

David A. Phoenix, Sarah R. Dennison, Frederick Harris

Antimicrobial Peptides

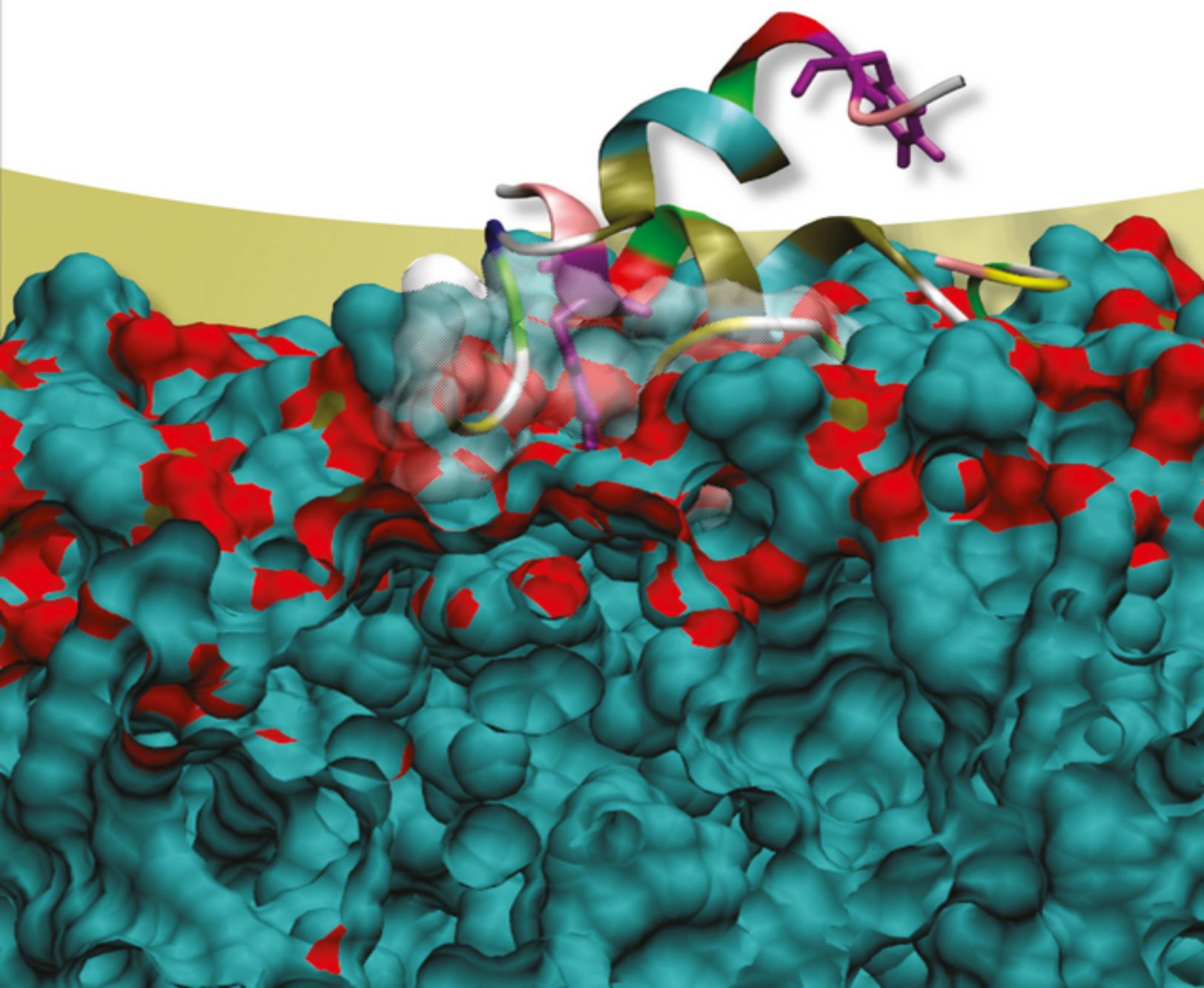


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Antimicrobial Peptides



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The cover shows aurein 2.3, a typical amphibian antimicrobial peptide in the presence of a lipid bilayer. The molecular dynamics simulation was undertaken by Dr. Manuela Mura, University of Central Lancashire. This MD simulation shows the potential of aurein 2.3 to use a pore-type mechanism of membrane interaction.

