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Animal Antimicrobial Peptides: An Overview

Abstract: *Antibiotic peptides are a key component of the innate immune systems of most multicellular organisms. Despite broad divergences in sequence and taxonomy, most antibiotic peptides share a common mechanism of action, i.e., membrane permeabilization of the pathogen. This review provides a general introduction to the subject, with emphasis on aspects such as structural types, post-translational modifications, mode of action or mechanisms of resistance. Some of these questions are treated in depth in other reviews in this issue. The review also discusses the role of antimicrobial peptides in nature, including several pathological conditions, as well as recent accounts of their application at the preclinical level.* © 1999 John Wiley & Sons, Inc. Biopoly 47: 415–433, 1998

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INTRODUCTION

Despite their structural and functional diversity, multicellular organisms have certain common features in their defense-and-surveillance systems against pathogens. Early concepts of nonspecific and specific defense systems for plants and animals, respectively, are being reevaluated and expanded in the light of growing evidence that plants are endowed with certain specific defense systems, while on the other hand innate (nonadaptive) immunity in animals largely depends on nonspecific effectors.[1–6] In particular, gene-encoded antimicrobial peptides are now clearly established as key players in both plant and animal defense systems. In the last two decades, a considerable number of peptides, either inducible or constitutive, and with activity against different types of microorganisms, have been found in almost all groups of animals. These discoveries were preceded by the finding of thionins in plants,[7] the earliest example of antimicrobial peptides related to host defense. At the moment of this writing, several hundreds of structures with some type of antimicrobial activity have been described. A quite comprehensive, periodically updated data base can be found on the Internet at http://www.bbcm.univ.tries.it/~tossi. The present chapter is meant to provide a general overview of the main families of animal host defense peptides, as well as selected details on their biological action, including

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also relevant synthetic analogues. A number of significant aspects of this emerging field have been covered by recent reviews[8–23] and by the accompanying articles in this issue.

STRUCTURAL ASPECTS

A considerable variety of peptide sizes and structures are associated to antimicrobial activity in eukaryotic hosts. The early classification of antimicrobial peptides on a taxonomical basis has been increasingly found inadequate, in view of the fact that very similar structural patterns are shared by peptides from widely different organisms. In contrast, the alternative classification based on chemical-structural criteria[1] is still quite useful for cataloging the different families of antimicrobial peptides. This classification defines two broad groups, corresponding to linear and cyclic structures, respectively. Within the first group, two subgroups can be distinguished (a) linear peptides tending to adopt α-helical amphipathic conformation (Table I); and (b) linear peptides of unusual composition, rich in amino acids such as Pro, Arg, or (occasionally) Trp (Table II). The second group, encompassing all cystine-containing peptides, can also be divided into two subgroups corresponding to single or multiple disulfide structures (Tables III and IV), respectively. Tables I–IV include sequences of some the earliest reported antimicrobial structures, as well as recent noteworthy additions, with no pretense to be exhaustive. Readers looking for additional structures are directed to the above Internet address and other general reviews of this field.[1–23]

A number of secondary structures of antimicrobial peptides, broadly representative of the above structural groups, have been determined by two-dimensional nmr, either in solution or in model membrane environments. They include cecropins,[89–92] magainins,[93–95] PGLa,[96] sarcotoxin,[97] buforin,[98] caerin,[99] bactenecins,[100] enkelytin,[101] histatin,[102] ranalexin,[103] thanatin,[104] protegrin,[105] tachyplesin,[106] different types of defensins,[107–115] and drosomycin.[116]

POSTTRANSLATIONAL MODIFICATIONS

Antimicrobial peptides display different types of posttranslational modifications that can modify their activity in a significant way. The following are among the most frequent.

Glycosylation

Glycosylation has been described in five antimicrobial peptides from insects, all of them belonging to the proline-rich structural subgroup: diptericin,[50] drosocin,[51] formaecin,[53] lebocin,[56] and pyrrhocorricin.[60] Occurrence of glycosylation is probably underestimated; for instance, a diptericin-like peptide from *Sarcophaga peregrina*[117] reported before the first report on drosocin glycosylation[51] has not been further investigated in this respect. Only *O*-glycosylation has been described to date in insects, with the oligosaccharide chain linked to the peptide backbone through a threonine residue. Although a common requirement for *O*-glycosylation seems to be the presence of nearby proline residues, especially in positions -1 and $+3$ from the glycosylated threonine,[118] one of the oligosaccharide chains of diptericin occurs in a glycine-rich region.[50] *O*-oligosaccharide chains are short and often microheterogeneous. Glycosylations with only one (GalNAc $\rightarrow$ Thr), two (Gal $\rightarrow$ GalNAc $\rightarrow$ Thr), or three (Glc $\rightarrow$ Gal $\rightarrow$ GalNAc $\rightarrow$ Thr) glycan residues have been described.[50,51,53,56,60] As a general rule, integrity of the oligosaccharide chain is necessary for optimal antimicrobial activity. Thus, treatment of diptericin with *O*-glycosydase abolished the antibacterial activity of diptericin against most bacteria tested[50] and the antibacterial activity of a nonglycosylated synthetic drosocin is several times lower than the native compound.[56]

Aside from its important role in antimicrobial activity, little is known about other roles of glycosylation in the lethal mechanisms of the corresponding peptides, though some ideas have been advanced, such as protection against proteinases, modification of secondary structure inhibition of enzymes involved in peptidoglycan biosynthesis or specific recognition between pathogen and peptide.[50,51,53,119] It is noteworthy that glycosylated peptides do not seem to have membrane permeabilization as their main mechanism of action. Evidence for nonmembrane mechanisms are as follows: (a) Deglycosylated all-D-diptericin is much less active than the deglycosylated natural form.[120] (b) Kinetics of the lethal process is usually quite slow for membrane permeabilization; long contact periods (usually hours) between peptide and microorganism are required for pyrrhocoricin, drosocin, and formaecins to produce a bactericidal effect.[50,51,53]

Disulfide Bonds

Intramolecular disulfide bonds are relatively common in antimicrobial peptides. Structures ranging from one to five disulfides have been reported (Tables III and

Table I Linear Antimicrobial Peptides with Helical Conformation

Peptide	Sequence	Source	Reference
Andropin	VFIDILDKVNAIHNAAQVGIGFAKPFEKLINPK	Fruit fly (*Drosophila melanogaster*)	24
BLP-1	GIGASILSAGKSALKGLAKGLAEHFAN[a]	Asian toad (*Bombina orientalis*)	25
Bombinin	GIGALSAKGALKGLAKGLAEHFAN[a]	Yellow-bellied toad (*Bombina variegata*)	26
Bombolitin	IKITTMLAKLGKVLAHV[a]	Bumblebee (*Megabombus pennsylvanicus*)	27
Cecropin A	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK[a]	Silk moth (*Hyalophora cecropia*)	28
Cecropin	RWKIFKKIEKMGRNIRDGIVKAGPAIEVIGSAKAI[a]	Silk moth (*Bombyx mori*)	29
Cecropin	WNPFKELERAGQRVRDAVISAAPAVATVGQAAAIARG[a]	Tobacco moth (*Manduca sexta*)	30
Cecropin C	GWLKKLGKRIERIGQHTRDATIQGLGIAQQAANVAATARG[a]	Fruit fly (*Drosophila melanogaster*)	31
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR[a]	Pig (*Sus scrofa*)	32
Ceratotoxin A	SIGSALKKALPVAKKIGKIALPIAKAALP	Mediterranean fruit fly (*Ceratitis capitata*)	33
Clavanin A	VFQFLGKIIHHVGNFVHGFSHVF[a]	Tunicate (*Styela clava*)	34
CRAMP	ISRLAGLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE	Mouse (*Mus musculus*)	35
Dermaseptin 1	ALWKTMLKKLGTMALHAGKAALGAAANTISQGTQ	Arboreal frog (*Phyllomedusa sauvageii*)	36
Enbocin	WNYFKEIERAVARTRDAVISAGPAVATVAAATSVAS[a]	Silk moth (*Bombyx mori*)	37
FALL-39	FALLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Man (*Homo sapiens*)	38
Lycotoxin I	IWLTALKFLGKHAAKHLAKQQLSKL[a]	Wolf spider (*Lycosa carolinensis*)	39
Magainin 1	GIGKFLHSAGKFGKAFVGEIMKS	South African clawed frog (*Xenopus laevis*)	40
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ[a]	Honeybee (*Apis mellifera*)	41
Misgurin	RQRVEELSKFSKKGAAARRRK	Mudfish (*Misgurnus anguillicaudatus*)	42
PGLa	GMASKAGAIAGKIAKVALKAL[a]	South African clawed frog (*Xenopus laevis*)	43
Pleurocidin	GWGSFFKKAAHVGKHVGKAALTHYL	Winter flounder (*Pleuronectes americanus*)	44
Seminalplasmin	SDEKASPDKHHRFSLSRYAKLANRLANPKLLETFLSKWIGDRGNRSV	Ox (*Bos taurus*)	45
Styelin	GWFGKAFRSVSNFYKKHKTYIHAGLSAATLLG	Tunicate (*Styela clava*)	46

[a] C-terminally amidated sequences.

Table II Linear Antimicrobial Peptides Rich in Certain Amino Acids

Peptide	Sequence	Source	Reference
Abaecin	YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY	Honeybee (*Apis mellifera*)	47
Apidaecin IA	GNNRPVYIPQPRPPHPRI[a]	Honeybee (*Apis mellifera*)	48
Bactenecin 5	RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPPFRPPLRFP	Ox (*Bos taurus*)	49
Diptericin	DEKPKLILPT[b] PAPPNLPQLVGGGGGNRKDGFGVSVDAHQKVW TSDNGRHSIGVT[b] PGYSQHLGGPYGNSRPDYRIGAGYSYNF[a]	Black blowfly (*Phormia terranovae*)	50
Drosocin	GKPRPYSPRPT[b] SHPRPIRV	Fruit fly (*Drosophila melanogaster*)	51
Enkelytin	KRFAEPLPSEEEGES[c] YS[c] KEVPEMEKRYGGFM[a]	Ox (*Bos taurus*)	52
Formaecin 1	GRPNPVNNKPT[b] PHPRL	Australian ant (*Myrmecia gulosa*)	53
Histatin I	DS[c] HEKRHHGYRRKFHEKHHSHKEFPFYGDYGSNYLYDN	Human (*Homo sapiens*)	54
Indolicidin	ILPWKWPWWPWRR[a]	Ox (*Bos taurus*)	55
Lebocin 1	DLRFLYPRGKLPVPT[b] PPPFNPKPIYIDMGNRY	Silk moth (*Bombyx mori*)	56
Metchnikowin	HRHQGPIFDTRPSPFNPNQPRPGPIY	Fruit fly (*Drosophila melanogaster*)	57
PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFP	Pig (*Sus scrofa*)	58
Prophenin	AFPPPNVPGPR(FPPPNFPGPR)$_3$FPPPNFPGPP FPPPIFPGPW FPPPPPFRPP PFGPPRFP[a]	Pig (*Sus scrofa*)	59
Pyrrhocoricin	VDKGSYLPRPT[b] PPRPIYNRN	Sap-sucking bug (*Pyrrhocorus apterus*)	60
Tenecin	HHDGHLGGHQTGHQGGQQGGHLGGQQGGHLGGHQGGQPG DGHLGGHQGGIGGTGGQQHGQHGPGTGAGHQGGYKTHGH	Yellow mealworm (*Tenebrio molitor*)	61

[a] C-terminally amidated sequences.
[b] Glycosylated Thr.
[c] Phosphoserine.

