.1365-2958.2007.05903.x>.
69. Mardirossian M, Grzela R, Giglione C, Meinel T, Gennaro R, Mergaert P, Scocchi M. 2014. The host antimicrobial peptide Bac71–35 binds to bacterial ribosomal proteins and inhibits protein synthesis. *Chem Biol* 21:1639–1647. <https://doi.org/10.1016/j.chembiol.2014.10.009>.
70. Ho YH, Shah P, Chen YW, Chen CS. 2016. Systematic analysis of intracellular-targeting antimicrobial peptides, bactenecin 7, hybrid of pleurocidin and dermaseptin, proline-arginine-rich peptide, and lactoferricin B, by using *Escherichia coli* proteome microarrays. *Mol Cell Proteomics* 15:1837–1847. <https://doi.org/10.1074/mcp.M115.054999>.
71. Ghiselli R, Giacometti A, Cirioni O, Circo R, Mocchegiani F, Skerlavaj B, D'Amato G, Scalise G, Zanetti M, Saba V. 2003. Neutralization of endotoxin *in vitro* and *in vivo* by Bac7(1–35), a proline-rich antibacterial peptide. *Shock* 19:577–581. <https://doi.org/10.1097/01.shk.0000055236.26446.c9>.
72. Cole AM, Weis P, Diamond G. 1997. Isolation and characterization of pleurocidin, an antimicrobial peptide in the skin secretions of winter flounder. *J Biol Chem* 272:12008–12013. <https://doi.org/10.1074/jbc.272.18.12008>.
73. Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock RE. 2002. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. *Antimicrob Agents Chemother* 46:614605–12013. <https://doi.org/10.1128/AAC.46.3.605-614.2002>.
74. Friedrich CL, Rozek A, Patrzykat A, Hancock RE. 2001. Structure and mechanism of action of an indolicidin peptide derivative with improved activity against Gram-positive bacteria. *J Biol Chem* 276:24015–24022. <https://doi.org/10.1074/jbc.M009691200>.
75. Durr UH, Sudheendra US, Ramamoorthy A. 2006. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochim Biophys Acta* 1758:1408–1425. <https://doi.org/10.1016/j.bbame.2006.03.030>.
76. Robinson WE, Jr, McDougall B, Tran D, Selsted ME. 1998. Anti-HIV-1 activity of indolicidin, an antimicrobial peptide from neutrophils. *J Leukoc Biol* 63:94–100.
77. Selsted ME, Tang YQ, Morris WL, McGuire PA, Novotny MJ, Smith W, Henschen AH, Cullor JS. 1993. Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. *J Biol Chem* 268:6641–6648.
78. van Abel RJ, Tang YQ, Rao VS, Dobbs CH, Tran D, Barany G, Selsted ME. 1995. Synthesis and characterization of indolicidin, a tryptophan-rich antimicrobial peptide from bovine neutrophils. *Int J Pept Protein Res* 45:401–409.
79. Le C-F, Yusof MYM, Hassan H, Sekaran SD. 2015. *In vitro* properties of designed antimicrobial peptides that exhibit potent antipneumococcal activity and produces synergism in combination with penicillin. *Sci Rep* 5:9761. <https://doi.org/10.1038/srep09761>.
80. Le C-F, Yusof MY, Hassan MA, Lee VS, Isa DM, Sekaran SD. 2015. *In vivo* efficacy and molecular docking of designed peptide that exhibits potent antipneumococcal activity and synergises in combination with penicillin. *Sci Rep* 5:11886. <https://doi.org/10.1038/srep11886>.
81. Le C-F, Gudimella R, Razali R, Manikam R, Sekaran SD. 2016. Transcriptome analysis of *Streptococcus pneumoniae* treated with the designed antimicrobial peptides, DM3. *Sci Rep* 6:26828. <https://doi.org/10.1038/srep26828>.
82. Agerberth B, Lee JY, Bergman T, Carlquist M, Boman HG, Mutt V, Jorvall H. 1991. Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. *Eur J Biochem* 202:849–854.
83. Shi J, Ross CR, Chengappa MM, Blecha F. 1994. Identification of a proline-arginine-rich antibacterial peptide from neutrophils that is analogous to PR-39, an antibacterial peptide from the small intestine. *J Leukoc Biol* 56:807–811.
