Bacteria and fungi coexist with all animals and plants in close physical association. Indeed, health depends on the existence of natural flora that provides vitamins and stimulates the development of specific immune responses in higher vertebrates. However, when particular bacteria or fungi are introduced into a host in a niche that permits their growth as well as the elaboration of virulence factors, disease can result. Given the number and diversity of organisms with which we are in constant contact, this result is thankfully rare—likely because of our defense systems. One such system is that of nonspecific immunity, which can proceed along either oxidative or nonoxidative antimicrobial pathways. These nonoxidative defense pathways comprise a wide variety of oligopeptides and proteins with potent antibacterial and antifungal activity. Such proteins occur in all animals and plants in which they have been sought. A recent paper sponsored by the Ciba Foundation (18 to 20 January 1994) brought together scientists working on these antimicrobial peptides. Despite the wide variety of sources and identities of these peptides, a number of common themes emerged.

On the basis of sequence and structural information, these antimicrobial agents can be arranged in groups, as indicated in the table. The low molecular weight (3000 to 5000) peptides are processed from larger precursors. Some are linear molecules and some contain highly conserved disulfide bonds. In addition, there are families within these two groups. Among the linear molecules, the cecropins, which have been isolated from both insects and mammals (1, 2), and the magainins (3) form well-defined groups on the basis of highly conserved sequence similarities. A specific motif characterized by proline and arginine occurs in the cecropins Bac-5 and Bac-7 (4) and in cecropin PR-39 (2) from pig intestine. This motif serves further attention. Coincidentally, this protein is obscure but certainly detectable antimicrobial activity (6, 7). Therefore, the serprocidins cathepsin G and proteinase 3 are proteases for connective tissue matrix components and are probably responsible for tissue damage during inflammation (6). Although protease activity per se is not required for microbial killing, many of the antimicrobial peptides are present in locations that are protease-rich. Proteolysis may allow the antimicrobial protein to gain easier access to the site of infection. Proteinase 3 is also a determinant of myeloid differentiation in the human leukemia HL-60 cell line (6). The relation among antimicrobial, proteolytic, and developmental activities of this protein is obscure but certainly deserves further attention. Coincidentally, saposins (insect defensins) have also been implicated as growth factors, for fly embryos (8). BPI, azurocidin, and the tachyplesins also bind lipopolysaccharide (LPS); this ability may facilitate their interactions with Gram-negative organisms, yet LPS binding alone is insufficient for antimicrobial activity because the major LPS-binding protein from mammalian serum, LBP, has no detectable antimicrobial activity (6, 9, 10). In addition to binding to LPS, BPI can neutralize LPS activity and thus must contribute to host defense against endotoxin.
The crucial point needing clarification is the mechanism of killing by antimicrobial proteins. How do these peptides and proteins recognize such diverse microorganisms? What are the targets for the lethal events? What is the structural basis for antimicrobial activity? In general, more is understood about the low molecular weight species, particularly the defensins and the cecropins. Virtually all antimicrobial peptides carry a net positive charge; thus, the initial contact between the antimicrobial peptide and the target organism is electrostatic, because many bacterial surfaces are anionic. In addition, there is an electrical potential on the order of ~200 mV across the cytoplasmic membrane and a smaller but significant negative potential across the outer membrane of Gram-negative organisms owing to the presence of fixed, high molecular weight anionic species in the periplasmic space. These potentials may contribute to entry of cationic antimicrobial peptides across the inner membrane (5). Both the linear and disulfide-containing low molecular weight peptides seem to be able to form pores in model test systems and in some cases can increase the permeability of bacterial outer and inner membranes (3, 5). The cecropins actually cause rapid lysis after a brief delay during which the peptide travels across the periplasmic space (1).

The mechanism of pore formation, however, differs substantially between the linear and disulfide-containing peptides. Most of the linear peptides can form amphipathic α-helices that are thought to self-assemble to form transmembrane channels with permeabilities exceeding the homeostatic capacities of the microorganism. A synthetic cecropin made of D-amino acids has properties indistinguishable from those of the native L-amino acid-containing molecule. Thus, cecropin activity does not require a stereospecific interaction with a target cell component (11). The proline-arginine-rich bactenecins and cecropin PR-39 must act differently, because the high concentrations of proline are incompatible with α-helix formation. Indeed, PR-39 does not have pore-forming activity but does inhibit macromolecular synthesis (12). The three-dimensional structure of the defensins has been determined both by crystallography and nuclear magnetic resonance (13). These molecules form dimers with a cat- phy and nuclear magnetic resonance (13).

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Microorganisms have coevolved with animals; most often the relationship is mutually beneficial. In instances where there is competition, both the microorganisms and the hosts have had ample opportunity to develop offensive and defensive strategies. Perhaps pathogens have developed resistance to antimicrobial proteins, and hosts have in turn compensated by producing a variety of related proteins. In spite of these endogenous antibiotics, we still succumb to infectious diseases and rely on exogenous antibiotics to turn the tide in our favor. As the availability of effective exogenous antibiotics decreases as a result of the increased resistance of the pathogens, we should explore the innate antibiotics of plants and animals as models for new therapeutic agents.

References
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