Table III Antimicrobial Peptides with a Single Cyclic Disulfide

Peptide	Sequence	Source	Reference
Bovine dodecapeptide	RLCRIVVIRVCR	Ox (*Bos taurus*)	62
Brevinin-1	FLPVLAGIAAKVVPALFCKITKC	Japanese frog (*Rana brevipoda porsa*)	63
Brevinin-1E	FLPLLAGLAANFLPKIFCKITRKC	European frog (*Rana esculenta*)	64
Esculentin	GIFSKLGRKKIKNLLISGLKNVGKEVGMDVVRTGIDIAGCKIKGEC	European frog (*Rana esculenta*)	64
Pipinin	FLPOOAGVAAKVFPKIFCAISKKC	Common frog (*Rana pipiens*)	63
Ranalexin	FLGGLIKIVPAMICAVTKKC	Bullfrog (*Rana catesbeiana*)	65
Thanatin	GSKKPVPIIYCNRRTGKCQRM	Hemipteran (*Podisus maculiventris*)	66
11-kD polypeptide[a]	(GLRKKFRKTRKRIQKLGRKIGKTGRKVWKAWREYGQIPYPCRI)$_2$	Guinea pig (*Cavia porcellus*)	67

[a] Homodimer, not cyclic disulfide (only structure of this type reported so far).

IV). As with other postranslational modifications, they do not have a unique function, and in some cases exhibit opposite effects, depending on the peptide and activity assayed. In some instances, reduction of disulfide bonds in native peptides or replacement of cysteine in synthetic analogues shows little or no effect on activity,[121,122] whereas the same process abrogates almost completely the activity of other structures.[123,124] Even for related peptides, sharp contrasts can be observed. Thus, reduction cancels the channel-forming activity of insect defensins on *Micrococcus luteus*[125] and the antibacterial activity of sapecin,[126] but not liposome permeabilization by mammalian defensins.[127] Reduction of both rabbit and human defensins modifies (but does not impair) the mechanism of permeabilization, from an all-or-none mechanism for the native peptides to a gradual leakage in the reduced forms.[128] Several studies on the relevance of disulfide bonds have been performed on tachyplesins and protegrins, a group of related peptides with predominantly β-sheet structures. While native tachyplesins act upon membranes by channel formation, substantial permeabilization by a detergent-like mechanism is observed for the reduced peptide, which is less antibacterial but still active.[129,130] Protegrins, in turn, require both disulfide bonds for channel formation but not for antibacterial activity; linear analogues with either free or acetylated thiol groups are less active on gram-negative but maintain lethal activity against gram-positive bacteria.[131]

The importance of disulfide bonds for peptide activity and stability is also relevant vis-à-vis the industrial production of antimicrobial peptides. If folding into native-like disulfide pairing can not be achieved spontaneously, either regioselective disulfide formation[132,133] or production by recombinant technologies must be applied.[134] Representative examples of antimicrobial peptides with correct disulfide pattern and full biological activity prepared by chemical synthesis are protegrins,[105,135] α-defensins,[136] androctonin,[68] rat cortistatin,[137] tachyplesin,[129] and thanatin.[66] Among those obtained by recombinant techniques are insect defensin,[112,138] drosomycin,[139] and α-[140] and β-defensins.[141,142]

Amidation

Perhaps the most common posttranslational modification of antimicrobial peptides, amidation occurs in a wide variety of peptides, such as melittin,[143] cecropins,[144] dermaseptins,[36] PGLa,[145] clavanin,[34] PR-39,[58] apidaecins,[48] diptericin,[50] prophenin,[59] polyphemusins,[69] or penaeidins.[146] The process in-

Table IV Antimicrobial Peptides with Several Internal Disulfides

Peptide	Sequence[a]	Cys Pairings	Source	Reference
A. Two disulfide bonds				
Androctonin	RSVCRQIKICRRRGGCYYKCTNRPY	1–4, 2–3	Scorpion (*Androctonus australis*)	68
Polyphemusin I	RRWCFRVCYRGFCYRKCR[a]	1–4, 2–3	Horseshoe crab (*Limulus polyphemus*)	69
Protegrin I	RGGRLCYCRRRFCVCVGR	1–4, 2–3	Pig (*Sus scrofa*)	70
B. Three disulfide bonds: The α-defensin family				
Cryptdin 1	LRDLVCYCRSRGCKGRERMNGTCRKGHLLYTLCCR	1–6, 2–4, 3–5	Mouse (*Mus musculus*)	71
Cryptdin 5	LSKKLICYCRIRGCKRRERVFGTCRNLFLTFVFCC	1–6, 2–4, 3–5	Mouse (*Mus musculus*)	72
HNP-1 (α-defensin)	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	1–6, 2–4, 3–5	Man (*Homo sapiens*)	73
NP-1 (α-defensin)	VVCACRRALCLPRERRAGFACRIRGRIHPLCCRR	1–6, 2–4, 3–5	Rabbit (*Oryctolagus cuniculus*)	74
RK-1	MPCSCKKYCDPWEVIDGSCGLFNSKYICCREK	1–6, 2–4, 3–5	Rabbit (*Oryctolagus cuniculus*)	75
C. Three disulfide bonds: The β-defensin family				
β-Defensin-1	DFASCHTNGGICLPNRCPGHMIQIGCIFRPRVKCCRSW	1–5, 2–4, 3–6	Ox (*Bos taurus*)	76
Big defensin	NPLIPAIYIGATVGPSVWAYLVALVGAAAVTAANIRRASSD NHSCAGNRGWCRSKCFRHEYVDTYYSAVCGRYFCCRSR	1–5, 2–4, 3–6	Horseshoecrab (*Limulus polyphemus*)	77
Gallinacin 1	GRKSDCFRKSGFCAFLKCPSLTLISGKCSRFYLCCKRIW	1–5, 2–4, 3–6	Chicken (*Gallus gallus*)	78
LAP	GFTQGVRNSQSCRRNKGICVPIRCPGSMRQIGTCLGAQVKCCRRK	1–5, 2–4, 3–6	Ox (*Bos taurus*)	79
TAP	NPVSCVRNKGICVPIRCPGSMKQIGTCVGRAVKCCRKK	1–5, 2–4, 3–6	Ox (*Bos taurus*)	80
D. Three disulfide bonds: Insect defensins				
Defensin	GFGCPLDQMQCHRHCQTITGRSGGYCSGPLKLTCTCYR	1–4, 2–5, 3–6	Dragonfly (*Aeschna cyanea*)	81
Defensin 4K	GFGCPLNQGACHRHCRSIRRRGGYCAGFFKQTCTCYRN	1–4, 2–5, 3–6	Scorpion (*Leiurus quinquestriatus*)	82
Formicin A	ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKGVCVCRN	1–4, 2–5, 3–6	Blowfly (*Phormia terranovae*)	83
Royalisin	VTCDLLSKFGQVNDSACAANCLSLGKAGGHCEKGVCICRK TSFKDLWDKYF	1–4, 2–5, 3–6	Royal jelly (*Apis mellifera*)	84
Sapecin	LTCEIDRSLCLLHCRLKGYLRAYCSQQKVCRCVQ	1–4, 2–5, 3–6	Flesh fly (*Sarcophaga peregrina*)	85
E. More than three disulfide bonds				
Drosomycin	DCLSGRYKGPCAVWDNETCRRVCKEEGRSSGHCSPSLKCWCEGC	1–8, 2–5, 3–6, 4–7	Fruit fly (*Drosophila melanogaster*)	86
ASABF	AVDFSSCARMDVPGLSKVAQGLCISSCKFQNCGTGHCEK RGGRPTCVCDRCGRGGGEWPSVPMPKGRSSRGRRHS	Unknown	Roundworm (*Ascaris suum*)	87
Tachycitin	YLAFRCGRYSPCLDDGPNVNLYSCCSFYNCHKCLARLENCPK GLHYNAYLKVCDWPSKAGCTSVNKECHLWKTGRK	1–6, 2–5, 3–9, 4–10, 7–8	Japanese horseshoe crab (*Tachypleus tridentatus*)	88

volves oxidative decarboxylation of an additional C-terminal glycine residue, in a two-step enzymatic process.[147] Amidation prevents cleavage by carboxypeptidases and provides an extra hydrogen bond for the formation of α-helices. The correlation between amidation and biological activity is not clear: the amidated and nonamidated forms do not differ substantially in a number of cases[135,148,149]; in other cases, however, activity is significantly impaired.[145,150] In several cases, synthetic C-terminally amidated analogues of peptides whose native forms are not amidated show increased antimicrobial activity.[151,152]

Halogenation

Bromination of the indole ring of tryptophan has been described in antibiotic peptides isolated from the hagfish *Mixina glutinosa.*[153] Mass spectral data also seem to support the presence of chlorine in misgurin from the mudfish *Misgurnus anguillicaudatus.*[154] The role of the halide atom is unclear. Whereas in the *Mixina* peptides it broadens the range of susceptible organisms, in misgurin the antibacterial activity of the synthetic analogue devoid of halogen is undistinguishable from the natural peptide.

D-Amino Acids

The occurrence of D-amino acids in eukaryotic peptides has been reported in several cases.[155,156] In antimicrobial peptides, the only examples of this modification are bombinins from the frog *Bombina variegata*,[157] which have D-alloisoleucine at position 2. Since no variation in the genetic code or codon use was observed for this residue, its presence was attributed to posttranslational epimerization of the corresponding L-Ile peptide, which is also present in the organism and does not show differences in antibiotic activity. A likely role is protection against aminopeptidases.

Other Modifications

Phosphorylation has been described for histatins,[54] although absence of phosphate does not preclude candidacidal activity.[158] Chromacin, a fragment from chromogranin A, requires both *O*-glycosylation and tyrosine phosphorylation for full antibiotic activity; the synthetic nonmodified peptide is completely inactive.[119] Enkelytin, an antibacterial peptide derived from proenkephalin A (209–237),[52] has two phosphoserines and an oxidized methionine required for activity. Other described modifications are hydroxylysine in cecropin B from silkworm[83] or methylated tyrosine in clavanins.[70]

MODE OF ACTION OF ANTIMICROBIAL PEPTIDES

Membrane Permeabilization

Studies on both live organisms and model membranes have indicated that most antimicrobial peptides provoke an increase in plasma membrane permeability. A direct correlation between antibiotic effect and permeabilization ability has been found for defensins,[82,128] magainins,[159–161] cecropins,[162–167] bactenecins,[168] or dermaseptins.[36,169]

A first step in the mechanism of action is the electrostatic interaction between the cationic peptide and the negatively charged components of the membrane of the pathogen; hence, an increase in positive charge of the peptide will increase microbicidal activity. A direct correlation between cationic character and activity has been established for magainin analogues[130,170–172] and for cecropins, where the less cationic cecropin D also shows the lowest microbicidal activity.[173] Similar correlations have been established for the interaction of cecropin A–melittin hybrids with either model membranes[91] or anionic lipopolisaccharides,[174] for cryptdins[175] or for rabbit defensins.[176] The correlation between charge and activity is less evident in other cases, such as rat defensins lacking one Arg residue but having the same antibacterial activity[177] or N-terminally acetylated cecropin A-melittin hybrids.[178] On the other hand, many positive charges can lead to a loss of activity. For instance, decreased activity of a highly cationic magainin analogue[130] has been attributed to either destabilization of the pore due to increased repulsion among peptide monomers, or to strong peptide association with the anionic lipids, which favors fast translocation into the inner leaflet of the membrane. The positive charges also influence specificity of the peptide toward the target membrane; variation in only one charge can lead to dramatic differences in hemolytic and antibacterial properties in pardaxin[179] and indolicidin[180] analogues, or in a seminalplasmin fragment where the Glu for Lys-5 replacement considerably increases microbicidal but not hemolytic activity.[181] In contrast, a more positively charged C-terminus of cecropin B decreases microbicidal but increases tumoricidal effects.[182]

While most antibiotic peptides described in the literature are strongly cationic, a few examples of anionic peptides have been reported. Examples in-

clude polyAsp-containing fragments from the ovine pulmonary surfactant[183] and propieces of ovine trypsinogen and PYLa frog activation peptide.[184] In these cases, the minimal inhibitory concentrations (MICs) against common bacterial strains are much higher than those of typical cationic peptides. On the other hand, Glu-rich enkelytin[52] shows activity in the submicromolar range against gram-positive organisms. Electron micrographs of bacteria exposed to peptide do not show the typical alterations observed for cationic peptides, suggesting a different mode of action.

An interesting group of antimicrobial peptides are those rich in His, whose net charge is pH dependent within the physiological range. Thus clavanins[34] from tunicates and histatins from saliva[54] show stronger activity at low pHs; the latter case is particularly relevant in view of the pH decrease caused by cariogenic pathogens.[185] Studies with histatin analogues[186–188] stress the importance of His residues, though the correlation with charge is not fully clarified.