84. Prnting M, Negrea A, Rhen M, Andersson DI. 2008. Mechanism and fitness costs of PR-39 resistance in *Salmonella enterica* serovar Typhimurium LT2. *Antimicrob Agents Chemother* 52:2734–2741. <https://doi.org/10.1128/AAC.00205-08>.
85. Boman HG, Agerberth B, Boman A. 1993. Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. *Infect Immun* 61:2978–2984.
86. Li WF, Ma GX, Zhou XX. 2006. Apidaecin-type peptides: biodiversity, structure-function relationships and mode of action. *Peptides* 27:2350–2359. <https://doi.org/10.1016/j.peptides.2006.03.016>.
87. Casteels P, Ampe C, Jacobs F, Vaecck M, Tempst P. 1989. Apidaecins: antibacterial peptides from honeybees. *EMBO J* 8:2387–2391.
88. Casteels P, Romagnolo J, Castle M, Casteels-Josson K, Erdjument-Bromage H, Tempst P. 1994. Biodiversity of apidaecin-type peptide antibiotics. Prospects of manipulating the antibacterial spectrum and combating acquired resistance. *J Biol Chem* 269:26107–26115.
89. Casteels-Josson K, Capaci T, Casteels P, Tempst P. 1993. Apidaecin multiprecursor structure: a putative mechanism for amplification of the insect antibacterial response. *EMBO J* 12:1569–1578.
90. Casteels P, Tempst P. 1994. Apidaecin-type peptide antibiotics function through a non-poreforming mechanism involving stereospecificity. *Biochem Biophys Res Commun* 199:339–345. <https://doi.org/10.1006/bbrc.1994.1234>.
91. Ho YH, Sung TC, Chen CS. 2012. Lactoferricin B inhibits the phosphorylation of the two-component system response regulators BasR and CreB. *Mol Cell Proteomics* 11:M111.014720. <https://doi.org/10.1074/mcp.M111.014720>.
92. Tu YH, Ho YH, Chuang YC, Chen PC, Chen CS. 2011. Identification of lactoferricin B intracellular targets using an *Escherichia coli* proteome chip. *PLoS One* 6:e28197. <https://doi.org/10.1371/journal.pone.0028197>.
93. Ganz T, Lehrer RI. 1997. Antimicrobial peptides of leukocytes. *Curr Opin Hematol* 4:53–58. <https://doi.org/10.1097/00062752-199704010-00009>.
94. Lehrer RI, Barton A, Daher KA, Harwig SS, Ganz T, Selsted ME. 1989. Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. *J Clin Invest* 84:553–561.
95. Jenssen H, Hamill P, Hancock RE. 2006. Peptide antimicrobial agents. *Clin Microbiol Rev* 19:491–511. <https://doi.org/10.1128/CMR.00056-05>.
96. Ellis RJ. 1990. The molecular chaperone concept. *Semin Cell Biol* 1:1–9.
97. Calloni G, Chen T, Schermann SM, Chang HC, Genevaux P, Agostini F, Tartaglia GG, Hayer-Hartl M, Hartl FU. 2012. DnaK functions as a central hub in the *E. coli* chaperone network. *Cell Rep* 1:251–264. <https://doi.org/10.1016/j.celrep.2011.12.007>.
98. Cociancich S, Dupont A, Hegy G, Lanot R, Holder F, Hetru C, Hoffmann JA, Bulet P. 1994. Novel inducible antibacterial peptides from a hemipteran insect, the sap-sucking bug *Pyrrhocoris apterus*. *Biochem J* 300:567–575.
99. Miura K, Ueno S, Kamiya K, Kobayashi J, Matsuoka H, Ando K, Chinzei Y. 1996. Cloning of mRNA sequences for two antibacterial peptides in a hemipteran insect, *Riptortus clavatus*. *Zoolog Sci* 13:111–117. <https://doi.org/10.2108/zsj.13.111>.
100. Otvos L, Jr, O I, Rogers ME, Consolvo PJ, Condie BA, Lovas S, Bulet P, Blaszczyk-Thurin M. 2000. Interaction between heat shock proteins and antimicrobial peptides. *Biochemistry* 39:14150–14159. <https://doi.org/10.1021/bi0012843>.
101. Chesnokova LS, Slepnev SV, Witt SN. 2004. The insect antimicrobial peptide, L-pyrrolicin, binds to and stimulates the ATPase activity of both wild-type and lidless DnaK. *FEBS Lett* 565:65–69. <https://doi.org/10.1016/j.febslet.2004.03.075>.