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Preface

The indiscriminate and widespread use of antibiotics for both medical and non-medical purposes [1] has led to the emergence of pathogenic bacteria with multidrug resistance, with reports of these pathogens increasing at an alarming rate [2, 3]. This is especially true in the hospital environment where microbes with resistance towards conventional antibiotics are becoming increasingly common [4, 5], with recent high-profile examples including vancomycin-resistant enterococci [6] and methicillin-resistant *Staphylococcus aureus* (MRSA) [7]. There is clearly an urgent need for new antibiotics with novel mechanisms of antimicrobial action and the focus of this book is antimicrobial peptides (AMPs), which show high potential to serve in this capacity. In [Chapter 1](#) we chart the discovery of AMPs, which is generally taken as the late 1980s when major research on these peptides began. This research revealed that AMPs are evolutionarily ancient molecules that are endogenous antibiotics produced by nearly all living organisms. However, it is now also becoming clear that in addition to their antimicrobial function, AMPs serve a variety of other roles, including modulation of the innate and adaptive immune systems via their ability to function as chemotactic agents. Over 2000 AMPs are now known and characterization has shown that the vast majority of these peptides are cationic, which are discussed in [Chapter 2](#), while the remaining AMPs are generally anionic and are considered in [Chapter 3](#). These chapters show that the capacity of AMPs to kill microbes depends upon a number of their structural and physiochemical characteristics such as charge and amphiphilicity, which facilitates their ability to partition into the membranes of the target organism. In

most cases, this action leads to membrane permeabilization and death of the host microbe, although in some cases AMPs are translocated across the membrane to attack intracellular targets such as DNA.

The most researched AMPs are those that form α -helices (α -AMPs), which may be their inherent secondary structure, although in most cases these peptides are unfolded in solution and require the membrane interface to adopt α -helical conformations. These α -helices are generally amphiphilic, which allows the apolar face of the peptide to interact with the membrane hydrophobic core while concomitantly permitting its polar face to engage in electrostatic interactions with the membrane lipid head-group region [8]. Based on the spatial regularity of the residues within these amphiphilic structures, a number of techniques have been developed that can predict the potential of peptides to form membrane interactive α -AMPs [9, 10]. A number of prediction techniques for other types of AMPs have also been presented [11, 12] and an overview of this area of research is discussed in [Chapter 4](#).

In addition to peptide-based properties, a number of membrane-based factors also contribute to the ability of AMPs to interact with membranes. Major examples of these factors include the transmembrane potential, lipid-packing characteristics, and the net negative charge generally carried by microbial membranes, which are targeted by cationic AMPs and thereby play fundamental roles in both the activity and selectivity of these peptides. Based on this research, a number of models to describe the antimicrobial activity of these peptides have been proposed, which are discussed in [Chapter 5](#). In addition, these models appear to describe the ability of AMPs to target and kill cancer cells, the membranes of which also carry a net negative charge, which is discussed in [Chapter 6](#). Currently, there is currently little evidence of microbial resistance to AMPs [13] and,

taken with the research described in the foregone chapters, this has led to the view that AMPs are attractive propositions as lead compounds to serve in a number of scenarios [14]. Major examples of this use include the treatment of cancer [15], along with infection control in the food industry, agriculture [16, 17], and healthcare [5, 18-20]. For example, the fungal defensin, plectasin, and its derivative, NZ2114, are currently under development by Novozymes as lead compounds for use against MRSA and *S. aureus* with resistance to vancomycin [21].