The antimicrobial activity is also affected by membrane characteristics such as phospholipid composition, sterol content, membrane potential, or the presence of polyanions (e.g., LPS, sialic acid residues). For instance, an *Escherichia coli* mutant lacking in cardiolipin is more resistant to sapecin than the wild type.[189] Similarly, resistance of *Serratia* to cecropin A has been related to lower levels of acidic phospholipids, closer to those of higher eukaryotes.[190] Also, the increased susceptibility of tumoral cells to some antibiotic peptides has been ascribed to a higher exposure of phosphatidylserine residues.[191] Manipulation of the cholesterol content of erythrocytes resulted in a reverse relationship between sterol levels and peptide susceptibility.[160] Similar results have been observed for cecropins and artificial membranes.[150]

Furthermore, the energetic status (membrane potential) of the microorganism contributes to the final outcome of the peptide–pathogen interaction. Thus, depolarized cells tend to be less susceptible to the action of antibiotic peptides.[192,193] Finally, another relevant but controversial aspect of the mechanism of action is the position of the peptide relative to the membrane, which bears directly on the permeabilization mechanism. Two extreme situations, described respectively as the barrel-stave and carpet models, have been proposed.[194] This question is discussed in detail elsewhere in this issue.

Other Mechanisms

There is substantial evidence that antimicrobial peptides exert their activities through mechanisms other than membrane permeabilization, though it is not easy to differentiate those other activities from secondary events arising from membrane permeabilization. Several findings point toward this direction. For instance, some peptides have enantio or retronenantio versions significantly less active upon certain organisms than the all-L forms,[47,57,66,120,195] in contrast with the more general observation of all-D analogues being either equally or more active (due to proteolytic resistance).[164,170,196,197]

Several pathways alternative to membrane permeabilization have been proposed, including inhibition of synthesis of specific membrane proteins by attacins or gloverin,[198,199] synthesis of stress proteins,[200] arrest of DNA synthesis by PR-39,[201] breakage of single-strand DNA by defensins,[202] interaction with DNA (without arrest of synthesis) by buforins,[203] or production of hydrogen peroxide.[204] Antimicrobial peptides can also act by triggering self-destructive mechanisms such as apoptosis in eukaryotic cells or autolysis in bacterial targets. Antimicrobial peptide-induced apoptosis has been described for lactoferricin[205] and the cecropin-melittin hybrid CA(1–8)M(1–18).[206] Autolysis, based on activation of amidases that degrade the peptidoglycan, has been observed on bacteria exposed to lantibiotics such as nisin and pep5.[207]

Antimicrobial peptides are also known to act as inbibitors of enzymes produced by pathogenic organisms, either by serving as pseudo-substrates or by tight binding to the active site that disturbs the access of substrate. Thus, histatins at the submicromolar range are capable of inhibiting a trypsin-like proteinase from the oral bacteria *Bacteroides gingivalis*,[208] and equine peptide eNAP-2[209] inhibits other microbial serine proteases. Alternatively, antimicrobial peptides can serve as a control for proteinases involved in inflammatory processes, such as the inhibition of furin by histatin 3,[210] which has close sequence homology with the prepropeptide, or proBac5 from bovine neutrophils,[211] which inhibits cathepsin L. That the recognition mechanism is due to the peptide sequence and/or conformation is demonstrated by the specificity of the proteinases and the inactivity of the enantiomers.[210] Inhibition of thrombin by defensins also helps in the contention of the pathogens, and consequently of the inflammatory process.[212,213] In contrast, upregulation of the inflammatory process is achieved by induction of IL-8 by defensins.[214] Other activities described so far for antimicrobial peptides include chemotaxis,[215,216] induction of syndecan synthesis,[217] histamine release,[218–220] and inhibition of steroidogenesis.[221,222]

MECHANISMS OF RESISTANCE TO ANTIMICROBIAL PEPTIDES

Inactivation and resistance are essential issues for both the understanding of action mechanisms and the potential therapeutic application of antimicrobial peptides. These phenomena have been largely documented on bacterial models through mutant generation and genetic rescue. In many cases, resistance is associated with virulence genes (reviewed in Ref. 223), though not always.[224] The following mechanisms can be distinguished.

Inactivation by the Incubation Media

Serum and its components have been described as inhibitors for different antimicrobial peptides, including LL-37[225] and defensin.[226] Inhibition of defensins by bovine serum albumin,[227] α_2-macroglobulins,[228] serpins,[229] and complement factor C1q[230] has been reported, though the latter finding is disputed.[231] Activated low density lipoproteins are the main inactivator for amphipathic cytolytic peptides C18G and 399.[232] Inactivation of the antimicrobial activity of lactoferricin in cow's milk has been reported.[233]

Inactivation by Oligosaccharide Barriers

Before reaching their final targets, antimicrobial peptides must cross barriers such as bacterial peptidoglycan (discussed below), extracellular matrix in eukaryotic cells, or anionic oligosaccharides bound to different membrane components. Heparin, a major component of the extracellular matrix, inhibits the tumoricidal activity of defensins[226] and the permeabilization activity of cecropin A-melittin hybrid peptides.[227,228] In the promastigote form of *Leishmania,* a parasitic protozoan, the plasma membrane contains a glycocalix formed by anionic lipophosphoglycan, which affords partial resistance against these peptides.[228] In erythrocytes, sialic acid residues from membrane glycoproteins induce minor resistance to magainin[160] or melittin; in the latter case resistance increases dramatically by certain substitutions.[236]

Inactivation by Bacterial Outer Membrane

In gram-negative bacteria, the main component of the outer leaflet is LPS (reviewed in Refs. 237–239). Any active peptide not recognized by peptide transporters must cross the outer membrane by the so-called self-promoting pathway (a term coined[238] to explain the bactericidal activity of polymixin), which consists of the displacement of divalent cations that keep LPS together, thus allowing passage of external molecules. The phenomenon has been observed for peptides such as indolicidin and analogues,[180] gloverin,[199] α-defensins,[240] bactenecin,[241] cecropin B,[242] or cecropin A-melittin hybrids.[174,243] Such studies have focused on either the microbicidal activity of the peptides, or on permeabilization to (otherwise excluded) fluorescent probes, hydrophobic antibiotics, or substrates for periplasmic enzymes. As peptide recognition by LPS takes place mainly by electrostatic interaction between cationic residues and phosphate groups, respectively, inactivation at the outer membrane level can be originated by either absence of LPS, such as in bactenecin-resistant *Borrelia burgdorferi*[244] or by decrease in overall negative charge, such as resulting from esterification of phosphate groups. This latter phenomenon takes place in naturally resistant bacteria such as *Proteus*[245] or *Burkholderia.*[246,247] Although LPS is the main constituent, other outer membrane components in gram-negative bacteria can play as well a role in this interaction, but their importance relative to LPS is not conclusively established. Thus, pathogenic strains of *Yersinia* are resistant to defensins[248] and to polyLys, polyOrn, cecropin P1, melittin, and polymixin B.[249] A role for the protein YadA, a main component of the outer membrane, was suggested by the former authors, but not found for the set of peptides tested by the second group.

Proteolysis of the Peptide

This perhaps most obvious of inactivation processes has important bearing on the practical application of antibiotic peptides. The high content in basic residues favors degradation by trypsin-like proteinases. However, pinpointing a given enzyme as responsible for a resistance mechanism is more difficult. Specific cecropin-degrading enzymes produced by pathogens such as *Bacillus larvae* in honeybees[250] and entomopathogenic strains of *Pseudomonas aeruginosa*[251] have been described. Virulence of the pathogenic strains correlates with the level of these proteinases. In *Salmonella,* a protein has been identified[223] with activity similar to magaininase, a metalloproteinase from the amphibian skin known to be involved in the degradation of magainins as well as other antibacterial peptides.[252]

Resistance by Plasma Membrane Components

Several membrane elements, both passive and active, have been described as contributing to resistance.

Lipid Composition. Lower levels of anionic phospholipids are associated to bacterial resistance, either in natural species such as *Serratia*,[253] or in spheroplasts from different gram-negative bacteria.[254] Higher activity of sapecin on *S. aureus* than on *E. coli* has been linked to cardiolipin content, since a mutant defficient in its biosynthesis is more resistant.[255] Similarly, treatment of erythrocytes with phospholipase D, which raises the phosphatidic acid content, is associated with higher susceptibility to antimicrobial peptides.[160]

Membrane Potential. Membrane potential has been associated with a higher permeabilization by antimicrobial peptides in both bacteria and model membranes. The amphipathic peptide H9c2[256] shows higher activity on rat embryo myoblasts depending on the cell cycle, in direct correlation to the variation in membrane potential. Similarly, hyperpolarization of erythrocytes increased their susceptibility to magainin but not to melittin[160] and collapse of the potassium gradient in *Leishmania* decreased the activity of cecropin A-melittin hybrids.[235] The *unc*A *E. coli* mutant, which relies on glycolysis rather than on oxidative phosphorylation for viability, and has therefore a lower membrane potential, is more resistant to sarcotoxin I.[257,258]

Reduction of Disulfide Bonds. Inactivation of NK-lysin is performed by human but not *E. coli* thioredoxin reductase; it was suggested that this system is located at the plasma membrane and partially responsible for the incomplete killing by this peptide.[124] Related forms of inactivation would be disulfide exchange, as described for thionins,[259] or binding of defensins to the activated thiol group of α-2 macroglobulin.[228]

Peptide Influx/Efflux Systems. Some transmembrane transport complexes, both in animal[260] and plant[261] pathogens, have been shown to be involved in peptide resistance, operating as intake systems that facilitate intracellular peptide degradation. In contrast, resistance to protegrins and LL-37 in *Neisseria gonorrhoeae* seems to be based on mtr, an efflux peptide system[262] working by mechanisms similar to other pumps involved in drug multiresistance. However, mdr1-transfected BRO melanoma cells, which show multiresistance to a variety of antitumoral drugs, do not decrease their susceptibility to magainin 2.[263] Likewise, melittin is not a substrate for protein P, from the same group of efflux pumps, though other peptides such as gramicidin and valinomycin are.[264,265]

FUNCTION AND PATHOLOGY OF ANTIMICROBIAL PEPTIDES

The variety of defensive mechanisms developed by multicellular organisms in nonspecific immunity raises questions on the role of antibiotic peptides as a deterrent against infection. In insects, specially in the *Drosophila* model, such a role was clearly demonstrated by the study of mutants lacking some of the induction pathways for the antibiotic peptide genes (reviewed in Refs. 3,5,6,19, and 266). For more evolved insects, it was clearly shown that mutations in the different signaling pathways of antimicrobial peptides rendered the organism very susceptible to infection.[267–269] In organisms endowed with highly developed antigen-specific immunity, the role of antibiotic peptides has been only partially ascertained; still, an increasing body of experimental evidence has accumulated during the last years.

