

## REVIEW ARTICLE

# Ribosomally synthesized peptides from natural sources

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There are many antibiotic-resistant microbial pathogens that have emerged in recent years causing normal infections to become harder and sometimes impossible to treat. The major mechanisms of acquired resistance are the ability of the microorganisms to destroy or modify the drug, alter the drug target, reduce uptake or increase efflux of the drug and replace the metabolic step targeted by the drug. However, in recent years, resistant strains have been reported from almost every environment. New antimicrobial compounds are of major importance because of the growing problem of bacterial resistance, and antimicrobial peptides have been gaining a lot of interest. Their mechanism of action, however, is often obscure. Antimicrobial peptides are widespread and have a major role in innate immunity. An increasing number of peptides capable of inhibiting microbial growth are being reviewed here. In this article, we consider the possible use of antimicrobial peptides against pathogens.

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## INTRODUCTION

Antibiotics have significantly improved our quality of health; however, the overuse of antibiotics has led to the emergence of multidrug-resistant microorganisms, and the subsequent constant demand for the discovery of novel antibiotics. To this end, antimicrobial peptides have attracted much attention as a novel class of antibiotics. Antimicrobial peptides are gene-encoded, ribosomally synthesized polypeptides. The natural antimicrobial peptides have been isolated from practically all living organisms, ranging from prokaryotes to humans. They have a key role in innate immunity of eukaryotes and are produced by many organisms including bacteria, fungi, insects, plants and higher animals. In mammals, antimicrobial peptides are generated by epithelial cells in the respiratory tract, gastrointestinal tract etc., the same is done by mucous cells in the mucous gland.<sup>1</sup> So far, over 800 antimicrobial peptides have been isolated and described. They have great ancient evolutionary importance preserved in mammals. They can protect against a broad array of infectious agents, such as bacteria, fungi, parasites and viruses, and show cytotoxicity against cancer cells. They are usually small peptides (30–60 a.a). They are commonly strongly cationic and heat stable (100 °C for 5 min). Their significant advantage resides in the global mechanism of their action, which is remarkably different from that of conventional antibiotics. Usage of these peptides will gain widespread increase, as more and more bacteria may develop the ability to resist conventional antibiotics owing to the abuse of these drugs worldwide.<sup>2</sup>

### Ribosomally synthesized peptides as new antimicrobials

Antimicrobial peptides are ribosomally synthesized antibiotic peptides produced by nearly all organisms, from bacteria to plants and

animals. Structures of antimicrobial peptides encompass molecules ranging in size from 6 to 60 amino-acid residues, being linear ( $\alpha$ -helical and non-helical), cyclic (Rhesus  $\beta$ -defensins, Rhesus  $\theta$ -defensins) or crosslinked by one, two, three or more internal disulfide bridges. Antimicrobial peptides consisting of three disulfide bonds include insect defensins and mammalian  $\alpha$  and  $\beta$  defensins. In terms of amino-acid composition and net surface charge, they may be rich in one type of amino-acid residue. Certain ribosomally synthesized antibiotic peptides show an exceptionally broad spectrum of activity against Gram-negative and -positive bacteria, fungi as well as viruses and parasites. Therefore, They or their derivatives may represent potentially new classes of antimicrobial drugs. At present, a few of them are in commercial development and some have advanced into clinical trials.<sup>3–5</sup>

There are several indications that new approaches are required to combat emerging infections. One is the pattern in the death rate from infection, for example, from 1900 to 1980, the rate dropped from 797 to 36 per 100 000 people.<sup>6</sup> However, owing to HIV and spread of drug-resistant bacteria, the death rate became double from 1980 to 2000. A second indication of the need for novel antibacterial therapeutics is the almost 40-year innovation gap between introductions of new molecular classes of antibiotics.<sup>7</sup> Third is the recent trend followed by pharmaceutical companies to leave the antibacterial and antifungal fields, suggesting a future decrease of developing new antibacterial drugs.<sup>8,9</sup>

### Importance of peptides

Many infectious diseases have been known for thousands of years, but over the past few years the number of infections (new) has increased. Some examples include the Lyme disease caused by *Borrelia*

*burgdorferi*, Legionnaires' disease caused by *Legionella pneumophila*, peptic ulcers caused by *Helicobacter pylori*, antibiotic-associated diarrhea caused by *Clostridium difficile* and AIDS caused by human immunodeficiency virus. In addition, microorganisms are constantly evolving, finding new places to live and new ways to survive, and adapting to new situations. In this process, microorganisms may shift their host or become pathogenic from non-pathogenic. Thus, it is important to continue to find anti-infective agents that can be used to treat these infections. Development of novel classes of drugs, drugs with fewer side effects and drugs with shorter lengths of treatment are key in continuing the fight against infectious disease.

### Understanding the biological process to devise new novel antibiotics

The aims of new criteria for successful anti-infective chemotherapeutics should be to preserve the efficiency of each anti-infective agent as long as possible by delaying the emergence of drug resistance and to spare the normal microbiota as much as possible. The normal microbiota are thought to be invaluable allies to prevent pathogenesis and protect their niches from new competitors, allow the sustainability of the species diversity that impedes virulence<sup>10</sup> and by helping to preserve the integrity and function of the epithelia and the immune system.<sup>11,12</sup>

Anyhow, killing all pathogens with an extrinsic antimicrobial agent may not be necessary or even desirable. For example, the eponymous gold pigment of *Staphylococcus aureus* is a carotenoid that protects the bacterium from oxidative injury by host immune cells. Interruption of bacterial pigment synthesis makes the pathogen much easier for host cells to kill but does not impair bacterial growth *in vitro*.<sup>13</sup> Thus, just stopping the spread of the pathogen would be sufficient for the host microbiota to prevent the invader.

### CLASSIFICATION OF ANTIMICROBIAL PEPTIDES

Other than microbes there are a wide variety of sources including humans, animals and plants that are being classified in four groups, that is:

1. Cationic peptide enriched with a specific amino acid: these are rich in proline, arginine, tryptophan, phenylalanine and glycine. For example, abaecin, apidaecin from honeybees and indolicidine from cattle (Table 1).
2. Linear cationic  $\alpha$ -helical peptides: these do not have cystein residues. For example, cecropins, andropin, moricin and mellitin from insects (Table 1).
3. Anionic peptide: these are rich in glutamic acid and aspartic acid. For example, maximin H5 from amphibian and dermcidin from humans (Table 2).
4. Anionic and cationic peptised having cysteine: contain the 1–3 disulfide bond. For example, brevinins, protegrin and defensins (Tables 1 and 2).

Apart from already mentioned peptides, there are neutral antimicrobial peptides that are less active than the cationic peptides and the anionic peptide complexed with zinc (Table 3). The amino-acid sequences are mentioned in Table 4.

### PROPERTIES OF RIBOSOMALLY SYNTHESIZED ANTIMICROBIAL PEPTIDES

#### Transmembrane potential

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. A fundamental difference between microbial and mammalian cells can be found in the charge separation between the extracellular and intracellular aspects of the cytoplasmic membrane. This electrochemical gradient, resulting from differing extents and rates of proton flux across the membrane, is termed the transmembrane potential ( $\Delta\psi$ ). The difference in  $\Delta\psi$  between certain microorganisms and host cells may provide a means of selective targeting of microorganisms by cationic antimicrobial peptides. For example, normal mammalian cells exhibit a  $\Delta\psi$  ranging from  $-90$  to  $-110$  mV. However, bacterial pathogens in the logarithmic growth phase commonly exhibit  $\Delta\psi$  of  $-130$  to  $-150$  mV. Such significant differences in membrane electrochemistry have been hypothesized as additional parameters guiding selective toxicity of antimicrobial peptides, through a mechanism that has been termed a self-promoted uptake.<sup>14</sup>

The carboxyl-terminal modification of indolicidin increases its antimicrobial activity against both Gram-negative and -positive bacteria presumably by increasing its net positive charge. This was consistent in part with subsequent outer membrane permeabilization by the carboxyl-terminal modification.<sup>15</sup> On the other hand, many positive charges can lead to a loss of activity. For example, the reduction in the activity of magainin analogs is either due to increased repulsion among peptide monomers or due to strong peptide association with the anionic lipids. The positive charges are also responsible for the specificity of the peptide toward the target membrane, as difference in only one charge can lead to marked differences in properties of pardaxin and indolicidin.<sup>16</sup>

The usually higher, negative-inside, transmembrane potential found in bacteria promotes their interaction while acting as a potential driving force for peptide insertion and translocation.