Although much has been learnt about AMPs and their various biological roles since they were first discovered, the factors underpinning their microbial selectivity and toxicity are still poorly understood. In a sense, the antimicrobial mechanisms of these peptides draw parallels to the “lock and key” model postulated for enzyme activity [22], where the “key” refers to characteristics of the peptide and the “lock” refers to those of the target membrane. For the action of AMPs to occur efficiently, the “lock” and “key” need to be fully engaged, and it is hoped that the discussion of these peptides and our current understanding of their function will further stimulate research into fully elucidating these structure-function relationships as well as draw attention to the importance of AMPs in the pharmaceutical industry.

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List of Abbreviations

α -ACPI	ACP with inactivity against cancer cells
[G-]	Active against Gram negative bacteria
[G+, G-]	Active against Gram positive and Gram negative bacteria
[G+, G-, F]	Active against Gram positive and Gram negative bacteria and fungi
[G+]	Active against Gram positive bacteria
ASP	agouti signal peptide
α -MSH	alpha-melanocyte stimulating hormone
AD	Alzheimer's Disease
ACE	angiotensin converting enzyme
AAMPs	Anionic antimicrobial proteins and peptides
ACPs	Anticancer peptides
APCs	antigen-presenting cells
AMPs	Antimicrobial peptides
ANN	Artificial neural networks
ATD	Atopic dermatitis
α -ACPs	α -helical anticancer peptides
α -AMPs	α -helical antimicrobial peptides
α -CAMPs	α -helical cationic antimicrobial peptides
α -LA	α -lactalbumin
ALA	5-aminolevulinic acid
BDs	Big defensins
β -LG	β -lactoglobulin
β -ACPs	β -sheet anticancer peptides
β -CAMPs	β -sheet cationic antimicrobial peptides
CL	Cardiolipin
CAMPs	Cationic antimicrobial peptides
CVC	Central venous catheters
CT	Chemotherapy
CS	Chondroitin sulfate
CF	Cystic fibrosis
CM	Cytoplasmic membranes
DCs	Dendritic cells
DCD	Dermcidin
EGF	epidermal growth factor
E-ACPs	extended structures
EPS	Extracellular polymeric substance
FPA	fibrinopeptide A

FPB	fibrinopeptide B
FDA	Food and Drug Administration
μG	Glycine moment
GAG	Glycosaminoglycan
[G+, G-, F, P]	Gram positive and Gram negative bacteria, and fungi and parasites
GPCR	G-protein-coupled receptor
HS	Heparan sulfate
HMMs	Hidden Markov models
HBDs	Human β -defensins
HIV	Human immunodeficiency virus
μH	Hydrophobic moment
H	Hydrophobicity
HAs	hylids of Australia
IFS	Incremental feature selection
ACPAO	Ineffective against non-cancerous cells and erythrocytes
LFM	Lactoferricin
LD50	lethal dose 50%
LPS	Lipopolysaccharide
LPC	Lysophosphatidylcholine
LysylPG	Lysylated PG
MIP-3 α	macrophage inflammatory protein-3 α
Mc1r	melanocortin 1 receptor
M-enk	methionineenkephalin
M-enk-RF	methionine-enkephalin-arginine phenylalanine
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MICs	Minimum inhibition concentration
mRM	Minimum redundancy method
MD	Molecular dynamics
MHP	Molecular hydrophobic potential
MLP	Molecular lipophilic potential
MDR	Multi-drug resistant
MDRPA	Multidrug-resistant <i>Pseudomonas aeruginosa</i>
PR-CAMPs	multiple arginine residues-CAMPs
MyD88	myeloid differentiation primary response gene 88
NEP	neutral endopeptidase
OM	Outer membrane
OA	Ovalbumin
PLUNC	Palate, lung, nasal epithelium clone
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol

PS	Phosphatidylserine
PACT	Photodynamic antimicrobial chemotherapy
PLS	Principle latent structures
PCA	Principal component analysis
PEA	Proenkephalin A
PGs	Proteoglycans
QSAR	Quantitative structure activity relationships
RT	Radiation therapy
RANAEs	ranids from Asia, North America, and Europe
RBD3	rat β -defensin 3
ROS	reactive oxygen species
SHM	Shai, Huang and Matsazuki
SP	Sphingomyelin
SAR	Structure-activity relationships
SAAPs	Surfactant-associated anionic peptides
TLRs	Toll-like receptors
ACPT	Toxic to both cancerous and non-cancerous cells
TM	Transmembrane
$\Delta\psi$	Transmembrane potential
TA-CAMPs	tryptophan and arginine residues-CAMPs
WHO	World Health Organization

1

Antimicrobial Peptides: Their History, Evolution, and Functional Promiscuity

Summary

Eukaryotic antimicrobial peptides (AMPs) first became a research focus in the middle decades of the twentieth century with the description of cecropins from moths and magainins from frogs. Since then, the number of reported AMPs has burgeoned to over 2000 with representatives in virtually all eukaryotic organisms. The availability of databases of AMPs has facilitated phylogenetic analyses, which have shown that the origins of β -defensins are traceable to an ancestral gene over half a billion years old, indicating the evolutionarily ancient nature of AMPs. An emerging theme from research on AMPs is their multifunctional nature, which we review here for β -defensins, and show that these peptides play roles in wound healing and modulation of both the innate and adaptive immune systems via the dual ability to promote and suppress the proinflammatory response to microbial infection.

1.1 Introduction: The History of Antimicrobial Peptides

Antimicrobial peptides (AMPs) have been recognized in prokaryotic cells since 1939 when antimicrobial substances, named gramicidins, were isolated from *Bacillus brevis*, and were found to exhibit activity both *in vitro* and *in vivo* against a wide range of Gram-positive bacteria [1, 2]. Gramicidins were later shown to successfully treat infected wounds on guinea-pig skin, indicating their therapeutic potential for clinical use [3], and were the first AMPs to be commercially manufactured as antibiotics [4]. In the case of

humans and other living creatures, which are constantly exposed to the threat of microbial infection, it had long been known that protection against these infections was provided by the adaptive immune system. However, this left the question as to why plants and insects, which lack an adaptive immune system, also remain free from infections for most of the time. The answer to this question is now known to be that similarly to prokaryotes, eukaryotes also produce AMPs and, historically, some sources attribute the discovery of eukaryotic AMPs to early work on plants [5] when in 1896 it was shown that a substance lethal to bread yeast was present in wheat flour [6]. At the end of the 1920s, lysozyme was identified by Alexander Fleming and is considered by some authors to be the first reported instance of a peptide with antimicrobial activity [7]. However, the mechanism of action used by lysozyme is now known to be enzymatic destruction of the bacterial cell wall, placing it at its time of discovery in a different category to AMPs, which utilize non-enzymatic mechanisms of antimicrobial activity [8, 9]. In 1928, Fleming discovered penicillin [10] and in the 1940s, along with Howard Florey and Ernst Chain, he brought the therapeutic use of penicillin to fruition, which led these three men to share the 1945 Nobel Prize for Medicine [11]. With the advent of penicillin and streptomycin in 1943, began the “Golden Age of antibiotics,” which led to a rapid loss of interest in the therapeutic potential of natural host antibiotics such as lysozyme and the importance of this immune defense strategy [12, 13]. However, in 1942, the antimicrobial substance that had previously been detected in wheat flour [6] was isolated from wheat endosperm (*Triticum aestivum*) and found to be a peptide that inhibited the growth of a variety of phytopathogens, such as *Pseudomonas solanacearum* and *Xanthomonas campestris* [14]. Later named purothionin in the mid-1970s [15, 16], this peptide is now known to be a member of the family of thionins, which