Antibiotic Peptides in Infection and Inflammation Processes

Increased levels of antibiotic peptides have been reported for several animal and human infections: for α-defensin in septicemia and bacterial meningitis,[270] for β-defensins in *Mycobacterium, Pasteurella*, or *Cryptosporidium* infections,[271,272] for PR-39 in salmonellosis,[273] for a variety of peptides in blisters and wound fluid,[274] or for lingual antibacterial peptide (LAP) in injured tongue.[79] Inflammatory situations or stimuli are also associated with induction of antibiotic peptides such as LL-37[274] TAP or LAP.[275,276] Levels of NK-lysin, a tumoricidal and antibacterial peptide, increase in NK cells or cytotoxic lymphocytes by stimulation with IL-2.[277]

Absence or Inactivity of Peptides

Depleted levels of antibiotic peptides are associated to several pathologies. Thus, patients of specific granule-deficiency syndrome, completely lacking in α-defensins,[278] suffer from frequent and severe bacterial infections. Low levels of histatins from saliva in a group of HIV patients correlated with a higher incidence of oral candidiasis[279] and fungal infection.[280] Perhaps the most compelling illustration of the implication of antimicrobial peptides in human pathology comes from cystic fibrosis, a genetic disease associated with recurrent bacterial infections of the airways. The defective chloride channel causing the disease increases salinity of the alveolar fluid, and thus impairs the bactericidal activity of β-defensins, which

are salt sensitive.[281,282] In diabetic patients, an interesting theory on defensin inactivation proposes binding of the peptide to advanced glycation end products.[283]

Pathology Caused by Antibiotic Peptides

As part of the defense reactions or inflammatory stimuli, neutrophils discharge their granule contents, releasing defensins that can damage surrounding tissues and cells. Damage to airway epithelia due to release of α-defensins from neutrophils in both cystic fibrosis[284] and respiratory distress syndromes[285] has been described. Localization of α-defensins in arterial wall vessels[286] can contribute to the inflammation process as well as to the formation of atherosclerotic plaque by favoring lipoprotein deposition in the vessel.[287]

ANTIBIOTIC PEPTIDES IN CLINICS

A great deal of work has been invested in recent years in localizing new antibiotic peptide sequences and improving their potency and selectivity, with the goal of expanding and/or refining resources against infection in an era of antibiotic resistance. These efforts may appear at first sight unrewarded, in view of the scarce examples of antibiotic peptides at advanced phases of clinical approval. Such a dismissal might be premature, however, especially considering the relative infancy of most research in the field. At present, advances in production (synthetic and recombinant) and development of antibiotic peptides are under way, clearing the way for cost-effective use. Therapeutical applications of antibiotic peptides have been largely envisaged in the treatment of bacterial[288] or viral[289] infections, and cancer.[290] While it must be admitted that most applications so far are confined to treatment of local infections, promising results may be forthcoming. A first step in this direction is a magainin analogue, termed pexiganan acetate, which has obtained approval for the treatment of diabetic foot ulcers.[291] Other fields where work is advancing are the following.

Buco-Dental Infections

Natural defenses of the oral cavity are based on the His-rich histatins from saliva, with a strong candidacidal activity,[185] and on antibiotic peptides from the epithelia such as LAP[79] or from neutrophils in the periodontium.[292] Many facultative oral gram-negative bacteria are killed by human defensins[293]; others such as *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens* are resistant, though not for rabbit NP-1 or the D forms of peptides such as protegrins.[294,295] Histatins can be adsorbed into polyacrylic material to reduce *Candida* adhesion to the denture.[296] C-terminal analogues of histatin C show good activity against oral pathogens other than *Candida.*[188] An additional effect of histatins is the inhibition of a proteinase from *Bacteroides gingivalis.*[208] In vivo efficacy of histatins has been assessed in an experimental gingivitis model in dogs.[297] A promising development in antimicrobial peptide-based gene therapy is the production of histatin 3 by infection with a histatin recombinant adenovirus, active on *Candida* strains.[298]

Ocular Infections

Antibiotic peptides such as rabbit (defensin) NP-1,[299] magainins,[300] cecropin-derived Shiva 11,[301] or cecropin D5C[302] have been proposed as preserving media for cornea storage,[303] contact lens disinfectants,[300,302] or ocular antiseptics. In vivo experiments have been performed with cecropin A–melittin hybrids on rabbits infected with *Pseudomonas aeruginosa;* the peptides were as effective as gentamycin in the clearance of the infection.[304]

Spermicidal Agents

The activity of magainins and their analogues on spermatozoids,[305,306] causing morphological and functional alterations led to propose them as contraceptive agents. The broad-range activity of protegrins against several sexually transmitted pathogens, including HIV virus, has suggested a possible combination of antibiotic and contraceptive activities.[307]

Antitumoral Activity

A number of studies have shown tumoral cells to be more susceptible to antibiotic peptides than their nontransformed counterparts. The basis for this difference is not fully clarified; changes in membrane potential due to higher metabolism,[308] higher exposure of acidic phospholipids in the outer leaflet of membrane,[191] or cytoskeleton alteration and possible alterations in the extracellular matrix[309] have been proposed as potentially implied in the process. In *ras*-transformed cells more susceptible to melittin action, a Ca^{2+} influx induced by hyperactivation of phospholipase A2 was proposed.[310,311] Preferential activity toward transformed cells has been described for cecropins and analogues,[182,309,312] magainin 2 and analogues,[313] cecropin A–magainin 2 and cecropin A–melittin hybrids,[314] and analogues derived from

human platelet factor.[315] Activity of magainin analogues was unchanged in a panel of human cancer cell lines with a broad range of susceptibility toward typical antitumor drugs,[316] or when a human melanoma cell line was transfected with the drug efflux transporter mdr1 that provides high resistance against usual antitumor agents.[263] As usual, extrapolation to an in vivo situation is hampered by partial inactivation by serum components[315] or by the increase in proteolytic activity of tumoral cells, a case where all-D analogues were shown to be quite successful.[290] Examples of the efficacy of antibiotic peptides against murine tumors include the complete cure of an induced melanoma in athymic nude mice with a single injection of all-D MSI-511 magainin analogue[290] or the 100% lifespan increase in mice with induced ascites and spontaneous ovarian tumors by intraperitoneal treatment with the magainin analogue MSI-238.[313] Colateral approaches tested in vitro for cancer therapy include the targetting of thionin conjugated to a monoclonal antibody toward lymphoma-causing CD5+ lymphocytes,[317] or the reversion and/or attenuation of the transformed phenotype by the internal expression of either cecropin or melittin inside tumoral cells.[318]

Antiviral Activity

Antibiotic peptides have been described to act upon viruses at three levels. Direct action related to peptide binding to the viral particle has been assessed on herpes virus for α-defensins,[227,319] modelin-1, an amphipathic model peptide,[289] and Hecate, a melittin analogue[320]; on HIV virus for polyphemusins and their analogues,[321]; and on viral stomatitis virus by tachyplesin I.[322]

Secondly, inhibition of virion production has been proposed to account for antiviral activity of melittin or cecropin A[323,324] on HIV. Virus production was arrested at sublethal peptide concentrations either with the peptide added externally or produced interacellularly by transfection with an expression vector.

Finally, mimicry of viral infective processes is a third mechanism by which antimicrobial peptides exert antiviral activity. Thus, melittin and its subK7I analogue, lacking antibiotic activity, inhibit infectivity of the tobacco mosaic virus by perturbing its assembly due to the similarity of melittin with a virus capsid region involved in RNA interaction.[325] Another interesting example is the T22 [Tyr,[5,12] Lys[7]] analogue of polyphemusin, with EC_{50} in the nanomolar range against HIV[326] (even AZT-resistant strains). The peptide, which requires intact disulfide bridges and Zn^{2+} for optimal activity,[321,327] seems to act through a nonmembrane-related mechanism, since its all-D version is 20-fold less active.[328] Though the molecular basis for T22 activity is not fully understood, it is known to bind gp120 and CD4 molecules[329–331] and thus to block virus-cell fusion; it also competes with the virus for the coreceptor of chemokines CXCR4 and fusin.[332] Recently, less cytotoxic (i.e., less cationic) analogs of this promising peptide have been developed.[333]

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REFERENCES

1. Boman, H. G. Annu Rev Immunol 1995, 13, 61–92.
2. Boman, H. G. Scand J Immunol 1996, 43, 475–482.
3. Boman, H. G. Scand J Immunol 1998, 48, 15–25.
4. Ganz, T.; Lehrer, R. I. Curr Opin Hematol 1997, 4, 53–58.
5. Hoffmann, J. A. Curr Op Immunol 1995, 7, 4–10.
6. Hoffmann, J. A.; Reichart, J. M. Trends Cell Biol 1997, 7, 309–316.
7. Fernández de Caleya, R.; González-Pascual, B.; García-Olmedo, F.; Carbonero, P. Appl Microbiol 1972, 23, 998–1000.
8. Hancock, R. E.; Lehrer, R. I. Trends Biotechnol 1998, 16, 82–88.
9. Hancock, R. E. W.; Falla, T.; Brown, M. Adv Microb Physiol 1995, 37, 135–175.
10. Harwig, S. S.; Eisenhauer, P. B.; Chen, N. P.; Lehrer, R. I. Adv Exp Med Biol 1995, 371A, 251–255.
11. Barra, D.; Simmaco, M. Trends Biotech 1995, 13, 205–209.
12. Maloy, W. L.; Kari, U. P. Biopolymers 1995, 37, 105–122.
13. Nicolas, P.; Mor, A. Annu Rev Microbiol 1995, 49, 277–304.
14. Brokaert, W. F.; Cammue, B. P. A.; De Bolle, M. F. C.; Thevissen, K.; De Samblanx, G. W.; Osborn, R. W. Crit Rev Plant Sci 1997, 16, 297–323.
15. White, S. H.; Wimley, W. C.; Selsted, M. E. Curr Opin Struct Biol 1995, 4, 521–527.
16. Lehrer, R. I.; Ganz, T. Ann NY Acad Sci 1996, 797, 228–239.
17. Levy, O. Eur J Haematol 1996, 56, 263–277.
18. Blondelle, S. E.; Pérez-Payá, E.; Houghten, R. A. Antimicrob Agents Chemother 1996, 40, 1067–1071.
19. Meister, M.; Lemaitre, B.; Hoffmann, J. A. Bioessays 1997, 19, 1019–1026.
20. Ganz, T.; Lehrer, R. I. Curr Opin Hematol 1997, 4, 53–58.

