
Preface

As our knowledge of the innate immune systems in multicellular organisms has grown steadily in the last two decades, so has our comprehension of the basic role played by gene-encoded, ribosomally synthesized antimicrobial peptides (AMPs) in this ancient line of defense against infections. Indeed, although the ability of many prokaryotes to produce and release peptidic or proteinaceous substances with antibacterial and antifungal activity has been known for quite a long time, the presence of AMPs in humans and animals (and later plants) has been recognized only more recently, in the 1980s, with the identification of cecropins in the pupae of *Hyalophora cecropia* moth, defensins in human neutrophils, and magainins in skin secretions of the frog *Xenopus laevis* (1–3); these findings, though, were grounded on previous work conducted in the 1960s and 1970s, for example, by Hussein Zeya and John Spitznagel. Since then, the quest for new AMPs has gifted us with many hundreds of biologically active peptides, extremely varied in sequence and structure, isolated from virtually every multicellular organism where they have been looked for (4). Visiting the several available web-based repositories dedicated to AMPs – such as AMSDB (<http://www.bbcm.univ.trieste.it/~tossi/pag1.htm>), ANTIMIC (<http://research.i2r.a-star.edu.sg/Templar/DB/ANTIMIC/>), and APD2 (<http://aps.unmc.edu/AP/main.php>) – can help in getting a more precise idea of what “diversity” means in this case. By the end of the 1990s, AMPs research was already blooming. The first Gordon Research Conference on Antimicrobial Peptides was held in 1997 in Ventura, California, and these meetings have since been a privileged point of discussion and the cradle of many ideas around these intriguing molecules. The same year the GRC series on AMPs started, the first *Methods in Molecular Biology* volume dedicated to AMPs, edited by William Shafer, was published (5).

From those early days, we have gone a long distance ahead, although the journey seems by no means to come to an end soon. Much has been learned about the mechanism of target selection and cell killing, always with the aim of transforming an evolutionary, successful, antimicrobial shield into the next century’s antibiotic drugs. Thanks to years of intense study in many public and private research institutions worldwide, many AMPs (in particular those of mammals) began to be recognized as endowed with more than simply direct antimicrobial properties, by acting at the interface between innate and adaptive immunity, for example, as immunomodulants, immunostimulants, and/or inducers of proinflammatory cytokines or chemokines, to the point that today many prefer to refer to these substances with the more general term of “host defense peptides.” Notwithstanding these major efforts, AMPs have not yet fulfilled their main promise, i.e., to be able to truly represent the next-generation antibiotics with new modes of actions. This is for a complex variety of causes that have collectively hampered clinical progress to date. Some of these are related with the peptidic nature of AMPs which translates, for instance, to poor bioavailability, poor proteolytic stability, and cost of goods issues, or with their mechanism of action, which might bear some potential for unwanted toxicity. A helping hand, in this sense, would probably come in the near future by the development of synthetic

analogues of AMPs, with improved characteristics (and lower cost) with respect to their natural counterparts (6).

For one that approaches this research area from outside, the truly multidisciplinary scenario it offers might at first sight be surprising and even a bit confusing. Immunologists, infectivologists, dermatologists, microbiologists, cell biologists, molecular geneticists, biochemists, peptide chemists, biophysicists, and many others with different scientific backgrounds have entered the arena, each one approaching the challenges on the table from a distinct point of view, while elaborating at the same time original perspectives for the field's development. The medical potential of which AMPs are inherently endowed with certainly lies at the core of this interest, but it does not explain everything. In our opinion, a good fraction of the AMPs' ability in catalyzing such a diversified attention relies on their privileged position at the center of a perfect storm, where scientifically mature fields such as antibiotics and immunology research on one side and membrane/peptide biochemistry and biophysics on the other one positively clash. On this stage, each field is in search of a new object of study for unleashing the potential of perfectionated equipment and theories, a reachable goal with important practical consequences, and, ultimately, an occasion for rejuvenation.