#### Signaling capacity

Antimicrobial peptides can have a possible role in cell signaling also. As human defensins (HNP-1, HNP-2 and NP-3) are potent inhibitors of protein kinase C, they do not have any role in other kinases.<sup>17</sup> The molecule, first being discovered as the epidermal growth factor receptor kinase inhibitor was also found to have antibacterial properties.<sup>18</sup> Another fact suggesting a possible role of antimicrobial peptides in the modulation of signaling pathways is the identification of defensins in lymphocyte nuclei. Defensins (HPN1–3) have also been found to be associated with the lymphocyte nuclear fraction, suggesting a cell function and regulation for these peptides.<sup>19</sup> PR-39 is a proline- and arginine-rich peptide that can bind to the adapter protein p130 (Cas)<sup>20</sup> involved in various cellular processes including cell adhesion, migration and transformation.<sup>21</sup> Moreover, it binds a subunit of phosphoinositide 3-kinase and inhibits kinase activity.<sup>20</sup> These natural antibiotics can also modulate the immune response through paracrine or endocrine pathways.

The cell-to-cell communication involved in these processes is dependent on the usage of small-signal molecules produced and released into the environment by the microorganisms. These communication molecules are referred to as pheromones and act as chemical messengers that transmit information across space. Explanation of the chemical structure of these pheromones reveals that most of them are unmodified peptides, post-translationally modified peptides, *N*-acyl homoserine lactones or butyrolactones. Lactone-based pheromones are the preferred communication signals in Gram-negative bacteria,<sup>22</sup> whereas peptide-based pheromones are the predominant extracellular signals among Gram-positive bacteria.<sup>23</sup>

**Table 1** Other sources of cationic ribosomally synthesized antimicrobial peptides

Name	Sources	Characteristics
Abaecin	<i>Apis mellifera</i> L., honeybee	34, Proline rich, highly active against an apidaecin-resistant <i>Xanthomonas</i> strain. Gram-positive and Gram-negative bacteria. <sup>89</sup>
AcAMP	<i>Aspergillus clavatus</i> ES1	94, 6 kDa size, cysteine rich, sensitive to proteolytic enzymes, thermostable, antifungal and antibacterial, biological control of plant diseases. <sup>71</sup>
Amino acid-enriched class	Insects, arthropods, mammals	Depending on the organism they are derived from.
Proline and glycine		
Cystine		
Histidine		
Antiviral protein Y3	<i>Pleurotus citrinopileatus</i> , golden oyster mushroom	97, Antiviral against TMV and antitumor against stomach cancer cells <i>in vitro</i> . <sup>90</sup>
Apidaecin IA	<i>Apis mellifera</i> L., honeybee	18, Rich in proline and active against Gram-negative bacteria. <sup>91</sup>
Aurein	<i>Litoria aurea</i> , <i>Litoria raniformis</i> , frog	13, Helix, active against HIV virus, fungi, cancer, Gram-positive and Gram-negative bacteria. <sup>92</sup>
Bactenecin	<i>Bos taurus</i> , cow bovine neutrophils	12, Bridge formation, S-S bond leads to cyclic dodecapeptide, act more against Gram-negative bacteria than the linearized version acting against Gram-positive bacteria. <sup>93</sup>
Bactericidin B2	<i>Manduca sexta</i> , tobacco hornworm	37, Induces synthesis of mRNA within 5 h of induction, act against Gram-positive, Gram-negative and mammalian cells. <sup>94</sup>
Beta-hairpin	Both vertebrates and invertebrates	2–8 kDa, contain beta-hairpin crosslinked by disulfide bridges.
Brevinin	<i>Rana brevipoda porsa</i> from Japan	24, Active against Gram-positive, Gram-negative, viruses and mammalian cells. <sup>95</sup>
Buforins	<i>B. bufo</i> , toad stomach	39, 90% Homology to histone H2A, contains helix-hinge-helix domain, anti-endotoxin and anticancer effect. <sup>96,97</sup>
Caerin 1.1	<i>Litoria splendid</i> , <i>Litoria rothii</i> , green tree frog	25 Helix, two well-defined helices from L2-K11 and V17-H24, inhibits HIV infection with little effect on T cells. <sup>98</sup>
Catestatin	<i>Homo sapiens</i>	21, Highly conserved across species, three single-nucleotide polymorphisms G364S, P370L and R374Q, P370L being most active, upregulated on skin injury and is a part of the cutaneous immune system. Chemotactic, fungicidal and bactericidal in action. <sup>99</sup>
Cecropins	Hemolymph of insects	3–4 kDa linear ampipathic peptide, no cysteine residue, two distinct helical segments. <sup>100</sup>
Ceratotoxin A	<i>Ceratitis capitata</i> , female reproductive accessory glands of medfly	29, Heat stable, highly basic, amphiphilic and active against Gram-positive and Gram-negative bacteria. <sup>101</sup>
Clavanins	Salmonids, fish	18, Anion-binding residue, iron-binding residue, cysteine residue for disulfide bonds. <sup>102</sup>
Conolysin-Mt	<i>Conus mustelinus</i> , weasel cone	23, Hemolytic to human erythrocytes, also called moonwalker peptide. Cytolytic to both prokaryotes and eukaryotes. <sup>103</sup>
Cupiennin ID	<i>Cupiennius salei</i> , spiders	35, Active against Gram-positive bacteria, Gram-negative bacteria and insects. <sup>104</sup>
Defensins	Molluscs, acari, arachnids, insects, mammals and plants	48 kDa, highly complex, open ended, cysteine rich, three and four disulfide bridges.
Dermaceptin B2	<i>Phyllomedusa bicolor</i> , south American frog	33, Helix, may contain D-amino acids, works against Gram-positive, Gram-negative bacteria, fungi and cancer cells. <sup>105</sup>
Discodermin A	<i>Discodermia kiiensis</i> , sea sponge	14, Tetradecapeptide containing D-amino acids, antitumor and toxic. <sup>106</sup>
Drosocin	<i>Drosophila</i> , fruit fly	19, Proline rich, small size, active against Gram-positive and Gram-negative bacteria. <sup>107</sup>
EcAMP 1	<i>Echinochloa crus-galli</i> , seeds	37, Helix, hairpin-like peptides, two S = S bonds, active against a variety of fungi. <sup>108</sup>
EcAMP 2	<i>Echinochloa crus-galli</i> L	31, Contains two S = S bonds, inhibits <i>Phytophthora infestans</i> and also many protists. <sup>109</sup>
EcLTP	<i>Echinochloa crus-galli</i> L	30, Lipid transfer proteins, active against Gram-positive as well as protest along with <i>P. infestans</i> . <sup>109</sup>
Esculentin 1	<i>Rana esculenta</i> , edible frog	46, Helix, residue 1–8 is an antimicrobial region, inhibits Gram-positive bacteria, Gram-negative bacteria, insects and mammalian cells.
Gomesin	<i>Acanthoscuria gomesiana</i> , spider hemocytes	18, Beta, bound to cell surface, N-terminal residue is a polyglutamate, disulfide bonds, works against Gram-positive bacteria, Gram-negative bacteria, fungi, parasites, cancer cells and mammalian cells. <sup>110</sup>
Hedistin	<i>Nereis diversicolor</i> , Coelomocytes	Contains bromo-tryptophans, activity against MRSA and <i>Vibrio alginolyticus</i> , present in NK cells, C-terminal amidation. <sup>111</sup>
Heliomicin	<i>Heliopsis virescens</i> , tobacco budworm	44, Combine helix and beta structure, three disulfide bridges, peptide expression in tobacco improves resistance to pathogens, inhibits <i>C. albicans</i> and <i>P. pastoris</i> . <sup>112</sup>
Hepcidin-25	<i>Homo sapiens</i> , liver of humans	25, Cysteine rich, master regulation of iron metabolism, antifungal activity, hairpin structure with four disulfide bonds.
Hinnavin II	<i>Artogeia rapae</i> , larval hemolymph of cabbage butterfly	38, 4.14 kDa, synergises with lysozymes, 78.9% identity with cercopin A, more active against Gram-negative than Gram-positive bacteria. <sup>113</sup>
Histone-derived compounds	Toad and fish epithelia	Structurally similar to cecropins, active against fungi and bacteria.
Hydracin	<i>Hydra vulgaris</i> , hydra	85 a.a, a disulfide bridge-stabilized $\alpha\beta$ motif, belt of positive residues sandwiched by hydrophobic areas, scaffold of knottin protein fold, active against bacteria with multiresistant-human pathogens. <sup>114</sup>
Im-1	<i>Isometrus maculatus</i> (scorpion), venom	56, helix, original thionin mutant was replaced to form this peptide, inhibits Gram-positive, Gram-negative and insects. <sup>115</sup>
Indolicidin	Bovine neutrophils	13, Tryptophan rich, bactericidal, carboxy terminal is amidated, so called tridecapeptide amide. <sup>116</sup>
KLAL	—	18 Residues, model compounds for potentially amphipathic $\alpha$ -helical peptides, random coil structure, $\beta$ -form in appropriate conditions, $\alpha$ -to- $\beta$ transition is a key step in Alzheimer's disease initiation and insoluble fibril formation, exhibits both antimicrobial and hemolytic activity. <sup>117</sup>
Lactoferricin B	<i>Bos taurus</i> , cow	25, Released by pepsin cleavage of lactoferrin, suppress candida cell growth and activate neutrophils. Inhibits Gram-positive bacteria, Gram-negative bacteria, HIV and cancer cells. <sup>118</sup>
Lumbricin I	<i>Lumbricus rubellus</i> , earthworm	62, Proline rich, active against a broad spectrum of microorganisms with hemolytic activity. <sup>119</sup>

