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## Cationic peptides: a new source of antibiotics

Robert E. W. Hancock and Robert Lehrer

Antimicrobial cationic peptides are an important component of the innate defenses of all species of life. Different peptides may have antibacterial, antiendotoxic, antibiotic-potentiating or antifungal properties, and so they are being developed for use as a novel class of antimicrobial agents and as the basis for making transgenic disease-resistant plants and animals.

As relative latecomers to a world inhabited by prokaryotic microorganisms, only those animal and plant species able to prevent and overcome infection were likely to survive. Given the multiplicity of potential pathogens, many host-defence mechanisms evolved, including the antimicrobial peptides. Although it is clearly an ancient mechanism, the production of gene-encoded antimicrobial peptides by animals was recognized only recently. Because many of these peptides show impressive *in vitro* activity against microorganisms resistant to conventional antibiotics, they may provide design templates for anti-infectious agents in humans. The best antimicrobial peptides kill susceptible bacteria *in vitro* at concentrations ranging from 0.25 to 4  $\mu\text{g ml}^{-1}$ . Although more-potent antibiotics exist, there are definite advantages to these peptides, including an ability to kill target cells rapidly, unusually broad activity spectra, activity against some of the more serious antibiotic-resistant pathogens in clinics and the relative difficulty in selecting resistant mutants *in vitro*. Thus these peptides offer exciting possibilities in the face of the declining efficacy of conventional antibiotics owing to the rise of antibiotic-resistant organisms. In addition, their potential to improve animal and (especially) plant husbandry has stimulated intense interest.

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### The nature of antimicrobial peptides

The endogenous antimicrobial peptides of plants and animals are typically cationic (i.e. contain excess lysine and arginine residues) amphipathic molecules composed of 12 to 45 amino acid residues<sup>1</sup> (Fig. 1). These peptides may be produced constitutively or only after infection or injury. Some are  $\alpha$ -helical, especially when placed in structure-promoting solvents such as trifluoroethanol or mixed with anionic phospholipid membranes. Others contain  $\beta$ -sheet elements (Fig. 2) stabilized by intramolecular cystine disulphide bonds, sometimes with an associated  $\alpha$ -helical domain. Yet others are unusually rich in proline, tryptophan or histidine residues. Although antimicrobial peptides manifest great structural diversity, certain common structural patterns are present.

### $\alpha$ -Helical peptides

Boman's discovery of cecropins in the pupae of the silkworm (*Hyalophora cecropia*) established the existence of antimicrobial peptides in invertebrates<sup>2</sup>. Over the next decade, these molecules were sequenced, synthesized, cloned, mutated and subjected to detailed structural and mechanistic analyses<sup>3</sup>. Although they have been most widely studied in lepidoptera (butterflies and moths) and diptera (flies, etc.), cecropin-like peptides also exist in other insect orders<sup>3</sup>, in the porcine intestine<sup>4</sup> and in the blood cells of a marine protochordate<sup>5</sup>. Synthetic cecropin-melittin chimeric peptides have been produced<sup>6</sup> (Fig. 3), and some have shown enhanced antibacterial activity.

About ten years ago, Zasloff isolated magainins ( $\alpha$ -helical, 23-residue antimicrobial peptides) from the skin of the African frog, *Xenopus laevis*<sup>7</sup>. Native magainins formed pores in the cell membranes of susceptible microorganisms but were relatively noncytotoxic towards eukaryotic cells. Their structures and interactions with microorganisms and model membranes have been analysed extensively<sup>8,9</sup>.

The leukocytes of many mammals contain cathelicidins, polypeptides with a conserved N-terminal precursor (cathelin) domain that contains approximately 100 amino acid residues and is followed by an antimicrobial peptide<sup>10</sup>. Cathelin-associated  $\alpha$ -helical peptides that contain 23–38 amino acid residues are found in the blood cells of humans, cattle, pigs, mice, rabbits and sheep. In addition to their antimicrobial properties, some of these peptides also bind bacterial lipopolysaccharide (endotoxin), a factor that contributes to the severity of Gram-negative bacterial infections.

### $\beta$ -Sheet peptides

Antimicrobial peptides with a substantial degree of  $\beta$ -sheet structure are produced in many animals and plants. Some of these are antifungal as well as antibacterial and some, such as protegrins and tachyplesins, retain efficacy in high-salt environments such as blood and tissue fluids<sup>11,12</sup>. This article will describe the defensins, protegrins and tachyplesins.