21. Iwanaga, S.; Kawabata, S.; Muta, T. J Biochem (Tokyo) 1998, 123, 1–15.
22. Ganz, T.; Lehrer, R. I. Curr Opin Immunol 1998, 10, 41–44.
23. Barra, D.; Simmaco, M.; Boman, H. G. FEBS Lett 1998, 430, 130–134.
24. Samakovlis, C.; Kylsten, P.; Kimbrell, D. A.; Engström, A.; Hultmark, D. EMBO J 1991, 10, 163–169.
25. Gibson, B. W.; Tang, D. Z.; Mandrell, R.; Kelly, M.; Spindel, E. R. J Biol Chem 1991, 266, 23103–23111.
26. Csordas, A.; Michl, H. Monatsh Chem 1970, 101, 182–189.
27. Argiolas, A.; Pisano, J. J. J Biol Chem 1985, 260, 1437–1444.
28. Steiner, H.; Hultmark, D.; Engström, Å.; Bennich, H.; Boman, H. G. Nature 1981, 292, 246–248.
29. Teshima, T.; Ueky, Y.; Nakai, T.; Shiba, T. Tetrahedron 1986, 42, 829–834.
30. Dickinson, L.; Russell, V.; Dunn, P. E. J Biol Chem 1988, 263, 19424–19429.
31. Tryselius, Y.; Samakovlis, C.; Kimbrell, D. A.; Hultmark, D. Eur J Biochem 1992, 204, 395–399.
32. Lee, J.-Y.; Boman, A.; Sun, C.; Andersson, M.; Jörnvall, H.; Mutt, V.; Boman, H. G. Proc Natl Acad Sci USA 1989, 86, 9159–9162.
33. Marchini, D.; Giordano, P. C.; Amons, R.; Bernini, L. F.; Dallai, R. Insect Biochem Mol Biol 1993, 23, 591–598.
34. Lee, I. H.; Zhao, C.; Cho, Y.; Harwig, S. S.; Cooper, E. L.; Lehrer, R. I. FEBS Lett 1997, 400, 158–162.
35. Gallo, R. L.; Kim, K. J.; Bernfield, M.; Kozak, C. A.; Zanetti, M.; Merluzzi, L.; Gennaro, R. J Biol Chem 1997, 272, 13088–13093.
36. Mor, A.; Nguyen, V. H.; Delfour, A.; Migliore-Samour, D.; Nicolas, P. Biochemistry 1991, 30, 8824–8830.
37. Kim, S. H.; Park, B. S.; Yun, E. Y.; Je, Y. H.; Woo, S. D.; Kang, S. W.; Kim, K. Y.; Kang, S. K. Biochem Biophys Res Commun 1998, 246, 388–392.
38. Agerberth, B.; Gunne, H.; Odeberg, J.; Kogner, P.; Boman, H. G.; Gudmundsson, G. Proc Natl Acad Sci USA 1995, 92, 195–199.
39. Yan, L.; Adams, M. E. J Biol Chem 1998, 273, 2059–2066.
40. Zasloff, M. Proc Natl Acad Sci USA 1987, 84, 5449–5453.
41. Habermann, E. Science 1972, 177, 314–322.
42. Park, C. B.; Lee, J. H.; Park, I. Y.; Kim, M. S.; Kim, S. C. FEBS Lett 1997, 411, 173–178.
43. Andreu, D.; Aschauer, H.; Kreil, G.; Merrifield, R. B. Eur J Biochem 1985, 149, 531–535.
44. Cole, A. M.; Weiss, P.; Diamond, G. J Biol Chem 1997, 272, 12008–12013.
45. Reddy, E. S. P.; Bhargava, P. M. Nature 1979, 279, 725–728.
46. Zhao, C.; Liaw, L.; Lee, I. H.; Lehrer, R. I. FEBS Lett 1997, 412, 144–148.
47. Casteels, P.; Ampe, C.; Riviere, L.; Damme, J. V.; Elicone, C.; Fleming, M.; Jacobs, F.; Tempst, P. Eur J Biochem 1990, 187, 381–386.
48. Casteels, P.; Ampe, C.; Jacobs, F.; Vaeck, M.; Tempst, P. EMBO J 1989, 8, 2387–2391.
49. Frank, R. W.; Gennaro, R.; Schneider, K.; Przybylski, M.; Romeo, D. J Biol Chem 1990, 265, 18871–18874.
50. Bulet, P.; Hegy, G.; Van Dorsselaer, A.; Hoffmann, J. A.; Hetru, C. Biochemistry 1995, 34, 7394–7400.
51. Bulet, P.; Dimarcq, J. L.; Hetru, C.; Lagueux, M.; Charlet, M.; Hegy, G.; Van Dorsselaer, A.; Hoffmann, J. A. J Biol Chem 1993, 268, 14983–14987.
52. Goumon, Y.; Strub, J. M.; Moniatte, M.; Nullans, G.; Poteur, L.; Hubert, P.; Van Dorsselaer, A.; Aunis, D.; Metz-Boutigue, M. H. Eur J Biochem 1996, 235, 516–525.
53. Mackintosh, J. A.; Veal, D. A.; Beattie, A. J.; Gooley, A. A. J Biol Chem 1998, 273, 6139–6143.
54. Oppenheim, F. G.; Xu, T.; McMillian, F. M.; Levitz, S. M.; Diamond, R. D.; Offner, G. D.; Troxler, R. F. J Biol Chem 1988, 263, 7472–7477.
55. Selsted, M. E.; Novotny, M. J.; Morris, W. L.; Tang, Y. Q.; Smith, W.; Cullor, J. S. J Biol Chem 1992, 267, 4292–4295.
56. Hara, S.; Yamakawa, M. Biochem J 1995, 310, 651–656.
57. Levashina, E. A.; Ohresser, S.; Bulet, P.; Reichhart, J.-M.; Hetru, C.; Hoffmann, J. A. Eur J Biochem 1995, 233, 694–700.
58. Agerberth, B.; Lee, J.-Y.; Bergman, T.; Carlquist, M.; Boman, H. G.; Mutt, V.; Jörnvall, H. Eur J Biochem 1991, 202, 849–854.
59. Harwig, S. L. S.; Kokryakov, V. N.; Swiderek, K. M.; Aleshina, G. M.; Zhao, C.; Lehrer, R. I. FEBS Lett 1995, 362, 65–69.
60. Cociancich, S.; Dupont, A.; Hegy, G.; Lanot, R.; Holder, F.; Hetru, C.; Hoffmann, J. A.; Bulet, P. Biochem J 1994, 300, 567–575.
61. Lee, K. H.; Hong, S. Y.; Oh, J. E.; Kwon, M.; Yoon, J. H.; Lee, J.; Lee, B. L.; Moon, H. M. Biochem J 1998, 334, 99–105.
62. Romeo, D.; Skerlavaj, B.; Bolognesi, M.; Gennaro, R. J Biol Chem 1988, 263, 9573–9575.
63. Morikawa, N.; Hagiwara, K.; Nakajima, T. Biochem Biophys Res Commun 1992, 189, 184–190.
64. Simmaco, M.; Mignogna, G.; Barra, D.; Bossa, F. FEBS Lett 1993, 324, 159–161.
65. Clark, D. P.; Durell, S.; Maloy, W. L.; Zasloff, M. J Biol Chem 1994, 269, 11956–11961.
66. Fehlbaum, P.; Bulet, P.; Chernysh, S.; Briand, J. P.; Roussel, J. P.; Letellier, L.; Hetru, C.; Hoffmann, J. A. Proc Natl Acad Sci USA 1996, 93, 1221–1225.
67. Yomogida, S.; Nagaoka, I.; Yamashita, T. Arch Biochem Biophys 1996, 328, 219–226.
68. Ehret-Sabatier, L.; Loew, D.; Goyffon, M.; Fehlbaum, P.; Hoffmann, J. A.; Van Dorsselaer, A.; Bulet, P. J Biol Chem 1996, 271, 29537–29544.

69. Miyata, T.; Tokunaga, F.; Yoneya, T.; Yoshikawa, K.; Iwanaga, S.; Niwa, M.; Takao, T.; Shimonishi, Y. J Biochem (Tokyo) 1989, 106, 663–668.
70. Kokryakov, V. N.; Harwig, S. S. L.; Panyutich, E. A.; Shevchenko, A. A.; Aleshina, G. M.; Shamova, O. V.; Korneva, H. A.; Lehrer, R. I. FEBS Lett 1993, 327, 231–236.
71. Ouellette, A. J.; Lualdi, J. C. J Biol Chem 1990, 265, 9831–9837.
72. Selsted, M. E.; Miller, S. I.; Henschen, A. H.; Ouellette, A. J. J Cell Biol 1992, 118, 929–936.
73. Lehrer, R. I.; Ganz, T.; Selsted, M. E. Cell 1991, 64, 229–230.
74. Ganz, T.; Rayner, J. R.; Valore, E. V.; Tumolo, A.; Talmadge, K.; Fuller, F. J Immunol 1989, 143, 1358–1365.
75. Bateman, A.; MacLeod, R. J.; Lembessis, P.; Hu, J.; Esch, F.; Solomon, S. J Biol Chem 1996, 271, 10654–10659.
76. Selsted, M. E.; Tang, Y.-Q.; Morris, W. L.; McGuire, P. A.; Novotny, M. J.; Smith, W.; Henschen, A. H.; Cullor, J. S. J Biol Chem 1993, 268, 6641–6648.
77. Saito, T.; Kawabata, S.; Shigenaga, T.; Takayenoki, Y.; Cho, J.; Nakajima, H.; Hirata, M.; Iwanaga, S. J Biochem (Tokyo) 1995, 117, 1131–1137.
78. Harwig, S. S.; Swiderek, K. M.; Kokryakov, V. N.; Tan, L.; Lee, T. D.; Panyutich, E. A.; Aleshina, G. M.; Shamova, O. V.; Lehrer, R. I. FEBS Lett 1994, 342, 281–285.
79. Schonwetter, B. S.; Stoltzenberg, E. D.; Zasloff, M. A. Science 1995, 267, 1645–1648.
80. Diamond, G.; Zasloff, M.; Eck, H.; Brasseur, M.; Maloy, L.; Bevins, C. L. Proc Natl Acad Sci USA 1991, 88, 3952–395.
81. Bulet, P.; Cociancich, S.; Reuland, M.; Sauber, F.; Bioschoff, R.; Hegy, G.; Van Dorsselaer, A.; Hetru, C.; Hoffmann, J. A. Eur J Biochem 1992, 209, 977–984.
82. Cociancich, S.; Ghazi, A.; Hetru, C.; Hoffmann, J. A.; Letellier, L. J Biol Chem 1993, 268, 19239–19245.
83. Lambert, J.; Keppi, E.; Dimarcq, J. L.; Wicker, C.; Reichhart, J. M.; Dunbar, B.; Lepage, P.; Van Dorsselaer, A.; Hoffmann, J. A.; Fothergill, J.; Hoffmann, D. Proc Natl Acad Sci USA 1989, 86, 262–266.
84. Fujiwara, S.; Imai, J.; Fujiwara, M.; Yaeshima, T.; Kawashima, T.; Kobayashi, K. J Biol Chem 1990, 265, 11333–11337.
85. Matsuyama, K.; Natori, S. J Biol Chem 1988, 263, 17112–17116.
86. Fehlbaum, P.; Bulet, P.; Michaut, L.; Lagueux, M.; Broekaert, W. F.; Hetru, C.; Hoffmann, J. A. J Biol Chem 1994, 269, 33159–33163.
87. Kato, Y.; Komatsu, S. J Biol Chem 1996, 271, 30493–30498.
88. Kawabata, S.; Nagayama, R.; Hirata, M.; Shigenaga, T.; Agarwala, K. L.; Saito, T.; Cho, J.; Nakajima, H.; Takagi, T.; Iwanaga, S. J Biochem (Tokyo) 1996, 120, 1523–1560.
89. Holak, T. A.; Engström, Å.; Kraulis, P. J.; Lindeberg, G.; Bennich, H.; Jones, T. A.; Gronnenborn, A. M.; Clore, G. M. Biochemistry 1988, 27, 7620–7629.
90. Sipos, D.; Andersson, M.; Ehrenberg, A. Eur J Biochem 1992, 209, 163–169.
91. Fernández, I.; Ubach, J.; Reig, F.; Andreu, D.; Pons, M. Biopolymers 1994, 34, 1251–1258.
92. Xia, W.; Liu, Q.; Wu, J.; Xia, Y.; Shi, Y.; Qu, X. Biochim Biophys Acta 1998, 1384, 299–305.
93. Marion, D.; Zasloff, M.; Bax, A. FEBS Lett 1988, 227, 21–26.
94. Bechinger, B.; Zasloff, M.; Opella, S. J. Protein Sci 1993, 2, 2077–2084.
95. Hirsh, D.; Hammer, J.; Maloy, W. L.; Blazyk, J.; Schaefer, J. Biochemistry 1996, 35, 12733–12741.
96. Bechinger, B.; Zasloff, M.; Opella, S. J. Biophys J 1998, 74, 981–987.
97. Iwai, H.; Nakajima, Y.; Natori, S.; Arata, Y.; Shimada, I. Eur J Biochem 1993, 217, 639–644.
98. Wong, H.; Bowie, J. H.; Carver, J. A. Eur J Biochem 1997, 247, 545–557.
99. Yi, G. S.; Park, C. B.; Kim, S. C.; Cheong, C. FEBS Lett 1996, 398, 87–90.
100. Raj, P. A.; Marcus, E.; Edgerton, M. Biochemistry 1996, 35, 4314–4325.
101. Kieffer, B.; Dillmann, B.; Lefevre, J. F.; Goumon, Y.; Aunis, D.; Metz-Boutigue, M. H. J Biol Chem 1998, 273, 33517–33523.
102. Raj, P. A.; Marcus, E.; Sukumaran, D. K. Biopolymers 1998, 45, 51–67.
103. Vignal, E.; Chavanieu, A.; Roch, P.; Chiche, L.; Grassy, G.; Calas, B.; Aumelas, A. Eur J Biochem 1998, 253, 221–228.
104. Mandard, N.; Sodano, P.; Labbe, H.; Bonmatin, J. M.; Bulet, P.; Hetru, C.; Ptak, M.; Vovelle, F. Eur J Biochem 1998, 256, 404–410.
105. Aumelas, A.; Mangoni, M.; Roumestand, C.; Chiche, L.; Despaux, E.; Grassy, G.; Calas, B.; Chavanieu, A. Eur J Biochem 1996, 237, 575–583.
106. Kawano, K.; Yoneya, T.; Miyata, T.; Yoshikawa, K.; Tokunaga, F.; Terada, Y.; Iwanaga, S. J Biol Chem 1990, 265, 15365–15367.
107. Bach, A. C.; Selsted, M. E.; Pardi, A. Biochemistry 1987, 26, 4389–4397.
108. Hanzawa, H.; Shimada, I.; Kuzuhara, T.; Komano, H.; Kohda, D.; Inagaki, F.; Natori, S.; Arada, Y. FEBS Lett 1990, 269, 413–420.
109. Bontems, F.; Roumestand, C.; Gilquin, B.; Menez, A.; Toma, F. Science 1991, 254, 1521–1523.
110. Zhang, X. L.; Selsted, M. E.; Pardi, A. Biochemistry 1992, 31, 11348–11356.
111. Pardi, A.; Zhangh, X. L.; Selsted, M. E.; Skalicky, J. J.; Yip, P. F. Biochemistry 1992, 31, 11357–11364.
112. Bonmatin, J. M.; Bonnat, J. L.; Gallet, X.; Vovelle, F.; Ptak, M.; Reichhart, J. M.; Hoffmann, J. A.; Keppi, E.; Legrain, M.; Achstetter, T. J Biomol NMR 1992, 2, 235–256.