Table 1 (Continued)

Name	Sources	Characteristics
Lycotoxin I	<i>Lycosa caroliensis</i> , wolf spider	25, Helix, amphipathic $\alpha$ -helical, inhibits Gram-positive bacteria, Gram-negative bacteria and insects. <sup>120</sup>
Magainins	<i>Xenopus</i> , frog	Magainin1 and magainin2—23 each, amphiphilic, nonhaemolytic peptides, analogs to hormones in GIT or CNS of mammals, small size, active against bacteria, fungus and protozoans. <sup>121</sup>
Maximin 1	<i>Bombina maxima</i> , Chinese red belly toad	27, Active against <i>E. coli</i> , <i>S. aureus</i> and has spermicidal effect, also inhibits Gram-positive bacteria, Gram-negative bacteria, viruses, fungi, cancer cells and mammalian cells. <sup>122</sup>
Mellitin	<i>Apis mellifera</i> , <i>A. cerana</i> , honeybee venom	26, Helix, forms tetramer at high peptide and salt concentration, $\alpha$ -helix with a bend between residues 11–15, inhibits HIV by suppressing viral gene expression, also active against colistin-resistant <i>A. baumannii</i> . <sup>123</sup>
Meucin	<i>Mesobuthuseupeus</i> , scorpion venom	Meucin-13 is 13 a.a and meucin-18 is 18 a.a long, similar to peptides of frog skin, mast cells, etc., $\alpha$ -helical amphipathic design, cytolytic effect on cells, meucin-18 being more potent. <sup>124</sup>
Myticin C	<i>Mytilus galloprovincialis</i> , mussel hemocytes	40, Four intramolecular disulfide bridges, highly polymorphic mRNA, antiviral and immunoregulatory, cysteine rich, has allelic variants, active against Gram-positive and Gram-negative bacteria, antifungal and chemotactic. <sup>125</sup>
Novicidin	Bovine cathelicidin SMAP-29, domestic sheep	18 a.a, derived from previous highly toxic antimicrobial peptide ovispirin, hydrophobic moment, acts by toroidal pore formation. <sup>126</sup>
Oncorhynchin III	<i>Oncorhynchus mykiss</i> , rainbow trout	6.7 kDa, 70 residue, one double methylation, active against bacteria in submicromolar concentrations, sensitive to NaCl. <sup>127</sup>
Paenibacillin	<i>Paenibacillus polymyxa</i> , Gram-positive bacteria	30, Lantibiotic with N-terminal acetyl group, against Gram-positive bacteria including MRSA, <i>Listeria monocytogenes</i> , and <i>Mycobacterium smegmatis</i> , acts by cytolysis. <sup>128</sup>
Parasin I	<i>Parasilurus asotus</i> , skin of cat fish	19, Amphipathic, 11% $\alpha$ -helical, 33% $\beta$ -sheet and 56% random coils, active against Gram-positive, Gram-negative bacteria and fungi. <sup>129</sup>
Pardaxins	<i>Pardachirus marmoratus</i> red sea mosses sole, teleost	35, Toxic and cytolytic, hydrophobic, rich in aspartic acid, serine, glycine and alanine. <sup>130</sup>
Perinerin	<i>Nereis aibuhitensis</i> , clamworm	51, 5.97 kDa, highly basic and hydrophobic, active against bacteria and fungi, serve as evaluation marker to study biochemistry of the host. <sup>131</sup>
Piscidin	Mast cells of fish	22, Rich in histidine and phenylalanine, amphipathic $\alpha$ -helical conformation, hemolytic activity. <sup>132</sup>
Ponerin G1	<i>Pachychondyla goeldii</i> , ants	30, Helix, adopt an amphipathic $\alpha$ -helical structure on cell membrane, inhibits Gram-positive bacteria, Gram-negative bacteria, fungi and insects. <sup>133</sup>
PR-39	<i>Sus</i> , pig bone marrow	173, 49% Proline and 24% arginine, important in innate immunity, can alter mammalian cell gene expression and behavior. <sup>134</sup>
Prolixicin	<i>Rhodnius prolixus</i> , hemipteran insect (adult fat body and midgut tissue)	21 a.a, 11 kDa, two putative phosphorylation sites and no glycosylation sites, active against Gram-negative and Gram-positive bacteria. <sup>135</sup>
PW2	Synthetic phage display library	12, Non-helix beta, rich in tryptophan and proline, anticoccidial peptide, active against fungi and parasites. <sup>136</sup>
Pyrrhocoricin	<i>Pyrrhocoris apterus</i> , Sap sucking bug	20, Inhibits ATPase activity of DnaK, proline rich, active against bacteria, fungi, parasites, insects and rodents. <sup>137</sup>
Ranalexin	<i>Rana catesbeiana</i> , bull frog	20, Contains intramolecular disulfide bond forming a heptapeptide ring, resembles polymyxin in structure, amphipathic in nature. <sup>138</sup>
Ranateurin	<i>Rana luteiventris</i> , <i>Rana pletiosa</i> , north American frog	28 a.a, contains a disulfide bridge, inhibits HIV at a concentration also toxic to T cells. Toxic to Gram-positive, Gram-negative bacteria and viruses. <sup>139</sup>
Strongylocin 1	<i>Strongylocentrotus droebachiensis</i> , sea urchin	83 a.a, 5.6 kDa, cysteine rich, three sulfide bridges, active against Gram-positive and Gram-negative bacteria. <sup>140</sup>
Stylisin 2	<i>Stylissa caribica</i> , Jamaican sponge	Proline rich, cycloheptapeptide, active against <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumonia</i> and <i>Candida albicans</i> , anticancer, antimalarial and anti-HIV. <sup>141</sup>
Tachyplepsin I	<i>Tachypleus tridentatus</i> , horse crab	17, $\beta$ -hairpin conformation stabilized by two disulfide bonds, active against Gram-positive and Gram-negative bacteria. <sup>142</sup>
Temporin	<i>Rana temporaria</i> , European common frog	13, C-terminal amidation, hydrophobic residues at position 1, 5 and 12. Active against Gram-positive, Gram-negative bacteria, viruses and parasites, chemotactic. <sup>143</sup>
Thionin	Plants	5 kDa, 6–8 conserved cysteine residues, toxic to plant pathogens and fungi.

Abbreviations: CNS, central nervous system; GIT, gastrointestinal tract; HIV, human immunodeficiency virus; MRSA, methicillin-resistant *S. aureus*; TMV, tobacco mosaic virus.