102. Kragol G, Lovas S, Varadi G, Condie BA, Hoffmann R, Otvos L, Jr. 2001.

- The antibacterial peptide pyrrolicorin inhibits the ATPase actions of DnaK and prevents chaperone-assisted protein folding. *Biochemistry* 40:3016–3026. <https://doi.org/10.1021/bi002656a>.
103. Rahnamaeian M, Cytrynska M, Zdybicka-Barabas A, Dobszlaff K, Wiesner J, Twyman RM, Zuchner T, Sadd BM, Regoes RR, Schmid-Hempel P, Vilcinskis A. 2015. Insect antimicrobial peptides show potentiating functional interactions against Gram-negative bacteria. *Proc Biol Sci* 282:20150293. <https://doi.org/10.1098/rspb.2015.0293>.
 104. Knappe D, Piantavigna S, Hansen A, Mechler A, Binas A, Nolte O, Martin LL, Hoffmann R. 2010. Oncocin (VDKPPYLPRPPRRRIYNR-NH₂): a novel antibacterial peptide optimized against Gram-negative human pathogens. *J Med Chem* 53:5240–5247. <https://doi.org/10.1021/jm100378b>.
 105. Knappe D, Zahn M, Sauer U, Schiffer G, Strater N, Hoffmann R. 2011. Rational design of oncocin derivatives with superior protease stabilities and antibacterial activities based on the high-resolution structure of the oncocin-DnaK complex. *ChemBiochem* 12:874–876. <https://doi.org/10.1002/cbic.201000792>.
 106. Scocchi M, Lüthy C, Decarli P, Mignogna G, Christen P, Gennaro R. 2009. The proline-rich antibacterial peptide Bac7 binds to and inhibits *in vitro* the molecular chaperone DnaK. *Int J Pept Res Ther* 15:9.
 107. Sorsa T, Suomalainen K, Uitto VJ. 1990. The role of gingival crevicular fluid and salivary interstitial collagenases in human periodontal diseases. *Arch Oral Biol* 35(Suppl):193S–196S.
 108. Troxler RF, Offner GD, Xu T, Vanderspek JC, Oppenheim FG. 1990. Structural relationship between human salivary histatins. *J Dent Res* 69:2–6. <https://doi.org/10.1177/00220345900690010101>.
 109. 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* 263:7472–7477.
 110. Puri S, Edgerton M. 2014. How does it kill?: understanding the candidacidal mechanism of salivary histatin 5. *Eukaryot Cell* 13:958–964. <https://doi.org/10.1128/EC.00095-14>.
 111. MacKay BJ, Denepitiya L, Iacono VJ, Krost SB, Pollock JJ. 1984. Growth-inhibitory and bactericidal effects of human parotid salivary histidine-rich polypeptides on *Streptococcus mutans*. *Infect Immun* 44:695–701.
 112. Forssten SD, Bjorklund M, Ouwehand AC. 2010. *Streptococcus mutans*, caries and simulation models. *Nutrients* 2:290–298. <https://doi.org/10.3390/nu2030290>.
 113. Gusman H, Travis J, Helmerhorst EJ, Potempa J, Troxler RF, Oppenheim FG. 2001. Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease. *Infect Immun* 69:1402–1408. <https://doi.org/10.1128/IAI.69.3.1402-1408.2001>.
 114. Gusman H, Grogan J, Kagan HM, Troxler RF, Oppenheim FG. 2001. Salivary histatin 5 is a potent competitive inhibitor of the cysteine proteinase clostripain. *FEBS Lett* 489:97–100. [https://doi.org/10.1016/S0014-5793\(01\)02077-4](https://doi.org/10.1016/S0014-5793(01)02077-4).
 115. Stevens DL, Bisno AL, Chambers HF, Everett ED, Dellinger P, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan EL, Montoya JG, Wade JC. 2005. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 41:1373–1406. <https://doi.org/10.1086/497143>.
 116. Nishikata M, Kanehira T, Oh H, Tani H, Tazaki M, Kuboki Y. 1991. Salivary histatin as an inhibitor of a protease produced by the oral bacterium *Bacteroides gingivalis*. *Biochem Biophys Res Commun* 174:625–630. [https://doi.org/10.1016/0006-291X\(91\)91463-M](https://doi.org/10.1016/0006-291X(91)91463-M).