113. Skalicky, J. J.; Selsted, M. E.; Pardi, A. Proteins 1994, 20, 52–67.
114. Zimmerman, G. R.; Legault, P.; Selsted, M. E.; Pardi, A. Biochemistry 1995, 34, 13663–13671.
115. Cornet, B.; Bonmatin, J. M.; Hetru, C.; Hoffmann, J. A.; Ptak, M.; Vovelle, F. Structure 1995, 3, 435–448.
116. Landon, C.; Sodano, P.; Hetru, C.; Hoffmann, J. A.; Ptak, M. Protein Sci 1997, 6, 1878–1884.
117. Ishikawa, M.; Kubo, T.; Natori, S. Biochem J 1992, 287, 573–578.
118. Wilson, I. B.; Gavel, Y.; Von Heijne, G. Biochem J 1991, 275, 529–534.
119. Strub, J. M.; Goumon, Y.; Lugardon, K.; Capon, C.; Lopez, M.; Moniatte, M.; Van Dorsselaer, A.; Aunis, D.; Metz-Boutigue, M. H. J Biol Chem 1996, 271, 28533–28540.
120. Bulet, P.; Urge, L.; Ohresser, S.; Hetru, C.; Otvos, L., Jr. Eur J Biochem 1995, 238, 64–69.
121. Hoek, K. S.; Milne, J. M.; Grieve, P. A.; Dionysius, D. A.; Smith, R. Antimicrob Agents Chemother 1997, 41, 54–59.
122. Yamashita, T.; Yomogida, S.; Nagaoka, I.; Saito, K. Biochim Biophys Acta 1995, 1243, 295–299.
123. Uchida, Y.; Shindo, M. Bull Chem Soc Jpn 1992, 65, 615–617.
124. Andersson, M.; Holmgren, A.; Spyrou, G. J Biol Chem 1996, 271, 10116–10120.
125. Cociancich, S.; Bulet, P.; Hetru, C.; Hoffmann, J. A. Parasitol Today 1994, 10, 132–139.
126. Kuzuhara, T.; Nakajima, Y.; Matsuyama, K.; Natori, S. J Biochem (Tokyo) 1990, 107, 514–518.
127. Fujii, G.; Selsted, M. E.; Eisenberg, D. Protein Sci 1993, 2, 1301–1312.
128. Wimley, W. C.; Selsted, M. E.; White, S. E. Protein Sci 1994, 3, 1362–1373.
129. Matsuzaki, K.; Nakayama, M.; Fukui, M.; Otaka, A.; Funakoshi, S.; Fujii, N.; Bessho, K.; Miyajima, K. Biochemistry 1993, 32, 11704–11710.
130. Matsuzaki, K.; Yoneyama, S.; Fujii, N.; Miyajima, K.; Yamada, K.; Kirino, Y.; Anzai, K. Biochemistry 1997, 36, 9799–9806.
131. Mangoni, M. E.; Aumelas, A.; Charnet, P.; Roumestand, C.; Chiche, L.; Despaux, E.; Grassy, G.; Calas, B.; Chavanieu, A. FEBS Lett 1996, 383, 93–98.
132. Andreu, D.; Albericio, F.; Solé, N. A.; Munson, M. C.; Ferrer, M.; Barany, G. Methods Mol Biol 1994, 35, 91–169.
133. Annis, I.; Hargitai, B.; Barany, G. Methods Enzymol 1997, 289, 198–221.
134. Valore, E. V.; Ganz, T. Methods Mol Biol 1997, 78, 115–131.
135. Gallis, B.; Mehl, J.; Prickett, K. S.; Martin, J. A.; Merriam, J.; March, C. J.; Cerretti, D. P. Biotechnol Ther 1990, 1, 335–346.
136. Rao, A. G.; Rood, T.; Maddox, J.; Duvick, J. Int J Pept Protein Res 1992, 40, 507–514.
137. Cervini, L. A.; Gray, W. R.; Kaiser, R.; Dykert, J.; Chan, R.; Solomon, S.; Rivier, C. L.; Rivier, J. E. Peptides 1995, 16, 837–842.
138. Lepage, P.; Bitsch, F.; Roecklin, D.; Keppi, E.; Dimarcq, J. L.; Reichhart, J. M.; Hoffmann, J. A.; Roitsch, C.; Van Dorseelaer, A. Eur J Biochem 1991, 196, 735–742.
139. Michaut, L.; Fehlbaum, P.; Moniatte, M.; Van Dorsselaer, A.; Reichhart, J. M.; Bulet, P. FEBS Lett 1996, 395, 6–10.
140. Porter, E. M.; van Dam, E.; Valore, E. V.; Ganz, T. Infect Immun 1997, 65, 2396–23401.
141. Valore, E. V.; Park, C. H.; Quayle, A. J.; Wiles, K. R.; McCray, P. B., Jr.; Ganz, T. J Clin Invest 1998, 101, 1633–1642.
142. Ouellette, A. J. Gastroenterology 1997, 113, 1779–1784.
143. Kreil, G. Eur J Biochem 1973, 33, 558–566.
144. Andreu, D.; Merrifield, R. B.; Steiner, H.; Boman, H. G. Proc Natl Acad Sci USA 1983, 80, 6475–6479.
145. Kuchler, K.; Kreil, G.; Sures, I. Eur J Biochem 1989, 179, 281–285.
146. Destoumieux, D.; Bulet, P.; Loew, D.; Van Dorsselaer, A.; Rodríguez, J.; Bachère, E. J Biol Chem 1997, 272, 28398–28406.
147. Bradbury, A. F.; Smyth, D. G. Trends Biochem Sci 1991, 16, 112–115.
148. Lee, I. H.; Cho, Y.; Lehrer, R. I. Infect Immun 1997, 65, 2898–28903.
149. Yasin, B.; Lehrer, R. I.; Harwig, S. S.; Wagar, E. A. Infect Immun 1996, 64, 4863–4866.
150. Nakajima, Y.; Qu, X-M.; Natori, S. J Biol Chem 1987, 262, 1665–1669.
151. Mor, A.; Nicolas, P. J Biol Chem 1994, 269, 1934–1939.
152. Fernández, R. C.; Weiss, A. A. Antimicrob Agents Chemother 1996, 40, 1041–1043.
153. Shinnar, A. E.; Uzzell, T.; Rao, M. N.; Spooner, E.; Lane, W. S.; Zasloff, M. A. In Peptides: Chemistry and Structural Biology; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: Kingswinford, (UK), 1996; pp 189–191.
154. Park, C. B.; Lee, J. H.; Park, I. Y.; Kim, M. S.; Kim, S. C. FEBS Lett 1997, 411, 173–178.
155. Mor, A.; Amiche, M.; Nicolas, P. Trends Biochem Sci 1992, 17, 481–485.
156. Kreil, G. Annu Rev Biochem 1997, 66, 337–345.
157. Mignona, G.; Simmaco, M.; Kreil, G.; Barra, D. EMBO J 1993, 12, 4829–4832.
158. Driscoll, J.; Zuo, Y.; Xu, T.; Choi, J. R.; Troxler, R. F.; Oppenheim, F. G. J Dent Res 1995, 74, 1837–1844.
159. Ludtke, S.; He, K.; Huang, H. Biochemistry 1995, 34, 16764–16769.
160. Matsuzaki, K.; Sugishita, K.-I.; Fujii, N.; Miyajima, K. Biochemistry 1995, 34, 3423–3429.
161. Tytler, E. M.; Anantharamaiah, G. M.; Walker, D. E.; Mishra, V. K.; Palgunachari, M. N.; Segrest, J. P. Biochemistry 1995, 34, 4393–4401.