### Ribosomally synthesized antibiotic peptide as mitogens

Initial evidence that antimicrobial peptides could possess mitogenic properties was provided by Murphy *et al.*<sup>24</sup> Defensins may perform a double role in skin injuries. Besides preventing microbial infection, they may also be involved in a wound healing process, which was found to be mediated by the mitogen-activated protein kinase pathway. The presence and expression of antimicrobial peptides is not restricted to particular cells or surfaces, as exemplified by granulocytes and epithelial cells in mammals. Moreover, biopsies of renal carcinoma tissue revealed the intracellular expression of these defensins and renal carcinoma cell lines studied *in vitro* demonstrated that defensins were present at a concentration not exceeding 25  $\mu\text{g ml}^{-1}$ , which stimulated DNA synthesis.<sup>25</sup> This may suggest a possible role of defensin proliferation of either normal or tumor cells in the kidney.

A cathelicidin was found first in the skin during analysis of wound fluid components.<sup>26</sup> Porcine wound fluid contains high levels of a molecule that induces synthesis of syndecans-1 and 4, proteoglycans involved in key aspects of wound repair. Purification and sequencing of this molecule revealed a peptide identical to proline/arginine-rich cathelicidin PR-39, an antimicrobial molecule previously identified in the pig gut and neutrophil azurophilic granules.<sup>27</sup>

The expression of a second cathelicidin, LL-37, was found in keratinocytes of human skin having inflammation. LL-37 is expressed at involved sites of psoriasis, nickel contact dermatitis and systemic lupus erythematosus. The absence of detectable LL-37 expression in uninflamed skin, suggested that this antimicrobial peptide functions mainly in injured cells rather than in modulating the surface colonization of an intact epithelium.<sup>28</sup>

**Table 2 Other sources of anionic ribosomally synthesized antimicrobial peptides**

Name	Sources	Characteristics
Aromatic dipeptides	Dipteran larvae, flesh fly, saw fly	573 Da, low MW.
Aspartic acid-rich molecules	Cattle pulmonary surfactants	Similar to charge-neutralizing propeptides, regulate activity of pulmonary enzyme systems in animals.
Citropins	<i>L. citropa</i> , Australian Blue Mountain tree frog	16, Simplest amphibian wide-spectrum antibacterial peptides. <sup>144</sup>
Neuropeptide-derived molecules	Infectious exudates of cattle and humans	From processed neuropeptide precursor to yield active peptide B and enkelytin, some are phosphorylated.
Oxygen-binding proteins	Hemolymph of arthropods and annelids, tick hemolymph	Derived from hemocyanin derivatives.

**Table 3 Other sources of neutral ribosomally synthesized antimicrobial peptides**

Name	Sources	Characteristics
Alloferron	<i>Calliphora vicina</i> , blow fly	13, Rich in glycine and histidine, active against viruses and cancer cells. <sup>145</sup>
Andropin	<i>Drosophila melanogaster</i> , fruit fly	34, Expressed in the adult male ejaculatory duct, less fungicidal than cercopins, inhibits Gram-positive bacteria. <sup>146</sup>
Gramicidin A	<i>Bacillus brevis</i> , soil bacteria	15, Helix, active for HIV and HSV infections other than Gram-positive and Gram-negative bacteria.
Kalata B1	<i>Oldenlandia affinis</i> , African herb	29, Beta, G6 is critical to bind phosphatidyl ethanolamine, active against viruses, insects, HIV and mammalian cells. <sup>147</sup>

### Antitumor activity

A growing number of studies have shown that some of the cationic antimicrobial peptides, which are toxic to bacteria but not to normal mammalian cells, exhibit a broad spectrum of cytotoxic activity against cancer cells. Such studies have considerably enhanced their significance, both synthetic and from natural sources, which have been of importance both for an increased understanding of the immune system and for their potential as clinical antibiotics. The basic difference between the cell membranes of malignant cells and normal cells accounts for the ability of certain antimicrobial peptides to show cytotoxic effects against the cancer cells (anticancer peptides) without causing any harm to the healthy cells.<sup>29</sup> Antimicrobial peptides have the capacity to interact with the negatively charged molecules that are present in the target, but tumor cells are different in their membrane composition.<sup>30</sup> The membrane fluidity of cancer cells is greater than that of untransformed cells, which may enhance the lytic activity of anticancer peptides by facilitating membrane destabilization. A large number of microvilli on the surface of cancer cells accounts for the greater surface area than normal cells; this may

allow them to bind to higher number of anticancer peptides. The microvilli are also more irregular in size and shape, which may be due to changes in structural proteins.<sup>29</sup> Defensins are also found to be effective against tumor cells by inducing tumor cell lysis, so the replacement of certain amino acids from L to D isomers in the peptide chain or modifying the peptide terminal (for example, amidation) will solve the problem and increase the compound's tumoricidal activity.<sup>31</sup> Putting the gene coding for the ribosomally synthesized antimicrobial peptide into the tumor cell can avoid this type of loss in activity.

Magainins are a group of cationic peptides derived from the skin of amphibians that are able to lyse many types of tumor cells at a concentration 5–10 folds lower than that for normal cell lysis.<sup>32</sup> Baker *et al.*<sup>33</sup> showed that magainin2 and magainins synthetic analogs exerted their antitumor activity toward murine ascites tumors, leukemia and spontaneous ovarian tumor cells, both *in vitro* and *in vivo*. All D-amino-acid analogs of magainin were found to be as effective as doxorubicin toward ovarian tumor cells. Insect-derived peptides, cecropins, are also able to lyse tumor cells. *In vitro* experiments proved their effectiveness in cell lines with multidrug-resistant phenotypes, where they destroyed tumor cells within 1 h. Expression of antimicrobial peptides has been reported in several tumor cell lines, where depending on their concentrations, they exert mitogenic or necrotic activity.<sup>31</sup>

### MECHANISM

Many antimicrobial peptides bind in a similar manner to negatively charged membranes and permeate them, resulting in the formation of a pathway for ions and solutes, but before reaching the phospholipid membrane it should cross the outer wall of the bacteria containing polysaccharides. However, a key weakness is that cationic charge leads to early recognition that salts close to physiological concentrations block antimicrobial peptide interaction with negatively charged surface groups.<sup>34</sup> In this mechanism, the peptides initially interact with the surface lipopolysaccharides, competitively displacing the divalent polyanionic cations and partly neutralize lipopolysaccharides. This causes disruption of the outer membrane and peptides pass through the disrupted outer membrane and reach the negatively charged phospholipid cytoplasmic membrane. The membrane-active properties of such peptides have been extensively studied using model membranes.

The interactions between an antimicrobial peptide and membranes, can be studied either by an *in vivo* approach using intact cells or, alternatively, by extensive use of the *in vitro* experiments with isolated or model membranes. Ribosomally synthesized peptides interact with microbial membranes, resulting in two possible modes of action, depending on the peptide and the microbial species. The peptides can be membrane-disruptive resulting in cell lysis or, alternatively, membrane interaction can lead to the formation of transient pores and the transport of peptides inside the cell, bringing them into contact with intracellular targets. Three models of peptide insertion and membrane permeabilization are reported: toroidal pore, carpet and barrel stave (helical bundle model). Following attraction,  $\alpha$ -helical peptide,  $\beta$ -sheet peptides and  $\theta$ -defensins are inserted into the lipid bilayer in accord to the peptide/lipid ratio and to one of these three models (reviewed by Brodgen).<sup>35</sup> In the 'toroidal-pore model',  $\alpha$ -helices of antimicrobial peptides such as magainin, protegrin and melittin insert into the membrane and induce lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid-head groups. In the 'carpet model', antimicrobial peptides accumulate on the lipid