 117. Couto MA, Harwig SS, Cullor JS, Hughes JP, Lehrer RI. 1992. eNAP-2, a novel cysteine-rich bactericidal peptide from equine leukocytes. *Infect Immun* 60:5042–5047.
 118. Couto MA, Harwig SS, Lehrer RI. 1993. Selective inhibition of microbial serine proteases by eNAP-2, an antimicrobial peptide from equine neutrophils. *Infect Immun* 61:2991–2994.
 119. Fogaça AC, Almeida IC, Eberlin MN, Tanaka AS, Bulet P, Daffre S. 2006. Ixodidin, a novel antimicrobial peptide from the hemocytes of the cattle tick *Boophilus microplus* with inhibitory activity against serine proteinases. *Peptides* 27:667–674. <https://doi.org/10.1016/j.peptides.2005.07.013>.
 120. Lutkenhaus J. 1990. Regulation of cell division in *E. coli*. *Trends Genet* 6:22–25. [https://doi.org/10.1016/0168-9525\(90\)90045-8](https://doi.org/10.1016/0168-9525(90)90045-8).
 121. Ishikawa M, Kubo T, Natori S. 1992. Purification and characterization of a dipterin homologue from *Sarcophaga peregrina* (flesh fly). *Biochem J* 287:573–578.
 122. Wanniarachchi YA, Kaczmarek P, Wan A, Nolan EM. 2011. Human defensin 5 disulfide array mutants: disulfide bond deletion attenuates antibacterial activity against *Staphylococcus aureus*. *Biochemistry* 50:8005–8017. <https://doi.org/10.1021/bi201043j>.
 123. Moser S, Chileveru HR, Tomaras J, Nolan EM. 2014. A bacterial mutant library as a tool to study the attack of a defensin peptide. *ChemBiochem* 15:2684–2688. <https://doi.org/10.1002/cbic.201402354>.
 124. Chileveru HR, Lim SA, Chairatana P, Wommack AJ, Chiang IL, Nolan EM. 2015. Visualizing attack of *Escherichia coli* by the antimicrobial peptide human defensin 5. *Biochemistry* 54:1767–1777. <https://doi.org/10.1021/bi501483q>.
 125. Navarre WW, Schneewind O. 1999. Surface proteins of Gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol Mol Biol Rev* 63:174–229.
 126. Vollmer W, Holtje JV. 2001. Morphogenesis of *Escherichia coli*. *Curr Opin Microbiol* 4:625–633. [https://doi.org/10.1016/S1369-5274\(01\)00261-2](https://doi.org/10.1016/S1369-5274(01)00261-2).
 127. Koch AL. 2003. Bacterial wall as target for attack: past, present, and future research. *Clin Microbiol Rev* 16:673–687. <https://doi.org/10.1128/CMR.16.4.673-687.2003>.
 128. Prasch T, Naumann T, Markert RL, Sattler M, Schubert W, Schaal S, Bauch M, Kogler H, Griesinger C. 1997. Constitution and solution conformation of the antibiotic mersacidin determined by NMR and molecular dynamics. *Eur J Biochem* 244:501–512. <https://doi.org/10.1111/j.1432-1033.1997.00501.x>.
 129. Brötz H, Bierbaum G, Leopold K, Reynolds PE, Sahl HG. 1998. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. *Antimicrob Agents Chemother* 42:154–160.
 130. Brötz H, Bierbaum G, Reynolds PE, Sahl HG. 1997. The lantibiotic mersacidin inhibits peptidoglycan biosynthesis at the level of transglycosylation. *Eur J Biochem* 246:193–199. <https://doi.org/10.1111/j.1432-1033.1997.t01-1-00193.x>.
 131. Chatterjee S, Lad SJ, Phansalkar MS, Rupp RH, Ganguli BN, Fehlhaber HW, Kogler H. 1992. Mersacidin, a new antibiotic from *Bacillus*. Fermentation, isolation, purification and chemical characterization. *J Antibiot (Tokyo)* 45:832–838.
 132. Hoffmann A, Pag U, Wiedemann I, Sahl HG. 2002. Combination of antibiotic mechanisms in lantibiotics. *Farmacol* 57:685–691. [https://doi.org/10.1016/S0014-827X\(02\)01208-9](https://doi.org/10.1016/S0014-827X(02)01208-9).
 133. Kruszewska D, Sahl HG, Bierbaum G, Pag U, Hynes SO, Ljungh A. 2004. Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. *J Antimicrob Chemother* 54:648–653. <https://doi.org/10.1093/jac/dkh387>.