162. Steiner, H.; Andreu, D.; Merrifield, R. B. Biochim Biophys Acta 1988, 939, 260–266.
163. Christensen, B.; Fink, J.; Merrifield, R. B.; Mauzerall, D. Proc Natl Acad Sci USA 1988, 85, 5072–5076.
164. Wade, D.; Boman, A.; Wåhlin, B.; Drain, C. M.; Andreu, D.; Boman, H. G.; Merrifield, R. B. Proc Natl Acad Sci USA 1990, 87, 4761–4765.
165. Gazit, E.; Lee, W.-J.; Brey, P. T.; Shai, Y. Biochemistry 1994, 33, 10681–10692.
166. Gazit, E.; Boman, A.; Boman, H. G.; Shai, Y. Biochemistry 1995, 34, 11479–11488.
167. Mchaourab, H.; Hyde, J. S.; Feix, J. B. Biochemistry 1994, 33, 6691–6699.
168. Gennaro, R.; Skerlavaj, B.; Romeo, R. Infect Immun 1989, 57, 3142–3146.
169. Pouny, Y.; Rapaport, D.; Mor, A.; Nicolas, P.; Shai, Y. Biochemistry 1992, 31, 12416–12423.
170. Besalle, R.; Kapitkovsky, A.; Gorea, A.; Shalit, I.; Fridkin, M. FEBS Lett 1990, 274, 151–154.
171. Matsuzaki, K.; Sugishita, K.; Harada, M.; Fujii, N.; Miyajima, K. Biochim Biophys Acta 1997, 1327, 119–130.
172. Iwahori, A.; Hirota, Y.; Sampe, R.; Miyano, S.; Takahashi, N.; Sasatsu, M.; Kondo, I.; Numao, N. Biol Pharm Bull 1997, 20, 805–808.
173. Fink, J.; Merrifield, R. B.; Boman, I. A.; Boman, H. G. J Biol Chem 1989, 264, 6260–6267.
174. Piers, K. L.; Brown, M. H.; Hancock, R. E. Antimicrob Agents Chemother 1994, 38, 2311–2316.
175. Aley, S. B.; Zimmerman, M.; Hetsko, M.; Selsted, M. E.; Gillin, F. D. Infect Immun 1994, 62, 5397–5403.
176. Hristova, K.; Selsted, M. E.; White, S. H. J Biol Chem 1997, 272, 24224–24233.
177. Eisenhauer, P.; Harwig, S. S.; Szklarek, D.; Ganz, T.; Lehrer, R. I. Infect Immun 1990, 58, 3899–3902.
178. Vunnam, S.; Juvvadi, P.; Rotondi, K. S.; Merrifield, R. B. J Pept Res 1998, 51, 38–44.
179. Oren, Z.; Hong, J.; Shai, Y. J Biol Chem 1997, 272, 14643–14649.
180. Falla, T. J.; Hancock, R. E. Antimicrob Agents Chemother 1997, 41, 771–775.
181. Sitaram, N.; Chandy, M.; Pillai, V. N.; Nagaraj, R. Antimicrob Agents Chemother 1992, 36, 2468–2472.
182. Chen, H. M.; Wang, W.; Smith, D.; Chan, S. C. Biochim Biophys Acta 1997, 1336, 171–179.
183. Brogden, K. A.; De Lucca, A. J.; Bland, J.; Elliott, S. Proc Natl Acad Sci USA 1996, 93, 412–416.
184. Brogden, K. A.; Ackermann, M.; Huttner, K. M. Antimicrob Agents Chemother 1997, 41, 1615–1617.
185. Xu, T.; Levitz, S. M.; Diamond, R. D.; Oppenheim, F. G. Infect Immun 1991, 59, 2549–2554.
186. Tsai, H.; Raj, P. A.; Bobek, L. A. Infect Immun 1996, 64, 5000–5007.
187. Tsai, H.; Bobek, L. A. Biochim Biophys Acta 1997, 1336, 367–369.
188. Helmerhorst, E. J.; Van't Hof, W.; Veerman, E. C.; Simmons-Smith, I.; Nieuw Amerongen, A. V. Biochem J 1997, 326, 39–45.
189. Matsuyama, K.; Natori, S. J Biochem (Tokyo) 1990, 108, 128–132.
190. Jacob, L.; Zasloff, M. In Antimicrobial Peptides. Ciba Foundation Symposium No. 186; Boman, H. G., Marsh, J., Goode, J. A., Eds.; Wiley: Chichester, 1994; pp 197–216.
191. Utsugi, T.; Schroit, A. J.; Connor, J.; Bucana, C. D.; Fidler, I. J. Cancer Res 1991, 51, 3062–3066.
192. Cruciani, R. A.; Barker, J. L.; Zasloff, M.; Chen, H. C.; Colamonici, O. Proc Natl Acad Sci USA 1991, 88, 3792–3796.
193. Díaz-Achirica, P.; Ubach, J.; Guinea, A.; Andreu, D.; Rivas, L. Biochem J 1998, 330, 453–460.
194. Shai, Y. Trends Biochem Sci 1995, 20, 460–464.
195. Merrifield, R. B.; Juvvadi, P.; Andreu, D.; Ubach, J.; Boman, A.; Boman, H. G. Proc Natl Acad Sci USA 1995, 92, 3449–3453.
196. Merrifield, E. L.; Mitchell, S. A.; Ubach, J.; Boman, H. G.; Andreu, D.; Merrifield, R. B. Int J Peptide Protein Res 1995, 46, 214–220.
197. Casteels, P. In Antimicrobial Peptides. Ciba Foundation Symposium No. 186; Boman, H. G., Marsh, J., Goode, J. A., Eds.; Wiley: Chichester, 1994; pp 136–139.
198. Engström, P.; Carlsson, A.; Engström, Å.; Tao, Z.-J.; Bennich, H. EMBO J 1984, 3, 3347–3351.
199. Axén, A.; Carlsson, A.; Engström, Å.; Bennich, H. Eur J Biochem 1997, 247, 614–619.
200. Groisman, E. A. Trends Microbiol 1996, 4, 127–128.
201. Boman, H. G.; Agerberth, B.; Boman, A. Infect Immun 1993, 61, 2978–2984.
202. Bateman, A.; Singh, A.; Congote, L. F.; Solomon, S. Regul Pept 1991, 13, 135–143.
203. Park, C. B.; Kim, H. S.; Kim, S. C. Biochem Biophys Res Commun 1998, 244, 253–257.
204. Leem, J. Y.; Nishimura, C.; Kurata, S.; Shimada, I.; Kobayashi, A.; Natori, S. J Biol Chem 1996, 271, 13573–13577.
205. Velasco, M.; Díaz-Guerra, J. M.; Díaz-Achirica, P.; Andreu, D.; Rivas, L.; Boscà, L. J Immunol 1997, 158, 4437–4443.
206. Yoo, Y. C.; Watanabe, R.; Koike, Y.; Mitobe, M.; Shimazaki, K.; Watanabe, S.; Azuma, I. Biochem Biophys Res Commun 1997, 237, 624–628.
207. Sahl, H. G. In Antimicrobial Peptides. Ciba Foundation Symposium No. 186; Boman, H. G., Marsh, J., Goode, J. A., Eds.; Wiley: Chichester, 1994; pp 27–53.
208. Nishikata, M.; Kanehira, T.; Oh, H.; Tani, H.; Tazaki, M.; Kuboki, Y. Biochem Biophys Res Commun 1991, 174, 625–630.
209. Couto, M. A.; Harwig, S. S.; Lehrer, R. I. Infect Immun 1993, 61,7 2991–2994.
210. Basak, A.; Ernst, B.; Brewer, D.; Seidah, N. G.; Munzer, J. S.; Lazure, C.; Lajoie, G. A. J Pept Res 1997, 49, 596–603.

211. Verbanac, D.; Zanetti, M.; Romeo, D. FEBS Lett 1993, 317, 255–258.
212. Higazi, A. A.; Barghouti, I. I.; Abu-Much, R. J Biol Chem 1995, 270, 9472–9477.
213. Higazi, A. A.; Ganz, T.; Kariko, K.; Cines, D. B. J Biol Chem 1996, 271, 17650–17655.
214. Van Wetering, S.; Mannesse-Lazeroms, S. P.; Van Sterkenburg, M. A.; Daha, M. R.; Dijkman, J. H.; Hiemstra, P. S. Am J Physiol 1997, 272, L888–L896.
215. Huang, H. J.; Ross, C. R.; Blecha, F. J Leukoc Biol 1997, 61, 624–629.
216. Chertov, O.; Michiel, D. F.; Xu, L.; Wang, J. M.; Tani, K.; Murphy, W. J.; Longo, D. L.; Taub, D. D.; Oppenheim, J. J. J Biol Chem 1996, 271, 2935–2940.
217. Gallo, R. L.; Ono, M.; Povsic, T.; Page, C.; Eriksson, E.; Klagsbrun, M.; Bernfield, M. Proc Natl Acad Sci USA 1994, 91, 11305–11039.
218. Yomogida, S.; Nagaoka, I.; Yamashita, T. Comp Biochem Physiol 1997, 116, 99–107.
219. Hook, W. A.; Tsuji, S.; Siraganian, R. P. Proc Soc Exp Biol Med 1990, 193, 50–55.
220. Cross, L. J.; Ennis, M.; Krause, E.; Dathe, M.; Lorenz, D.; Krause, G.; Beyermann, M.; Bienert, M. Eur J Pharmacol 1995, 291, 291–300.
221. Zhu, Q.; Solomon, S. Endocrinology 1992, 130, 1413–1423.
222. Tominaga, T.; Fukata, J.; Naito, Y.; Nakai, Y.; Funakoshi, S.; Fujii, N.; Imura, H. J Endocrinol 1990, 125, 287–292.
223. Groisman, E. A. Trends Microbiol 1994, 2, 444–449.
224. Porter, E. M.; Van Dam, E.; Valore, E. V.; Ganz, T. Infect Immun 1997, 65, 2396–401.
225. Johansson, J.; Gudmundsson, G. H.; Rottenberg, M. E.; Berndt, K. D.; Agerberth, B. J Biol Chem 1998, 273, 3718–3724.
226. Lichtenstein, A.; Ganz, T.; Selsted, M. E.; Lehrer, R. I. Blood 1986, 68, 1407–1410.
227. Daher, K. A.; Selsted, M. E.; Lehrer, R. I. J Virol 1986, 60, 1068–1074.
228. Panyutich, A.; Ganz, T. Am J Respir Cell Mol Biol 1991, 5, 101–106.
229. Panyutich, A. V.; Hiemstra, P. S.; Van Wetering, S.; Ganz, T. Am J Respir Cell Mol Biol 1995, 12, 351–357.
230. Prohászka, Z.; Német, K.; Csermely, P.; Hudecz, F.; Mezõ, G.; Füst, G. Mol Immunol 1997, 34, 809–816.
231. Panyutich, A. V.; Szold, O.; Poon, P. H.; Tseng, Y.; Ganz, T. FEBS Lett 1994, 356, 169–173.
232. Peck-Miller, K. A.; Blake, J.; Cosand, W. L.; Darveau, R. P.; Fell, H. P. Int J Pept Protein Res 1994, 44, 143–151.
233. Jones, E. M.; Smart, A.; Bloomberg, G.; Burgess, L.; Millar, M. R. J Appl Bacteriol 1994, 77, 208–214.
234. Díaz-Achirica, P.; Prieto, S.; Andreu, D.; Rial., E.; Rivas, L. Eur J Biochem 1994, 224, 257–264.
235. Díaz-Achirica, P.; Ubach, J.; Guinea, A.; Andreu, D.; Rivas, L. Biochem J 1998, 330, 453–460.
236. Pérez-Payá, E.; Houghten, R. A.; Blondelle, S. E. J Biol Chem 1995, 270, 1048–1056.
237. Vaara, M. Microbiol Rev 1992, 56, 395–411.
238. Hancock, R. E. Annu Rev Microbiol 1984, 38, 237–264.
239. Hancock, R. E. Lancet 1997, 349, 418–422.
240. Sawyer, J. G.; Martin, N. L.; Hancock, R. E. Infect Immun 1988, 56, 693–698.
241. Skerlavaj, B.; Romeo, D.; Gennaro, R. Infect Immun 1990, 58, 3724–3730.
242. Vaara, M.; Vaara, T. Antimicrob Agents Chemother 1994, 38, 2498–2501.
243. Piers, K. L.; Hancock, R. E. Mol Microbiol 1994, 12, 951–958.
244. Scocchi, M.; Romeo, D.; Cinco, M. Infect Immun 1993, 61, 3081–3083.
245. Sidorczyk, Z.; Zähringer, U.; Rietschel, E. T. Eur J Biochem 1983, 137, 15–22.
246. Cox, A. D.; Wilkinson, S. G. Mol Microbiol 1991, 5, 641–646.
247. Jones, A. L.; Beveridge, T. J.; Woods, D. E. Infect Immun 1996, 64, 782–790.
248. Visser, L. G.; Hiemstra, P. S.; Van den Barselaar, M. T.; Ballieux, P. A.; Van Furth, R. Infect Immun 1996, 64, 1653–1658.
249. Bengoechea, J. A.; Díaz, R.; Moriyón, I. Infect Immun 1996, 64, 4891–4899.
250. Jarosz, J.; Glinski, Z. J Invertebr Pathol 1990, 56, 143–149.
251. Jarosz, J. Cytobios 1997, 89, 73–80.
252. Resnick, N. M.; Maloy, W. L.; Guy, H. R.; Zasloff, M. Cell 1991, 66, 541–554.
253. Zasloff, M. In Phylogenetic Perspectives in Immunity: The Insect Host Defense; Hoffmann, J. A., Janeway, C. A., Jr., Natori, S., Eds.; R. G. Landes: Austin, TX, 1994; pp 31–41.
254. Matsuzaki, K.; Nakamura, A.; Murase, O.; Sugishita, K.; Fujii, N.; Miyajima, K. Biochemistry 1997, 36, 2104–2111.
255. Matsuyama, K.; Natori, S. J Biochem (Tokyo) 1990, 108, 128–132.
256. Pellegrin, P.; Menard, C.; Mery, J.; Lory, P.; Charnet, P.; Bennes, R. FEBS Lett 1997, 418, 101–105.
257. Okada, M.; Natori, S. Biochem J 1984, 222, 119–124.
258. Okada, M.; Natori, S. Biochem J 1985, 229, 453–458.
259. Piñeiro, M.; Díaz, I.; Rodríguez-Palenzuela, P.; Titarenko, E.; García-Olmedo, F. FEBS Lett 1995, 369, 239–242.
260. Parra-López, C.; Baer, M. T.; Groisman, E. A. EMBO J 1993, 12, 4053–4062.
261. López-Solanilla, E.; García-Olmedo, F.; Rodríguez-Palenzuela, P. Plant Cell 1998, 10, 917–924.
262. Shafer, W. M.; Qu, X.; Waring, A. J.; Lehrer, R. I. Proc Natl Acad Sci USA 1998, 95, 1829–1833.
263. Lincke, C. R.; Van der Bliek, A. M.; Schuurhuis, G. J.; Van der Velde-Koerts, T.; Smit, J. J.; Borst, P. Cancer Res 1990, 50, 1779–1785.