**Table 4 Amino-acid sequence of the peptides**

<i>Origin</i>	<i>Name (antimicrobial peptide)</i>	<i>Amino-acid sequence</i>
Cationic	Abacin	YVPLPNVPQGRPFPTFGQGFNPKIKWPQGY
	AcAMP	MKVVSLASLGFALVAALGVAASVPVDADSLAAGGLDARDESAVQATYDGKCYKDKDNICKYKAQSGKTAICKCYVK VCPRDGAKCEFDSYKGCYC
	Antiviral protein Y3	AACARFIDDFCDTLTPNIYRPRDNGQRCYAVNGHRCDFTVFNTNNGGNPIRASTPNCKTVLRTAANRCPTGGRG KINPNAPFLFAIDPNDGDCSTNF
	Apidaecin IA	GNNRPVYIPQPRPPHRI
	Aurein 1.1	GLFDIHKIAESI
	Bactenecin	RLCRIVVIRVCR
	Bactericidin B2	WNPFKELERAGQVRDAVISAAPAVATVGQAAAIARG
	Brevinin	FLPVLAGIAAKVVPALFCKITKCC
	Buforins	AGRKGQGGKVRAKAKTRSSRAGLQFPVGRVHLLLRKGNV
	Carein 1.1	GLLSVLGSAKHVLPHPVPIAEHL
	Catestatin	SSMKLSFRARAYGFRGPGPQL
	Cecropins	KWKLFKIKFLHSAKFF
	Ceratotoxin A	SIGSALKKALPVAKKIGKIALPIAKAALP
	Conolysin-Mt	FHPSLWVLI PQYIQLIRKILKSG
	Cupiennin ID	GFGLFKFLAKKVAKTAKQAQKQAKYVANKHME
	Dermaceptin B2	GLWSKIEVKGKAAKAAKAAAGKAALGAVSEAV
	Drosocin	GKPRPYSRPTSHPRPIRV
	EcAMP 1	GSGRGSCRSQCMRRHEDEPWRVQECVQCRRRRGGGD
	EcAMP 2	DRCSQQCQHHRDPDRKQCMRECRRHQGRSD
	EcLTP	AISCGQVSSAIGPCLSYARGQGSAPSAGCC
	Esculentin 1	GIFSKLGRKKIKNLLISGLKNVKGVEGMDVVRTGIDIAGCKIKGEC
	Gomesin	ECRRLCYKQRCVTYCRGR
	Heliomicin	DKLIGSCVWGAVNYTSDCNGECKRRGYKGGHCGSFANVNCWCET
	Hepcidin-25	DTHFPICIFCCGCCRRSKCGMCCCT
	Hydramacin	MRSIVGFIVSIFLVALMPSDTQAGTLLGCWETWSRCKTWSQRGTGILWKNCNNRCKELGHSSGL CEKRRSKCRFSKNAWACICY
	Im-1	FSFKRLKGFALKLWNSKLARKIIRTGLKYVKNFAKDMLSEGEAEPPAEPPVEAPQ
	Indolicidin	ILPWKWPWWPWR—NH <sub>2</sub>
	KLAL	KLALKLALKALKAAALKLA—NH <sub>2</sub>
	Lactoferricin B	FKCRRWQWRMKKLGAPISITCVRRAF
	Lumbricin I	FSKYERQDKRPSYERKNQYTPQFLYPPERIPPQKVIKWNEEGLPIYEIPGEGGHAEPAAA
	Lycotoxin 1	IWLTALKFLGKHAAKHLAKQLSKL
	Magainins	Magainin1—GIGKFLHSAGKFGKAFVGEIMKS Magainin2—GIGKFLHSAKKFGKAFVGEIMNS
	Maximin 1	GIGTKILGGVKTALKGALKELASTYAN
	Melittin	GIGAVLKVLTGLPALISWIKRKRQQ
	Meucin	Meucin-13—IFGAIAGLLKNIF-NH <sub>2</sub> Meucin-18—FFGHFLKATKIIPSLFQ
	Oncorhynchin	MPKRKATKGDPEARRSARLSARPVPKPAKPKKAAAPKAVKGGKAAENGDAKAEAKVQAAGDGAGNAK
	Parasin I	KGRGKQGGKVRAKATRSS
	Paenibacillin	ASIIKTTIKVSKAVCKLTCTCTGSCSNCK
	Perinerin	FNKLGKQSSKRTCAKCFRKIMPVHELDERRRGANRWAAGFRKCVSSICRY
	Ponericin G1	GWKDWAKKAGGWLKKKPGMAKAAKAAAMQ
	PW2	HPLKQYWRPSI
	Pyrrhocoricin	VDKGSYLPRPTPPRIYNNR
	Ranalexin	FLGGLIKIVPAMICAVTKCC
	Ranateurin	GLMDTVKNVAKNLAGHMLDKLCKITGC
	Tachyplesin I	KWCFRVCYRGICYRRCR
	Temporin	FLPLIGRVLGSL
	Anionic	Citropins
Neutral	Alloferon	HGVSGHGQHGTVHG
	Andropin	VFIDILDKVENAIHNAAQVIGIFAKPFKELINPK
	Gramicidin A	VGALAVVWLWLWLW
	Kalata B1	GLPVCGETCVGGTCNTPGCTCSWPVCTR

bilayer surface, which are orientated in the plane of membrane. When high concentration is attained, peptides re-orient and their aggregates get inserted into the membrane and disrupt it by producing micelles in a detergent-like manner. Antimicrobial peptides that act by the 'carpet model' are represented by cecropin, dermaseptin and melittin. Finally, in the 'barrel-stave model', antimicrobial peptide  $\alpha$ -helices form a bundle in the membrane, where the hydrophobic stretches of inserted peptides align with the lipid core region of the bilayer, and the hydrophilic peptide stretches form the pore lining. Thus, these three mechanisms contribute to form pores, channels and to provoke membrane disruption and cell killing.<sup>36</sup> Certain peptides may also be actively taken up by transporters.<sup>35,37</sup> Antimicrobial peptides can have multiple intracellular targets. They can bind DNA, RNA and proteins, inhibit cell wall synthesis and DNA, RNA or protein synthesis.<sup>38–40</sup> FtsZ, a conserved tubulin-like bacterial protein critical for division septum formation, has recently been identified as the intracellular target for an antimicrobial peptide.<sup>41</sup> Other known intracellular targets of antimicrobial peptides are DNA gyrase and the heat-shock protein DnaK. In addition, some peptides have non-protein targets such as the peptidoglycan precursor lipid II.<sup>42,43</sup>

### ANTIBIOTIC RESISTANCE

Microbes can occupy and exploit a vast variety of tissues and niches, but still microbial pathogens may be able to resist antimicrobial peptides. This can happen by constitutive (passive) or inducible (adaptive) mechanisms. The constitutive mechanism is an inherent property of the microorganisms and is expressed normally even when the peptides are not present in the vicinity. Many microorganisms resist killing by diverse types of microorganisms, for example, *Serratia* sp., *Proteus* sp. and *Burkholderia* sp. This may be due to the stable structure or functional property or pathogenesis. Cashman *et al.*<sup>44</sup> demonstrated that *Enterococcus* species exhibit broad resistance to a panel of cationic antimicrobial peptides and heavy metal ions. Furthermore, these investigators identified an inverse correlation between glycopeptide susceptibility and resistance to cationic antimicrobial peptides or oxidized metal ions among enterococcal clinical isolates.

Inducible mechanism or resistance is triggered in response to the antimicrobial peptide or any kind of stress caused due to them. Many pathogenic microbes have to come across a number of host defense systems and they have to deal with them in order to survive and grow. So many of them have evolved some or the methods to protect themselves from these systems. For example, in bacteria, inducible resistance to antimicrobial peptides is largely controlled through sophisticated sensor–transducer response systems. Among the first to observe this phenomenon were Fields *et al.*,<sup>45</sup> who determined that a specific genetic locus of the pathogen *Salmonella typhimurium* is integral to intracellular survival in macrophages and virulence in mice by conferring resistance to a defensin peptide. Oh *et al.*<sup>46</sup> demonstrated that sublethal levels of cecropin induce hyperosmotic stress response systems in *Escherichia coli*. However, sublethal concentrations of cecropin A prompted a pattern of genomic responses in *E. coli* that is distinguishable from that induced by lethal concentrations, and distinct from bacterial stress response systems such as heat-shock, hyperosmotic or oxidative response paradigms. The following (Figure 1)<sup>47</sup> shows the various mechanisms of resistance.