 134. de Leeuw E, Li C, Zeng P, Diepeveen-de Buin M, Lu WY, Breukink E, Lu W. 2010. Functional interaction of human neutrophil peptide-1 with the cell wall precursor lipid II. *FEBS Lett* 584:1543–1548. <https://doi.org/10.1016/j.febslet.2010.03.004>.
 135. Sass V, Schneider T, Wilmes M, Korner C, Tossi A, Novikova N, Shamova O, Sahl HG. 2010. Human beta-defensin 3 inhibits cell wall biosynthesis in staphylococci. *Infect Immun* 78:2793–2800. <https://doi.org/10.1128/IAI.00688-09>.
 136. Breukink E, de Kruijff B. 2006. Lipid II as a target for antibiotics. *Nat Rev Drug Discov* 5:321–332. <https://doi.org/10.1038/nrd2004>.
 137. Hsu ST, Breukink E, Tischenko E, Lutters MA, de Kruijff B, Kaptein R, Bonvin AM, van Nuland NA. 2004. The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. *Nat Struct Mol Biol* 11:963–967. <https://doi.org/10.1038/nsmb830>.
 138. Hasper HE, Kramer NE, Smith JL, Hillman JD, Zachariah C, Kuipers OP, de Kruijff B, Breukink E. 2006. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science* 313:1636–1637. <https://doi.org/10.1126/science.1129818>.
 139. Wescombe PA, Tagg JR. 2003. Purification and characterization of streptin, a type A1 lantibiotic produced by *Streptococcus pyogenes*. *Appl Environ Microbiol* 69:2737–2747. <https://doi.org/10.1128/AEM.69.5.2737-2747.2003>.
 140. Stein T, Borchert S, Conrad F, Feesche J, Hofemeister B, Hofemeister J, Entian KD. 2002. Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A1/3. *J Bacteriol* 184:1703–1711. <https://doi.org/10.1128/JB.184.6.1703-1711.2002>.
 141. McAuliffe O, Ross RP, Hill C. 2001. Lantibiotics: structure, biosynthesis and mode of action. *FEMS Microbiol Rev* 25:285–308. <https://doi.org/10.1111/j.1574-6976.2001.tb00579.x>.
 142. Bonelli RR, Schneider T, Sahl HG, Wiedemann I. 2006. Insights into *in vivo* activities of lantibiotics from gallidermin and epidermin mode-of-action studies. *Antimicrob Agents Chemother* 50:1449–1457. <https://doi.org/10.1128/AAC.50.4.1449-1457.2006>.

143. Furmanek B, Kaczorowski T, Bugalski R, Bielawski K, Bohdanowicz J, Podhajska AJ. 1999. Identification, characterization and purification of the lantibiotic staphylococcin T, a natural gallidermin variant. *J Appl Microbiol* 87:856–866. <https://doi.org/10.1046/j.1365-2672.1999.00937.x>.
144. Essig A, Hofmann D, Munch D, Gayathri S, Kunzler M, Kallio PT, Sahl HG, Wider G, Schneider T, Aebi M. 2014. Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. *J Biol Chem* 289:34953–34964. <https://doi.org/10.1074/jbc.M114.599878>.
145. Schneider T, Kruse T, Wimmer R, Wiedemann I, Sass V, Pag U, Jansen A, Nielsen AK, Mygind PH, Raventos DS, Neve S, Ravn B, Bonvin AM, De Maria L, Andersen AS, Gammelgaard LK, Sahl HG, Kristensen HH. 2010. Plectasin, a fungal defensin, targets the bacterial cell wall precursor lipid II. *Science* 328:1168–1172. <https://doi.org/10.1126/science.1185723>.
146. Mygind PH, Fischer RL, Schnorr KM, Hansen MT, Sonksen CP, Ludvigsen S, Raventos D, Buskov S, Christensen B, De Maria L, Taboureau O, Yaver D, Elvig-Jorgensen SG, Sorensen MV, Christensen BE, Kjaerulff S, Frimodt-Moller N, Lehrer RI, Zasloff M, Kristensen HH. 2005. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature* 437:975–980. <https://doi.org/10.1038/nature04051>.
147. Sahl HG, Brandis H. 1981. Production, purification and chemical properties of an antistaphylococcal agent produced by *Staphylococcus epidermidis*. *J Gen Microbiol* 127:377–384.