are AMPs distributed across the plant kingdom [5]. At the time this work was undertaken there was also the realization that the “Golden Age of antibiotics” had ended and with the rise of multidrug-resistant microbial pathogens in the early 1960s, an awakened interest in host defense molecules was prompted [17, 18]. It is this point in time that some sources consider to be the true origin of research into AMPs [19], beginning with studies that were conducted in the 1950s and 1960s, when it was shown that cationic proteins were responsible for the ability of human neutrophils to kill bacteria via oxygen-independent mechanisms – clearly not activity associated with the adaptive immune system [20, 21]. In 1962, in what some consider to be the first description of an animal AMP [22], bombinin was reported in the orange speckled frog *Bombina variegata* [23]. Also in the 1960s, the antimicrobial protein, lactoferrin, was isolated from milk [24] and small antimicrobial molecules were observed to be induced in the hemolymph of wax moth larvae after challenging with *Pseudomonas aeruginosa* [25]. In the late 1970s and 1980s several groups reported a number of AMPs and antimicrobial proteins from leukocytes [26], including what are now known to be α -defensins from rabbits [27–29] and humans [30]. Along with purothionin described above, these defensins were among the first cysteine-stabilized AMPs to be reported ([Chapter 2](#)). In 1981, in what are now generally considered as landmark studies, Boman *et al.* injected bacteria into the pupae of the silk moth, *Hyalophora cecropia*, and isolated the inducible cationic antimicrobial proteins, P9A and P9B, from the hemolymph of these pupae ([Chapter 2](#)) [31]. Soon after, these peptides were sequenced, characterized, and renamed as the more familiar “cecropins,” thereby constituting the first major α -helical AMPs to be reported [32]. In 1987, another landmark study occurred when Zasloff *et al.* (1987) isolated and characterized cationic AMPs from the African clawed frog, *Xenopus laevis*, and,

reflecting their defense role, named these peptides magainins after the Hebrew word for “shield” [33]. A few years later, β -defensins and θ -defensins, which differ from α -defensins with respect to their cysteine pairings, were characterized after isolation, respectively, from bovine granulocytes [34] and leukocytes of the rhesus monkey [35]. In the mid-1990s, Brogden *et al.* identified the first anionic AMPs in *X. Laevis* [36] and characterized several other such peptides in ruminants, including sheep and cattle [37]. Ironically, also in the early 1990s, evidence began to accumulate that led to the current view that lysozyme possesses antimicrobial activity involving non-enzymatic mechanisms that are similar to AMPs, thereby substantiating the view that it was one of the first of these peptides to be discovered [8]. Based on these results, a number of investigators considered the possibility that AMPs may play a role in the defense systems of organisms lacking an adaptive immune system [38]. In the mid-1990s, this was confirmed for the fruit fly, *Drosophila melanogaster*, when it was shown that the deletion of a gene encoding an AMP rendered the insect susceptible to a massive fungal infection [39]. Since these earlier studies, AMPs have been extensively studied not only in plants [40, 41] and insects [42–44], but also other invertebrate organisms that lack an adaptive immune system [45–48] although most of the current understanding of AMPs has been obtained from studies on those isolated from amphibian skin secretions, which is a rich source of these peptides [49–52]. In combination, these studies have established that AMPs exist in virtually all multicellular organisms [53] and it is increasingly being recognized that that these peptides play an important role in the immune system of mammals, including humans [54–57]. These peptides have been identified at most sites of the human body normally exposed to microbes such as the skin and mucosae [54, 55], and are produced by a number of blood cell types, including