Human antimicrobial peptide LL-37 induces the *mefE* promoter and confers resistance to erythromycin and LL-37. Macrolide resistance is a major concern in the treatment of *Streptococcus pneumoniae*. Inducible macrolide resistance in *S. pneumoniae* is mediated by the efflux pump MefE/Mel. The MefE/Mel efflux pump

can further contribute to cathelicidin resistance and may thereby contribute to increased survival in the human host.<sup>48</sup> In *Drosophila*, the antifungal response is induced by the toll signaling pathway that is very similar to the dorso-ventral morphogenic pathway as well as to the acute phase response in mammals, which involves the cytokine interleukin-1. The antibacterial response involves a less extensively characterized *imd* gene (immune deficiency gene) system. In pigs cathelicidins are responsible to avoid bacterial proliferation in skin wounds.<sup>49</sup>

### NATURAL ALTERNATIVE TO CHEMICAL ANTIBIOTICS

Antimicrobial peptides are a defense mechanism that can remain over long evolutionary time spans and can act rapidly to neutralize a broad range of microorganisms, such as bacteria, fungi and viruses.<sup>50</sup> The most common mechanism of action against bacteria is the so-called 'carpet' mechanism, in which the peptide accumulates on the bacterial membrane up to a threshold concentration causing permeabilization/disintegration.<sup>51</sup>

In case of neonatal diarrheal disease in cattle, host defense antimicrobial peptides present in body secretions, such as tears and saliva, are naturally active against bacteria, viruses and parasites. They also stimulate an immune response against an infectious disease.<sup>52</sup> Unlike antibiotics, these are not specific to a particular disease, thus protect against a number of pathogens. Similarly, bacteriocins produced by many bacteria, for example, lactic acid bacteria (LAB) (produce a high diversity of bacteriocins), can target many food pathogens such as *E. coli* and *Salmonella* without affecting any other parameters of food. Moreover, consumption of food that is chemically preserved has created a need for more safe and naturally preserved food.

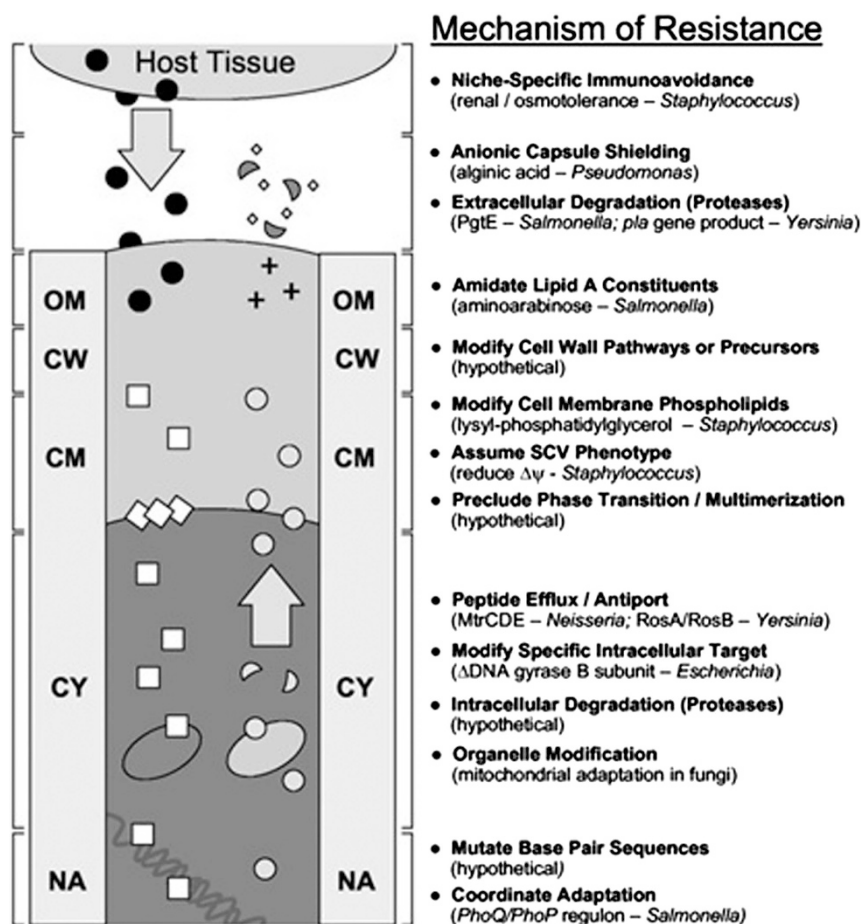
### SOURCES OF RIBOSOMALLY SYNTHESIZED ANTIMICROBIAL PEPTIDES

#### Bacteria

A wide range of bacteria are found to produce antimicrobial peptides. The peptides produced by bacteria are also termed 'bacteriocins'.<sup>47</sup> LAB can produce a number of ribosomally synthesized proteins and peptides, which have antimicrobial activity to compete with other bacteria of the same species as well as of other genera. The first description of bacteriocin-mediated growth inhibition was reported 85 years ago, when antagonism between strains of *E. coli* was first discovered.<sup>53</sup> The inhibitory substances were called 'colicins', to reflect the producer organism, whereas gene-encoded antibacterial peptides produced by bacteria are now referred to as 'bacteriocins'. Some of the bacteriocins being produced by LAB are listed in the Table 5 below.<sup>54</sup>

LAB also produces acidocin that is active against methicillin-resistant *S. aureus* and *C. difficile*, which are common hospital pathogens. All the antimicrobial peptides produced by LAB are heat stable, active at pH 3 to 6.5 and are effective against a number of Gram-positive and -negative pathogens. Several bacteriocins are produced by enterococci, especially by *E. faecium*. Till now, many enterococins from *E. faecium* have been characterized: enterocin A,<sup>55</sup> enterocin B,<sup>55</sup> enterocin P,<sup>56</sup> enterocin I,<sup>57</sup> enterocin Q<sup>58</sup> and enterocin M (a new variant of enterocin P).<sup>59</sup> In general, most enterococins belong to the class 2a bacteriocins, are like pediocin and are thermostable.

Peptides derived from bacteria are important antimicrobial compounds owing to their inherent ability to defend against potential pathogens and their low toxicity in mammalian cells.<sup>60</sup> The iturin and bacillomycin families produced by *Bacillus* species and viscosin, amphisin, tolaasin and syringomycin produced by *Pseudomonas* sp. all have cyclic peptidolipid structures and display antimicrobial



**Figure 1** Parallels in antimicrobial peptide mechanisms of action and resistance. OM, outer membrane; CW, cell wall; CM, cytoplasmic membrane; CY, cytoplasm; NA, nucleic acid. Specific examples of mechanisms of action or resistance, listed in parenthesis are representative only and not to be considered exhaustive.<sup>47</sup>

activity against a wide range of plant pathogens.<sup>61</sup> *Brevibacillus laterosporus*, previously classified as *B. laterosporus*,<sup>62</sup> can produce different virulence factors: parasporal crystalline,<sup>63</sup> extracellular protease<sup>64</sup> and lipopeptide antibiotics.<sup>65</sup> In addition, *B. laterosporus* also secretes short-sequence peptides with broad antibiotic spectra, such as loloatin A.<sup>66</sup> These antimicrobial peptides show thermostability in antimicrobial activity (90% activity at 100 °C for 15 min) and are unaffected by protease K, trypsin and pepsin after 60 min at 28 °C.<sup>67</sup> They are also of low MW and stable at vast pH range.