148. Bierbaum G, Sahl HG. 1987. Autolytic system of *Staphylococcus simulans* 22: influence of cationic peptides on activity of *N*-acetylmuramoyl-L-alanine amidase. *J Bacteriol* 169:5452–5458. <https://doi.org/10.1128/jb.169.12.5452-5458.1987>.
149. Yeaman MR, Puentes SM, Norman DC, Bayer AS. 1992. Partial characterization and staphylocidal activity of thrombin-induced platelet microbicidal protein. *Infect Immun* 60:1202–1209.
150. Yeaman MR, Norman DC, Bayer AS. 1992. Platelet microbicidal protein enhances antibiotic-induced killing of and postantibiotic effect in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 36:1665–1670. <https://doi.org/10.1128/AAC.36.8.1665>.
151. Yeaman MR, Ibrahim AS, Edwards JE, Jr, Bayer AS, Ghannoum MA. 1993. Thrombin-induced rabbit platelet microbicidal protein is fungicidal *in vitro*. *Antimicrob Agents Chemother* 37:546–553. <https://doi.org/10.1128/AAC.37.3.546>.
152. Yeaman MR, Tang YQ, Shen AJ, Bayer AS, Selsted ME. 1997. Purification and *in vitro* activities of rabbit platelet microbicidal proteins. *Infect Immun* 65:1023–1031.
153. Koo SP, Bayer AS, Sahl HG, Proctor RA, Yeaman MR. 1996. Staphylocidal action of thrombin-induced platelet microbicidal protein is not solely dependent on transmembrane potential. *Infect Immun* 64:1070–1074.
154. Shimoda M, Ohki K, Shimamoto Y, Kohashi O. 1995. Morphology of defensin-treated *Staphylococcus aureus*. *Infect Immun* 63:2886–2891.
155. Yeaman MR, Bayer AS, Koo SP, Foss W, Sullam PM. 1998. Platelet microbicidal proteins and neutrophil defensin disrupt the *Staphylococcus aureus* cytoplasmic membrane by distinct mechanisms of action. *J Clin Invest* 101:178–187. <https://doi.org/10.1172/JCI562>.
156. Xiong YQ, Yeaman MR, Bayer AS. 1999. *In vitro* antibacterial activities of platelet microbicidal protein and neutrophil defensin against *Staphylococcus aureus* are influenced by antibiotics differing in mechanism of action. *Antimicrob Agents Chemother* 43:1111–1117.
157. Viljanen P, Koski P, Vaara M. 1988. Effect of small cationic leukocyte peptides (defensins) on the permeability barrier of the outer membrane. *Infect Immun* 56:2324–2329.
158. Raetz CR, Reynolds CM, Trent MS, Bishop RE. 2007. Lipid A modification systems in Gram-negative bacteria. *Annu Rev Biochem* 76:295–329. <https://doi.org/10.1146/annurev.biochem.76.010307.145803>.
159. Raetz CR, Whitfield C. 2002. Lipopolysaccharide endotoxins. *Annu Rev Biochem* 71:635–700. <https://doi.org/10.1146/annurev.biochem.71.110601.135414>.
160. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A. 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 330: 662–664. <https://doi.org/10.1038/330662a0>.
161. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ, Jr, Recombinant Human Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) Study Group. 2001. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 344:699–709. <https://doi.org/10.1056/NEJM200103083441001>.
162. Esmon CT. 2000. Regulation of blood coagulation. *Biochim Biophys Acta* 1477:349–360. [https://doi.org/10.1016/S0167-4838\(99\)00266-6](https://doi.org/10.1016/S0167-4838(99)00266-6).
163. van Deuren M, Brandtzaeg P, van der Meer JWM. 2000. Update on meningococcal disease with emphasis on pathogenesis and clinical management. *Clin Microbiol Rev* 13:144–166. <https://doi.org/10.1128/CMR.13.1.144-166.2000>.
164. Piers KL, Brown MH, Hancock RE. 1994. Improvement of outer membrane-permeabilizing and lipopolysaccharide-binding activities of an antimicrobial cationic peptide by C-terminal modification. *Antimicrob Agents Chemother* 38:2311–2316. <https://doi.org/10.1128/AAC.38.10.2311>.