264. Sharom, F. J.; Diodato, G.; Yu, X.; Ashbourne, K. J. J Biol Chem 1995, 270, 10334–10341.
265. Sharom, F. J.; Lu, P.; Liu, R.; Yu, X. Biochem J 1998, 333, 621–630.
266. Hoffmann, J. A.; Reichhart, J.-M.; Hetru, C. Curr Opin Immunol 1996, 8, 8–13.
267. Lemaitre, B.; Kromer-Metzger, E.; Michaut, L.; Nicolas, E.; Meister, M.; Georgel, P.; Reichhart, J. M.; Hoffmann, J. A. Proc Natl Acad Sci USA 1995, 92, 9465–9469.
268. Lemaitre, B.; Nicolas, E.; Michaut, L.; Reichhart, J. M.; Hoffmann, J. A. Cell 1996, 86, 973–983.
269. Lemaitre, B.; Reichhart, J. M.; Hoffmann, J. A. Proc Natl Acad Sci USA 1997, 94, 14614–14619.
270. Panyutich, A. V.; Panyutich, E. A.; Krapivin, V. A.; Baturevich, E. A.; Ganz, T. J Lab Clin Med 1993, 122, 202–207.
271. Stolzenberg, E. D.; Anderson, G. M.; Ackermann, M. R.; Whitlock, R. H.; Zasloff, M. Proc Natl Acad Sci USA 1997, 94, 8686–8690.
272. Tarver, A. P.; Clark, D. P.; Diamond, G.; Russell, J. P.; Erdjument-Bromage, H.; Tempst, P.; Cohen, K. S.; Jones, D. E.; Sweeney, R. W.; Wines, M.; Hwang, S.; Bevins, C. L. Infect Immun 1998, 66, 1045–1056.
273. Zhang, G.; Ross, C. R.; Dritz, S. S.; Nietfeld, J. C.; Blecha, F. Clin Diagn Lab Immunol 1997, 4, 774–777.
274. Frohm, M.; Gunne, H.; Bergman, A. C.; Agerberth, B.; Bergman, T.; Boman, A.; Lidén, S.; Jörnvall, H.; Boman, H. G. Eur J Biochem 1996, 237, 86–92.
275. Russell, J. P.; Diamond, G.; Tarver, A. P.; Scanlin, T. F.; Bevins, C. L. Infect Immun 1996, 64, 1565–1568.
276. Diamond, G.; Russell, J. P.; Bevins, C. L. Proc Natl Acad Sci USA 1996, 93, 5156–5160.
277. Andersson, M.; Gunne, H.; Agerberth, B.; Boman, A.; Bergman, T.; Sillard, R.; Jörnvall, H.; Mutt, V.; Olsson, B.; Wigzell, H.; Dagerlind, Å.; Boman, H. G.; Gudmunsson, G. H. EMBO J 1995, 14, 1615–1625.
278. Ganz, T.; Metcalf, J. A.; Gallin, J. I.; Boxer, L. A.; Lehrer, R. I. J Clin Invest 1988, 82, 552–556.
279. Mandel, I. D.; Barr, C. E.; Turgeon, L. J. Oral Pathol Med 1992, 21, 209–213.
280. Lal, K.; Pollock, J. J.; Santarpia, R. P., III; Heller, H. M.; Kaufman, H. W.; Fuhrer, J.; Steigbigel, R. T. J Acquir Immune Defic Syndr 1992, 5, 904–914.
281. Smith, J. J.; Travis, S. M.; Greenberg, E. P.; Welsh, M. J. Cell 1996, 85, 229–236.
282. Goldman, M. J.; Anderson, G. M.; Stolzenberg, E. D.; Kari, U. P.; Zasloff, M.; Wilson, J. M. Cell 1997, 88, 553–560.
283. Li, Y. M.; Tan, A. X.; Vlassara, H. Nature Med 1995, 1, 1057–1061.
284. Soong, L. B.; Ganz, T.; Ellison, A.; Caughey, G. H. Inflamm Res 1997, 46, 98–102.
285. Ashitani, J.; Mukae, H.; Ihiboshi, H.; Taniguchi, H.; Mashimoto, H.; Nakazato, M.; Matsukura, S. Nihon Kyobu Shikkan Gakkai Zasshi 1996, 34, 1349–1353.
286. Barnathan, E. S.; Raghunath, P. N.; Tomaszewski, J. E.; Ganz, T.; Cines, D. B.; Higazi, A. A. Am J Pathol 1997, 150, 1009–1020.
287. Higazi, A. A.; Lavi, E.; Bdeir, K.; Ulrich, A. M.; Jamieson, D. G.; Rader, D. J.; Usher, D. C.; Kane, W.; Ganz, T.; Cines, D. B. Blood 1997, 89, 4290–4298.
288. Chopra, I. J Antimicrob Chemother 1993, 32, 351–353.
289. Aboudy, Y.; Mendelson, E.; Shalit, I.; Bessalle, R.; Fridkin, M. Int J Pept Protein Res 1994, 43, 573–582.
290. Soballe, P. W.; Maloy, W. L.; Myrga, M. L.; Jacob, L. S.; Herlyn, M. Int J Cancer 1995, 60, 280–284.
291. Magainin Pharmaceuticals, Inc., press release, July 27, 1998.
292. Miyasaki, K. T.; Bodeau, A. L.; Ganz, T.; Selsted, M. E.; Lehrer, R. I. Infect Immun 1990, 58, 3934–3940.
293. Miyasaki, K. T.; Bodeau, A. L.; Selsted, M. E.; Ganz, T.; Lehrer, R. I. Oral Microbiol Immunol 1990, 5, 315–319.
294. Miyasaki, K. T.; Iofel, R.; Lehrer, R. I. J Dent Res 1997, 76, 1453–1459.
295. Miyasaki, K. T.; Iofel, R.; Oren, A.; Huynh, T.; Lehrer, R. I. J Periodontal Res 1998, 33, 91–98.
296. Edgerton, M.; Raj, P. A.; Levine, M. J. J Biomed Mat Res 1995, 29, 1277–1286.
297. Paquette, D. W.; Waters, G. S.; Stefanidou, V. L.; Lawrence, H. P.; Friden, P. M.; O'Connor, S. M.; Sperati, J. D.; Oppenheim, F. G.; Hutchens, L. H.; Williams, R. C. J Clin Periodontol 1997, 24, 216–222.
298. O'Connell, B. C.; Xu, T.; Walsh, T. J.; Sein, T.; Mastrangeli, A.; Crystal, R. G.; Oppenheim, F. G.; Baum, B. J Human Gene Ther 1996, 7, 2255–2261.
299. Cullor, J. S.; Mannis, M. J.; Murphy, C. J.; Smith, W. L.; Selsted, M. E.; Reid, T. W. Arch Ophthalmol 1990, 108, 861–864.
300. Schuster, F. L.; Jacobs, L. S. Antimicrob Agents Chemother 1992, 36, 1263–1271.
301. Gunshefski, L.; Mannis, M. J.; Cullor, J. S.; Schwab, I. R.; Jaynes, J.; Smith, W. L.; Mabry, E.; Murphy, C. J. Cornea 1994, 13, 237–242.
302. Sousa, L. B.; Mannis, M. J.; Schwab, I. R.; Cullor, J.; Hosotani, H.; Smith, W.; Jaynes, J. CLAO J 1996, 2, 114–117.
303. Schwab, I. R.; Dries, D.; Cullor, J.; Smith, W.; Mannis, M.; Reid, T.; Murphy, C. J. Cornea 1992, 11, 370–375.
304. Nos-Barberá, S.; Portolés, M.; Morilla, A.; Ubach, J.; Andreu, D.; Patterson, C. A. Cornea 1997, 16, 101–106.
305. De Waal, A.; Gomes, A. V.; Mensink, A.; Grootegoed, J. A.; Westerhoff, H. V. FEBS Lett 1991, 293, 219–223.
306. Edelstein, M. C.; Gretz, J. E.; Bauer, T. J.; Fulgham, D. L.; Alexander, N. J.; Archer, D. F. Fertil Steril 1991, 55, 647–649.

307. Qu, X. D.; Harwig, S. S.; Oren, A. M.; Shafer, W. M.; Lehrer, R. I. Infect Immun 1996, 64, 1240–1245.
308. Zasloff, M. Curr Opin Immunol 1992, 4, 3–7.
309. Jaynes, J. M.; Julian, G. R.; Jeffers, G. W.; White, K. L.; Enright, F. M. Pept Res 1989, 2, 157–160.
310. Sharma, S. V. Oncogene 1992, 7, 193–201.
311. Sharma, S. V. Oncogene 1993, 8, 939–947.
312. Moore, A. J.; Devine, D. A.; Bibby, M. C. Pept Res 1994, 7, 265–269.
313. Baker, M. A.; Maloy, W. L.; Zasloff, M.; Jacob, L. S. Cancer Res 1993, 53, 3052–3057.
314. Shin, S. Y.; Lee, M. K.; Kim, K. L.; Hahm, K. S. J Pept Res 1997, 50, 279–285.
315. Peck-Miller, K. A.; Darveau, R. P.; Fell, H. P. Cancer Chemother Pharmacol 1993, 32, 109–115.
316. Ohsaki, Y.; Gazdar, A. F.; Chen, H. C.; Johnson, B. E. Cancer Res 1992, 52, 3534–3538.
317. Gasanov, S. E.; Rael, E. D.; Gasanov, N. E.; Vernon, L. P. Cancer Immunol Immunother 1995, 41, 122–128.
318. Winder, D.; Günzburg, W. H.; Erfle, V.; Salmons, B. Biochem Biophys Res Commun 1998, 242, 608–612.
319. Lehrer, R. I.; Daher, K.; Ganz, T.; Selsted, M. E. J Virol 1985, 54, 467–472.
320. Baghian, A.; Jaynes, J.; Enright, F.; Kousoulas, K. G. Peptides 1997, 18, 177–183.
321. Tamamura, H.; Murakami, T.; Masuda, M.; Otaka, A.; Takada, W.; Ibuka, T.; Nakashima, H.; Waki, M.; Matsumoto, A.; Yamamoto, N.; et al. Biochem Biophys Res Commun 1994, 205, 1729–1735.
322. Murakami, T.; Niwa, M.; Tokunaga, F.; Miyata, T.; Iwanaga, S. Chemotherapy 1991, 37, 327–334.
323. Wachinger, M.; Saermark, T.; Erfle, V. FEBS Lett 1992, 309, 235–241.
324. Wachinger, M.; Kleinschmidt, A.; Winder, D.; Von Pechmann, N.; Ludvigsen, A.; Neumann, M.; Holle, R.; Salmons, B.; Erfle, V.; Brack-Werner, R. J Gen Virol 1998, 79, 731–740.
325. Marcos, J. F.; Beachy, R. N.; Houghten, R. A.; Blondelle, S. E.; Perez-Payá, E. Proc Natl Acad Sci USA 1995, 92, 12466–12469.
326. Nakashima, H.; Masuda, M.; Murakami, T.; Koyanagi, Y.; Matsumoto, A.; Fujii, N.; Yamamoto, N. Antimicrob Agents Chemother 1992, 36, 1249–1255.
327. Tamamura, H.; Otaka, A.; Murakami, T.; Ibuka, T.; Sakano, K.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. Biochem Biophys Res Commun 1996, 229, 648–652.
328. Otaka, A.; Tamamura, H.; Terakawa, Y.; Masuda, M.; Koide, T.; Murakami, T.; Nakashima, H.; Matsuzaki, K.; Miyajima, K.; Ibuka, T.; et al. Biol Pharm Bull 1994, 17, 1669–1672.
329. Weeks, B. S.; Nomizu, M.; Otaka, A.; Weston, C. A.; Okusu, A.; Tamamura, H.; Yamamoto, N.; Fujii, N. Biochem Biophys Res Commun 1995, 215, 626–631.
330. Tamamura, H.; Ishihara, T.; Otaka, A.; Murakami, T.; Ibuka, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. Biochim Biophys Acta 1996, 1298, 37–44.
331. Tamamura, H.; Otaka, A.; Murakami, T.; Ishihara, T.; Ibuka, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. Biochem Biophys Res Commun 1996, 219, 555–559.
332. Murakami, T.; Nakajima, T.; Koyanagi, Y.; Tachibana, K.; Fujii, N.; Tamamura, H.; Yoshida, N.; Waki, M.; Matsumoto, A.; Yoshie, O.; Kishimoto, T.; Yamamoto, N.; Nagasawa, T. J Exp Med 1997, 186, 1389–1393.
333. Tamamura, H.; Arakaki, R.; Funakoshi, H.; Imai, M.; Otaka, A.; Ibuka, T.; Nakashima, H.; Murakami, T.; Waki, M.; Matsumoto, A.; Yamamoto, N.; Fujii, N. Bioorg Med Chem Lett 1998, 6, 231–238.