There are several spermicidal antimicrobial peptides being produced by bacteria such as Gramicidin A produced by the soil bacteria *B. brevis*. It is the first peptide antibiotic used clinically. It is 15-amino-acid long and helical in shape, synthesized non-ribosomally by a multienzyme complex. It is active against HIV and HSV infections along with Gram-positive, Gram-negative bacteria and other viruses. Amino acids 4,6,8,10 and 12 are D-forms, allowing formation of a special helical structure that dimerizes into a cation channel in the lipid bilayer with the C-terminal exposed.<sup>68</sup> Another peptide produced by *B. subtilis*, known as subtilisin A is also a 35-amino-acid helix containing an N and C-circulated peptide. It also contains three rare crosslinks between the S of cysteine 13, 7 and 4 and  $\alpha$ -C of phe 22, thr 28 and phe 31. It shows chemical modifications and three thioether links.<sup>69</sup> Similarly a peptide known as lactacin 3147 produced by *Lactococcus lactis* also comes in the spermicidal group with 30 amino acids, where two polypeptide chains work synergistically. When it

comes in contact with bacteria, A1 first binds to lipid II followed by binding of chain A2 leading to pore formation.<sup>70</sup>

### Fungi

Plectasin is the first defensin to be isolated from a fungus, the saprophytic Ascomycete *Pseudoplectanina nigrella*. Plectasin has primary, secondary and tertiary structures that closely resemble those of defensins found in spiders, scorpions, dragonflies and mussels. Recombinant plectasin was produced at a very high and commercially viable yield and purity. *Aspergillus clavatus* ESI produces a peptide AcAMP with high thermostability and antibacterial property. It is 6.0 kDa and shows characteristics common to the group of small, basic, cysteine-rich antifungal proteins from molds.<sup>71</sup> It is also pH tolerant and active against bacteria and fungi. The open-reading frame is of 282 bp encoding a peptide of 94 amino-acid residues consisting of a 21-amino-acid signal peptide, a 22-amino-acid pro-peptide and a 51-amino-acid mature peptide.

### Protozoans

Several antimicrobial peptides in *Hydra*, a simple Eumetazoan organism, living in fresh water ponds is found to possess antimicrobial peptides, which are highly active against human pathogenic bacteria. *Hydra* holds promise for the detection of novel antimicrobial molecules and the treatment of infections caused by the multiresistant bacteria. Hydramacin from *H. vulgaris* is isolated as a



**Table 5 Classification of bacteriocins produced by lactic acid bacteria<sup>54</sup>**

Category	Subcategory	Characteristics	Examples
Class 1 Lantibiotics, lanthionine containing	Type A	Elongated molecules: molecular mass <4 kDa	Nisin
	Type B	Globular molecules: molecular mass 1.8–2.1 kDa	Mersacidin
Class 2 Non-modified heat-stable bacteriocins	Subclass 2a	Molecular mass <10 kDa	Pediocin PA 1
	Subclass 2b		Plantaricin EF
	Subclass 2c		Lactococcin 972
Class 3 Protein bacteriocins		Molecular mass >30 kDa	Helveticin J

disulfide bridge-stabilized  $\alpha\beta$  motif, which is a scaffold of knottin protein fold.

Arminin 1a is a novel antimicrobial peptide discovered during investigations of the epithelial defense of the ancient metazoan *Hydra*. It is 31-amino-acid long, positively charged and the C-terminal part of arminin 1a exhibits potent and broad-spectrum activity against bacteria, including multidrug-resistant human pathogenic strains, such as methicillin-resistant *S. aureus* strains. Arminin 1a is a selective antibacterial agent that does not affect human erythrocyte membranes. It shows no sequence homology to any known antimicrobial peptide,<sup>72</sup> making it a candidate for further studies regarding antimicrobial properties.

### Insects

There have been a number of antimicrobial peptides isolated from insects. Insect defensins are 36–46-amino-acid long with the exception of the 51 residue bee and bumblebee defensins. These include the phormicins, sapecins, royalisin and spodoptericin. Defensins, were first reported from cell cultures of the flesh fly *Sarcophaga peregrine*. Some of the antifungal defensins are as follows: drosomycin from *Drosophila*; heliomyacin from the tobacco budworm *Heliothis virescens* (Lepidoptera), gallerimycin from the greater wax moth larvae *Galleria mellonella* (Lepidoptera), termicin from the isopteran *P. spiniger* and Alo13 from the harlequin beetle *Acrocinus longimanus*.<sup>73</sup>

Thanatin has been isolated from the spine soldier bug *Podisus maculiventris* containing 21 amino acids with a broad spectrum of activity, both Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts without measurable toxicity for red blood cells. It is a novel therapeutic agent against multidrug-resistant bacteria because of its different modes of action, small size and non-toxicity to eukaryotic cells.

Linear proline-rich antimicrobial peptides are isolated from insects such as, abaecins and apidaecins from bees and wasps, formaecins from ants, drosocins and metchnikowins from the *D. melanogaster*, lebecins from the silkworm *Bombyx mori*, the cabbage looper moth *Trichoplusia*, pyrrocoricin and metalnikowins from bugs.<sup>73</sup> Andropin is a peptide secreted by male ejaculatory duct of male *D. melanogaster*. It contains a hydrophobic amino terminus with striking similarity to the signal peptide of the cecropins. It is for the protection of the seminal fluid and the male reproductive tract against microbial infections. In *Drosophila*, the cecropin genes are found in a dense cluster of several genes and pseudogenes at 99E on the third chromosome, encoding at least two different types of cecropins. These are expressed mainly in the fat body and to some extent in hemocytes, and their antibacterial products accumulate in the hemolymph.<sup>74</sup> Drosocin is a novel 19-residue proline-rich inducible antibacterial peptide from *Drosophila*. The peptide has an *O*-glycosylation that is necessary for its full biological activity.

Four antimicrobial peptides were purified from the royal jelly of honeybees, called jelleines (I–IV). They are capable of antimicrobial activity, mast cell degranulating activity and hemolysis. The Jelleines I–III present antimicrobial activity against both Gram-positive and -negative bacteria. These peptides do not present any similarity with the other antimicrobial peptides from the honeybees and they are produced constitutively by the workers and secreted into royal jelly.<sup>75</sup>

Scorpion venom contains series of bioactive peptides, among which the antibacterial peptide is an important part, and a new antimicrobial peptide StCT2 is characterized from the venomous gland cDNA library of the *Scorpiops tibetanus*.<sup>76</sup> It is active against methicillin-resistant *S. aureus*, methicillin-resistant coagulase-negative *Staphylococci* and penicillin-resistant *S. aureus* and has both cationic and amphiphilic nature.

### Fishes

The peptide antibiotics isolated from fish, reside in mast cells, an immune cell of uncertain function. Piscidins from the tissues of an important aquacultured fish, hybrid striped bass (*Morone saxatilis* and *M. chrysops*). Piscidins are 22-amino-acid peptides with a highly conserved amino terminus that is rich in histidine and phenylalanine. The peptides probably adopt an amphipathic  $\alpha$ -helical conformation. Piscidins have potent, broad-spectrum antibacterial activity against fish pathogens, which is comparable to that of many peptides from aquatic animals. A 6.7 kDa antimicrobial peptide was isolated from trout skin secretions. The peptide is a potent antibacterial agent against both Gram-positive and -negative bacteria and is sensitive to NaCl.

Parasin I isolated from catfish *Parasilurus asotus* has a MW of 2000.4 Da, strong antimicrobial activity and has no hemolytic ability. It is 19 residues long and produced only when the fish is injured. Another antimicrobial peptide named Misgurin is isolated from loach, *Misgurnus anguillicaudatus*. It has 21 amino acids with a weight of 2502 Da and binds specifically to negatively charged phospholipids.

The cod cathelicidin antimicrobial peptide was isolated from the head and kidney of the Atlantic cod *Gadus morhua*, 67 amino acids long with serine, arginine and glycine predominance. It acts through rapid lysis of bacterial cells.

### Amphibians

Tachyplesins and their analogs are antimicrobial peptides composed of 17 or 18 amino-acid residues present abundantly in acid extracts of hemocyte debris of horseshoe crabs. Tachyplesins is isolated from two species of southeast Asian horseshoe crab *Carcinoscorpius rotundicauda* and *Tachyplesus gigas gigas*. Ranalexin is a novel antimicrobial peptide from the bullfrog *R. catesbeiana*. It is structurally similar to polymyxin.

Nineteen citropin peptides are present in the secretion from the granular dorsal glands of the Blue Mountain tree frog *Litoria citropa*; 15 of these peptides are also present in the secretion from the submental gland. Two major peptides, citropin 1.1, citropin 1.2 and a minor peptide, citropin 1.3 are wide-spectrum antibacterial peptides. Citropin 1.1 is an amphipathic  $\alpha$ -helix with well-defined hydrophobic and hydrophilic regions.<sup>77</sup> These peptides show minimal antibacterial activity; their role in the amphibian skin is not known.