165. Piers KL, Hancock RE. 1994. The interaction of a recombinant cecropin/melittin hybrid peptide with the outer membrane of *Pseudomonas aeruginosa*. *Mol Microbiol* 12:951–958. <https://doi.org/10.1111/j.1365-2958.1994.tb01083.x>.
166. Gough M, Hancock RE, Kelly NM. 1996. Antiendotoxin activity of cationic peptide antimicrobial agents. *Infect Immun* 64:4922–4927.
167. Gee K, Kozlowski M, Kumar A. 2003. Tumor necrosis factor- α induces functionally active hyaluronan-adhesive CD44 by activating sialidase through p38 mitogen-activated protein kinase in lipopolysaccharide-stimulated human monocytic cells. *J Biol Chem* 278:37275–37287. <https://doi.org/10.1074/jbc.M302309200>.
168. Kubo Y, Fukuishi N, Yoshioka M, Kawasoe Y, Iriguchi S, Imajo N, Yasui Y, Matsui N, Akagi M. 2007. Bacterial components regulate the expression of Toll-like receptor 4 on human mast cells. *Inflamm Res* 56:70–75. <https://doi.org/10.1007/s00011-006-6064-4>.
169. Mukhopadhyay S, Herre J, Brown GD, Gordon S. 2004. The potential for Toll-like receptors to collaborate with other innate immune receptors. *Immunology* 112:521–530. <https://doi.org/10.1111/j.1365-2567.2004.01941.x>.
170. Sun Y, Shang D. 2015. Inhibitory effects of antimicrobial peptides on lipopolysaccharide-induced inflammation. *Mediators Inflamm* 2015: 167572. <https://doi.org/10.1155/2015/167572>.
171. Freccer V, Ho B, Ding JL. 2000. Interpretation of biological activity data of bacterial endotoxins by simple molecular models of mechanism of action. *Eur J Biochem* 267:837–852. <https://doi.org/10.1046/j.1432-1327.2000.01069.x>.
172. Muhle SA, Tam JP. 2001. Design of Gram-negative selective antimicrobial peptides. *Biochemistry* 40:5777–5785. <https://doi.org/10.1021/bi0100384>.
173. Maria-Neto S, Candido Ede S, Rodrigues DR, de Sousa DA, da Silva EM, de Moraes LM, Otero-Gonzalez Ade J, Magalhaes BS, Dias SC, Franco OL. 2012. Deciphering the magainin resistance process of *Escherichia coli* strains in light of the cytosolic proteome. *Antimicrob Agents Chemother* 56:1714–1724. <https://doi.org/10.1128/AAC.05558-11>.
174. Jindal HM, Le CF, Mohd Yusof MY, Velayuthan RD, Lee VS, Zain SM, Isa DM, Sekaran SD. 2015. Antimicrobial activity of novel synthetic peptides derived from indolicidin and ranalexin against *Streptococcus pneumoniae*. *PLoS One* 10:e0128532. <https://doi.org/10.1371/journal.pone.0128532>.
175. Lum KY, Tay ST, Le CF, Lee VS, Sabri NH, Velayuthan RD, Hassan H, Sekaran SD. 2015. Activity of novel synthetic peptides against *Candida albicans*. *Sci Rep* 5:9657. <https://doi.org/10.1038/srep09657>.
176. Becucci L, Valensin D, Innocenti M, Guidelli R. 2014. Dermcidin, an anionic antimicrobial peptide: influence of lipid charge, pH and Zn²⁺ on its interaction with a biomimetic membrane. *Soft Matter* 10: 616–626. <https://doi.org/10.1039/C3SM52400K>.
177. Harris F, Dennison SR, Phoenix DA. 2009. Anionic antimicrobial peptides from eukaryotic organisms. *Curr Protein Pept Sci* 10:585–606. <https://doi.org/10.2174/138920309789630589>.
178. Noore J, Noore A, Li B. 2013. Cationic antimicrobial peptide LL-37 is effective against both extra- and intracellular *Staphylococcus aureus*. *Antimicrob Agents Chemother* 57:1283–1290. <https://doi.org/10.1128/AAC.01650-12>.
179. Chen L, Todd R, Kiehlbauch J, Walters M, Kallen A. 2017. Notes from the field: pan-resistant New Delhi-metallo-beta-lactamase-producing *Klebsiella pneumoniae*—Washoe County, Nevada, 2016. *MMWR Morb Mortal Wkly Rep* 66:33. <https://doi.org/10.15585/mmwr.mm6601a7>.