neutrophils, eosinophils, and platelets [58–60]. However, as research into the expression of AMPs progressed, it became clear that the production of these peptides may be either constitutive or induced by inflammation or injury [38]. Typically, for example, α -defensins and dermcidin (the precursor of AMPs involved in skin defense) tend to be produced constitutively, whereas the majority of β -defensins are inducible [61–63]. Moreover, although particular AMPs may predominate at specific body sites only a small minority are exclusively produced by a certain cell type or tissue and each tissue has its own spectrum of AMPs that may vary in composition depending upon the prevailing physiological conditions [55]. For example, peptides derived from dermcidin are the major AMPs in human sweat but show differing profiles between the body sites of a given individual in response to exercise [64]. One question that has puzzled investigators since the discovery of AMPs is the fact that the minimal inhibitory concentrations (MICs) required for their *in vitro* antimicrobial activity are generally much higher than the physiological concentrations of these peptides found *in vivo* [65]. Two major explanations proposed for this observation are that at sites of inflammation, AMPs can accumulate at high local concentrations sufficiently above their MIC to exert their antimicrobial effect or that these peptides may act synergistically with other AMPs [65]. Over the last decade, these synergistic effects have been demonstrated for a variety of AMPs [66], including those that are structurally similar and from the same host organism, such as magainin and PGLa, which is another α -helical peptide from *X. Laevis* [67, 68], and those that are structurally dissimilar and from differing host organisms, such as LL-37, an α -helical human peptide, and indolicidin, an extended bovine peptide ([Chapter 2](#)) [69]. Studies over the last decade have also established that some organisms produce AMPs as suites of closely related peptides that synergize to produce a broad

spectrum of antimicrobial activity, such as maximins, which are α -helical AMPs produced in the brains of amphibians [70], and cyclotides, which are cyclic cystine knot AMPs produced in the leaves, flowers, stems, and roots of various plants [71, 72]. As more has been learnt about AMPs, it has become somewhat arbitrary as to their precise definition. For example, perforin and the complement component C9 are large proteins of approximately 60 kDa that under physiological conditions insert into membranes and form pores as result of highly regulated immune processes, and are thus not classified as AMPs [73, 74]. In contrast, lactoferrin, which is around 80 kDa, is generally included as an AMP, based on the fact that it is ubiquitous in various body fluids and utilizes a non-specific mode of antimicrobial action similar to other AMPs ([Chapter 5](#)) [75, 76]. Moreover, some “AMPs” do not appear to exert direct antimicrobial activity such as the PLUNC (palate, lung, nasal epithelium clone) proteins that appear to primarily play a role in neutralizing endotoxins, promoting the agglutination of bacteria, and modulating cytokine production [77]. Nonetheless, it is now well established that the production of AMPs is a defense strategy used across eukaryotes, evidenced by the list of databases dedicated to these peptides that have appeared almost every year over the last decade ([Table 1.1](#)). Examination of these databases shows that in excess of 2000 AMPs have now been listed and the number of these peptides being reported is increasing rapidly [87, 89]. The availability of these databases has allowed comparisons to be made between AMPs based on a variety of criteria, most often structure-function relationships and mechanisms of antimicrobial action, which are discussed in later chapters of this book. However, two less well discussed aspects of AMPs are reviewed in the remainder of this chapter, namely their ancient origins and evolution along with their functional

promiscuity, encompassing a number of biological roles in addition to antimicrobial activity.

Table 1.1 Representative databases dedicated to AMPs.