### Reptiles

Cathelicidin-like antimicrobial peptide, named cathelicidin-BF, was isolated from the crude venom of the snake *Bungarus fasciatus*, commonly known as banded krait and the peptide sequence was characterized. It exerts strong and rapid antimicrobial activities against many microorganisms including Gram-negative, Gram-positive bacteria and fungi, especially some clinically isolated drug-resistant microorganisms. Besides, cathelicidin-BF has no hemolytic and cytotoxic activity on human cells.<sup>78</sup> It could significantly inhibit *Propionibacterium acnes*, induced secretion of several pro-inflammatory factors including TNF- $\alpha$ , interleukin-8, interleukin-1b and MCP-1.

*Naja* antimicrobial peptide purified from *Naja naja* venom is a potent antibacterial agent against Gram-negative organisms such as *E. coli*, *P. aeruginosa* and *Vibrio cholerae*, and Gram-positive bacterial strains such as *S. aureus*, *E. faecalis*, *S. pneumoniae*, *S. pyogenes* and *B. subtilis*. The most potent activity was toward Gram-negative bacteria. The amphipathic alpha-helical conformation has an important role in their biological activities.<sup>79</sup>

### Aves

Five bactericidal peptides (chicken heterophil peptides CHP1 and CHP2; turkey heterophil peptides THP1, THP2 and THP3) are purified from avian heterophil granules. All peptides are cationic and rich in cysteine, arginine and lysine. The complete amino-acid sequence, consist of 39 amino acids, as determined for CHP1. This peptide had a MW of 4481. They have antibacterial properties against *E. coli* and *S. aureus*. These peptides were extracted from cells pooled from numerous birds.<sup>80</sup>

Common pheasant *Phasianus colchicus* gives three deduced mature cathelicidin antimicrobial peptides, Pc-CATH1, 2 and 3 that are composed of 26, 32 and 29 amino-acid residues, respectively. Unlike the mammalian cathelicidins that are highly divergent even within the same genus, it shows hemolytic activity and serum stability. *Yak* also produces a peptide from polymorphonuclear leukocytes (PMN). The crude extraction from PMN was isolated, respectively, through a method of extraction by using 5% acetic acid. The crude extraction showed feeble hemolysis activity to red blood cells of sheep and rabbit. The crude extraction from PMN of yak showed feeble hemoblast anticoagulation activity to blood cell of rabbit, whereas to blood cell of sheep, they were less effective.

### Mammals

Humans express only one cathelicidin, a prepropeptide called hCAP-18. LL-37, a 37-residue  $\alpha$ -helical peptide at the carboxy-terminal domain of hCAP-18, is released after hCAP-18 is processed by neutrophil elastase. Human cathelicidin antimicrobial peptide, LL-37, is shown to exhibit potent activity against microbes and is salt sensitive. Protegrins are small (~2 kDa) broad-spectrum antimicrobial peptides found in porcine neutrophils, where they are stored as cathelin-containing precursors. Mature, fully processed protegrins contain 16–18 amino-acid residues and are released from their

precursors by neutrophil elastase.<sup>81</sup> Unlike the larger defensins, protegrins retain activity in physiological concentrations of sodium chloride, whereas truncated protegrins with 12 residues manifest potent antibacterial activity.

Granulysin, a peptide stored in the cytoplasmic granules of human natural killer cells and cytolytic T cells, was shown to kill *Mycobacterium tuberculosis*. The combination of granulysin and perforin-like molecules may equip T cells to kill many intracellular pathogens. Histatins are small, histidine-rich, human salivary proteins that display moderate activity against *Candida albicans*, especially at an acidic pH, under conditions of relatively low ionic strength. Because histatins have little or no toxicity and are active against azole-resistant fungal species, there are considerable efforts to design histatin-like peptides with enhanced activity and stability.<sup>82</sup> Histatins are not obviously membranolytic, and histatin-mediated killing may be mediated by binding to a 67-kDa *C. albicans* protein, which is found in lysates and crude membrane fractions.<sup>83</sup>

Antimicrobial beta-defensins, such as tracheal antimicrobial peptides in epithelial cells of the bovine respiratory tract and lingual antimicrobial peptides in the tongue, are stimulated by lipopolysaccharides, interleukin-1b and tumor necrosis factor. Defensins are widely distributed in mammalian epithelial cells and phagocytes, often in high concentrations. Other antimicrobial peptides are restricted to a few animal species and tissues, such as histatins, dermicidins and anionic peptides.

### Plants

Thionins constitute a family of basic peptides (pI > 8), with low MW (~5 kDa), rich in basic and sulfur-containing residues (arginine, lysine and cysteine). Its diverse members have high sequential and structural similarities besides presenting toxic effects against bacteria, fungi, yeast and animal and plant cells.  $\alpha$ -Purothionins are extracted from flour milled from hexaploid wheat. The  $\alpha$ 1 and  $\alpha$ 2 purothionin molecules each consists of 45 amino acids differing only at six positions.<sup>84</sup>

Plant defensins, which are also known as  $\gamma$ -thionins, are one of the most important and very well-studied class of antimicrobial peptides. With an approximate size of 5 kDa, they have been reported to show inhibitory activities against fungi, bacteria and insects. All plant defensins share a typical three-dimensional folding pattern, stabilized by four disulfide bridges, that incorporate the cysteine-stabilized  $\alpha\beta$  motif. The first floral defensin that was characterized structurally by NMR was NaD1, isolated from *Nepenthes alata*.

Lipid transfer proteins, a family of proteins is characterized by facilitating the lipid transfer among natural or artificial membranes and by linking to fatty acids *in vitro*. Owing to the accumulated information about plant lipid transfer proteins, various functions have been suggested for this family of proteins, as well as its participation in cuticle formation and embryogenesis.<sup>85</sup>

### CHALLENGES IN DEVELOPMENT OF RIBOSOMALLY SYNTHESIZED ANTIMICROBIAL PEPTIDES

Antimicrobial peptides have high level of chemical and biological diversity, are highly specific and are difficult to identify in a specific screen. They are small, but the amount of antimicrobial peptides required is much more compared with antibiotics.<sup>86</sup> Their potency is also not as strong. Although they do have good *in vitro* effects, they may have different *in vivo* effects as peptides with L-amino acids are potential immunogens.<sup>87</sup> They are active against multidrug-resistant strains at similar concentrations, are rapid and involve multiple targets, still production cost is not economically feasible and is nearly

5–20 times higher than typical antibiotic drugs. There is also a lack of knowledge in thoroughly examining the systemic peptide, pharmacodynamics and pharmacokinetics, thus its not patient compliant.

In the process of developing, the half-life of a peptide is very important and can be extended by modifications such as glycosylation, conjugation and converting D-forms of amino acids to L-forms.<sup>88</sup> For example, polyethylene glycol can conjugate with an amino acid and thus protect it from proteolysis by stearic hindrance. This can also be harmful, as more modification can lead to more resistance and thus avoid its contact with a specific target. Peptides may also be designed to fuse with serum albumin (as it is the most abundant protein in serum with 19 days life) with non-covalent association and increase the half-life.

The nanoparticle approach can also be followed to enhance the effect and make it economical. The biomolecule and nanoparticle hybrid system is highly active, stable and an efficient alternative to enhance their therapeutic application.<sup>88</sup> It protects from degradation, enhances absorption by diffusion and, most importantly, the drug-targeting system can be given to a precise location, thus reducing the amount required and also the side effects, also making it economically feasible. Owing to small size, it is also possible to search for an alternative to give peptides i.v. Much more developments have to be done with respect to antimicrobial peptides or ribosomally synthesized antibiotic peptides.

## CONCLUSION

Ribosomally synthesized antimicrobial peptides are a great answer to the problems caused by increasing drug resistance, as they have multiple functions against bacteria, fungi, cancer etc. As they are effective with both innate and adaptive immune responses, they are toxic to a number of pathogens. They are stable at a wide range of temperature and pH values making them an effective natural remedy to many diseases. Their synthesis and mode of action distinguishes them from clinical antibiotics. Moreover, microbes showing resistance to antibiotics are generally not resistant to them. Also they are safe to use, so can be used for various purposes such as food preservatives. Their multifunctional ability has increased the scope of exploiting their nature for the welfare of humans.

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