In the present volume of *Methods in Molecular Biology*, we have attempted to provide an updated overview of this burgeoning field, by offering snapshots of the many different approaches leading scientists in the field follow to crack AMPs' most hidden attributes and properties, with the ultimate end of harnessing the potential these compounds do display for applicative purposes. As in the series' style (and name), most of the contributing chapters deal with experimental protocols, presented "in readily reproducible, step-by-step fashion." In addition, several review chapters discuss in a more classic manner selected issues pertaining to different aspects of AMPs, namely the forms of interaction of AMP's with the lipopolysaccharide (LPS) component of Gram-negative bacteria (Chapter 10), the diverse dynamic transitions peptides (not only AMPs, but also, for example, fusogenic peptides) may undergo in membranes (Chapter 13), and the therapeutic potential of host defense peptides (Chapters 19 and 20).

As a reading guide, chapters are organized around three main themes: the isolation, purification, and production in recombinant form of AMPs from natural sources and the design and synthesis of unnatural compounds; the methodologies – of either biophysical, biochemical, or computational nature – usually applied to define AMPs' molecular features and to explore their mechanism(s) of action; the studies focused on the biological activities (antimicrobial, anti-inflammatory, immunomodulatory, and so on) of AMPs, and on their use as therapeutic agents. Contributions gathered in the first part will lead the reader through the discovery of novel AMPs from sources as different as frog skin secretions (Chapter 1) and human skin (Chapter 2), and of lantibiotics from Gram-positive bacteria (Chapter 3). The production of recombinant antimicrobial peptides in bacteria is one of the possible routes to achieve an in-depth structure–activity analysis of AMPs, through selective and systematic removal and/or substitution of given residue positions, and a suitable strategy for the development of a less expensive peptide-production platform. Chapters 4 and 5 will thus discuss the production of AMPs by recombinant approaches in *Escherichia coli*. Approaches for the rational design of AMPs and for the production of peptide variants by solid-phase synthesis will be described in Chapters 6 and 7, respectively. Finally, the SPOT synthesis technique – a powerful tool offering the opportunity of synthesizing and screening a large number of peptides arrayed on a planar cellulose support – comes under focus in two different contributions (Chapters 8 and 9). With membranes apparently being an obligate passage in the action of all AMPs – whether

exerting their activity through destabilization of the membrane itself or directed toward an intracellular target – it is not surprising that all contributions of the second part of the volume deal, in a way or the other, with membrane interactions of peptides. These can be characterized by taking advantage of sophisticated membrane model systems or live cells, tryptophan and lipid fluorescence, and a few selected fluorescent dyes, a combination that permits to carefully measure several aspects of membrane-binding and membrane-perturbing activities of selected AMPs and peptidomimetics, as described in Chapters 11 and 12. Structural studies of AMPs, either alone or when embedded in a membrane environment, are unquestionably of pivotal importance, and in this context solid-state NMR is gaining momentum as a very informative technique, as underlined in Chapter 14 (see also Chapter 13 for more details on NMR studies). Besides NMR, microscopic techniques can also offer important clues in order to disclose more on AMPs function and cell specificity, providing a “whole-cell” perspective. Chapter 15 depicts how atomic force microscopy can be used to dissect the behavior of some AMPs (in this specific case, the so-called Sushi peptides from horseshoe crab), whereas Chapter 16 describes the use of a mix of fluorescence/electron microscopy techniques to get insight into the damage caused by AMPs on the morphology and membrane structure of intact bacterial cells. This section terminates with the important contributions coming from computational resources, in the form of molecular dynamics simulations (Chapter 17) and quantitative structure–activity relationships (QSAR) analysis of antimicrobial peptides (Chapter 18 for this approach see also Chapter 6). The third and last section of the book sketches just a few, although very significant, approaches put in force so far to disclose the medical and therapeutic potential of AMPs as anti-infective and immunomodulant agents. Single contributions deal with infection models and activity assay systems (Chapters 21, 22, 23, and 24), and with the characterization of the antimicrobial activity of peptides against the protozoan pathogen *Leishmania* (Chapter 25).

Within the limitations inherent to the selection process of topics to be included in the present volume, which necessarily left over many important issues that would have deserved the same covering, we hope that the readers – either expert of the field or newcomers – will find in these pages both an authoritative guide for their own lab work on AMPs or related substances and a good load of thought stimuli to inspire their scientific endeavors.

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