Year	Database	Website	Content	Key reference
2002	AMSDb	http://www.bbcm.univ.trieste.it/~tossi/amsdb.html	Animal/plant AMPs	–
2002	SAPD	http://oma.terkko.helsinki.fi:8080/~SAPD	Synthetic AMPs	[78]
2003	NAD	http://www.nih.go.jp/~jun/NADB/search.html	General AMPs	–
2004	A/OL	http://www.atoapps.nl/AOLKnowledge/	Antimicrobial compounds	–
2004	Peptaibol	http://www.cryst.bbk.ac.uk/peptaibol/home.shtml	Fungal AMPs	[79]
2006	CyBase	http://researcht.imb.uq.edu.au/cybase	Plant AMPs	[80]
2006	PenBase	penbase.immunaqua.com	Shrimp AMPs	[81]
2007	BACTIBASE	bactibase.pfba-lab.org	Bacterial AMPs	[82]
2007	Defensins	defensins.bii.a-star.edu.sg	Defensins across eukarya	[83]
2007	AMPer	http://marray.cmdr.ubc.ca/cgi-bin/amp.pl	Animal/plant AMPs	[84]
2008	PhytAMP	phytamp.pfba-lab-tun.org	Plant AMPs	[85]
2008	RAPD	http://faculty.ist.unomaha.edu/chen/rapd/index.php	Recombinant AMPS	[86]
2009	APD2	http://aps.unmc.edu/AP	Natural AMPs	[87]
2010	CAMP	http://www.bicnirrh.res.in/antimicrobial	General AMPs	[88]
2012	YADAMP	www.yadamp.unisa.it	General AMPs	[89]
2012	DAMPD	http://apps.sanbi.ac.za/dampd	General AMPs	[90]
2012	DADP	http://split4.pmfst.hr/dadp/	Amphibian AMPS	[91]

1.2 AMPs: Evolutionarily Ancient Molecules

Since the discovery of magainins in *X. laevis* [33], it has become clear that the skin secretions of many anurans include a spectrum of AMPs that ranges between 10 and 20

Pseudoplectania nigrella

psoriasis

psyle A – psyle F

puroindoline A

purothionin

pyrrhocoricins

q

quantitative structure – activity relationship (QSAR) models

quinupristin-dalfopristin

r

radiation therapy (RT)

rat β -defensins (RBDs)

reactive oxygen species (ROS)

receptor tyrosine kinases

residue composition

respiratory tract

retrocyclins

RGD-tachyplesin-1

rhabdomyosarcoma

rhabdosarcoma

rifampicin

rifamycin

s

Saccharomyces cerevisiae

sakacin P

Salmonella typhimurium

salt bridging

“sand in a gearbox” model

Segrest method

selectivity of ACPs

sepsis
sequence length
serine
sessile bacteria
sexually transmitted diseases
Shai-Huang-Matsuzuki (SHM) model
sialic acid
SK84
SMAP-29
snorkeling mechanism
specific residues
sphingolipids
sphingomyelin (SM)
spider β -ACP_T
Staphylococcus spp
Staphylococcus aureus
- see also MRSA
Staphylococcus epidermidis
STAT proteins
stereo-selective antimicrobial mechanism
sterols
S-thanatin
stomach carcinoma (AGS)
Streptococcus
Streptococcus mutans
Streptococcus pneumoniae
Streptomyces roseosporus
streptomycin
structure - function relationships
surfactant associated anionic peptides (SAAPs)
styelin D
synovial sarcoma

t

T-cell epitopes
T-cells
TA-CAMPs
tachyplesin-1
teichoic acid
teichuronic acid
teicoplanin
temozolomide
temporin A
temporin B
temporin-1DRa
temporin L
 θ -defensins
thiamine biosynthesis
thiol
thionins
thrombin
thymosin- β_4
tilted α -helices
tilted peptides

- α -ACPs
- amphiphilicity

Toll-like receptors (TLRs)
Tomich model
topical biocides
toroidal pore model

- AAMPs
- membrane interaction

toxicity of ACPs
transactivation model
transforming growth factor- α

translocation

- AAMPs
- anticancer

transmembrane (TM) α -helices

transmembrane (TM) pores

transmembrane (TM) potential ($\Delta\Psi$)

- CAMPs

trans proline

Trichoderma

Triticum aestivum

tritrpticin

tropomyosin

trypanosomiasis

trypsin

tryptophan

tryptophan and arginine CAMPs (TA-CAMPs)

tumor necrosis factor- α

V

V13K

vaccines

valine

vancomycin

varv A and varv F

varv A, varv E and vitri A

vhl-2

vibi D, vibi E, vibi G and vibi H

Vigna radiate

Viola odorata

violacin A

viruses

- AAMPs
- CAMPs