#### Vertebrate $\alpha$ and $\beta$ defensins

The defensins of vertebrates contain 29 to approximately 40 amino acid residues, including six invariant cysteine residues that form three intramolecular disulphide bonds (Fig. 2). The  $\alpha$ - and  $\beta$ -defensin peptides differ with respect to the placement and connectivity of their cysteines, the nature of their precursors and their sites of expression. However,  $\alpha$  and  $\beta$  defensins have identical 'folds' and the human  $\alpha$ - and  $\beta$ -defensin gene clusters are located within 150 kb of each other. This indicates that  $\alpha$  and  $\beta$  defensins are branches of a single gene family that has undergone repeated duplications.

Human neutrophils are rapidly deployed phagocytic blood cells that constitute a first line of defense against microorganisms that enter the tissues. Four  $\alpha$  defensins (HNP 1–4) are stored in the cytoplasmic granules of neutrophils and make up over 5% of the total cellular protein. Because neutrophils are attracted to and accumulate in infected tissue sites, they provide an extremely appropriate defensin delivery system. Paneth cells also produce  $\alpha$ -defensins in the mouse<sup>13</sup> and human<sup>14</sup> small intestine. The  $\beta$  defensin HBD-1 is produced by epithelial cells throughout the human body<sup>15</sup> and is especially prominent in the kidney and the female genitourinary tract<sup>16</sup>. Another human  $\beta$  defensin, HBD-2, is made by inflamed skin<sup>17</sup>, but little is known about its properties. Production of  $\alpha$  defensins by neutrophils is constitutive and regulated by the cell's intrinsic maturation program; the intestinal Paneth cells secrete their defensin-containing granules after bacteria enter the intestine or following

Rabbit defensin (NP-1)	VVC <sub>1</sub> AC <sub>2</sub> <b>RR</b> ALC <sub>3</sub> LPRERRAGFC <sub>3</sub> <b>RIRRGRI</b> HLC <sub>2</sub> C <sub>1</sub> <b>RR</b>
Crab tachyplesin	<b>RRWC</b> <sub>1</sub> <b>FRVC</b> <sub>2</sub> YRGFC <sub>2</sub> YRKC <sub>1</sub> <b>R</b>
Bovine bactenecin	<b>RLC</b> <sub>1</sub> <b>RIVVIRVC</b> <sub>1</sub> <b>R</b>
Silk-moth cecropin A	<b>KWKFKKIEKMGRNIRDGIVKAGPAIEVIGSAKAI</b>
Bovine indolicidin	<b>ILPWKWPWWPWR</b>
Bacterial nisin	<b>IXA</b> <sub>1</sub> <b>IULA</b> <sub>2</sub> <b>Z</b> <sub>2</sub> <b>PGA</b> <sub>2</sub> <b>KZ</b> <sub>3</sub> <b>GLAMGA</b> <sub>3</sub> <b>NMKZ</b> <sub>4</sub> <b>AZ</b> <sub>5</sub> <b>A</b> <sub>4</sub> <b>HA</b> <sub>5</sub> <b>SIHVUK</b>

Figure 1

Examples of the primary sequence of natural cationic antimicrobial peptides (modified amino acids are: U = 2,3-didehydroalanine, X = 2,3-didehydrobutyrine, Z =  $\alpha$ -amino butyrate). Positively charged residues are in bold type; subscripts refer to residues that are covalently linked via disulphide (for cysteines) or thioether (for nisin) bonds.

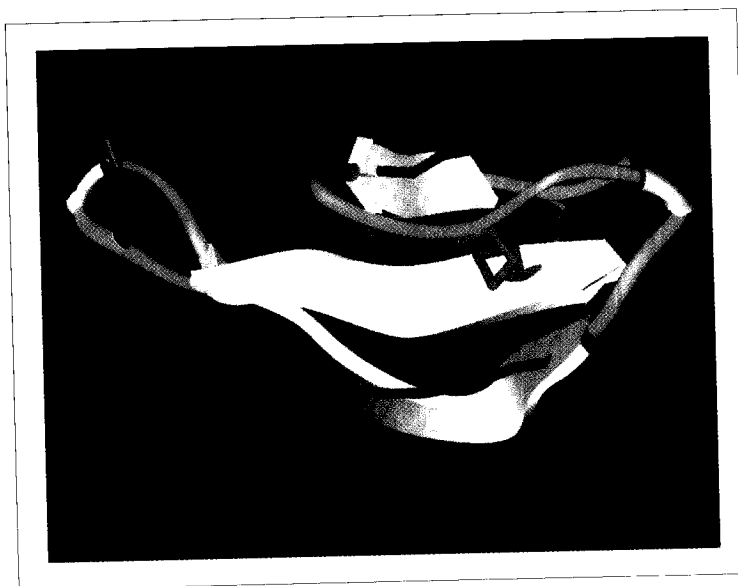


Figure 2

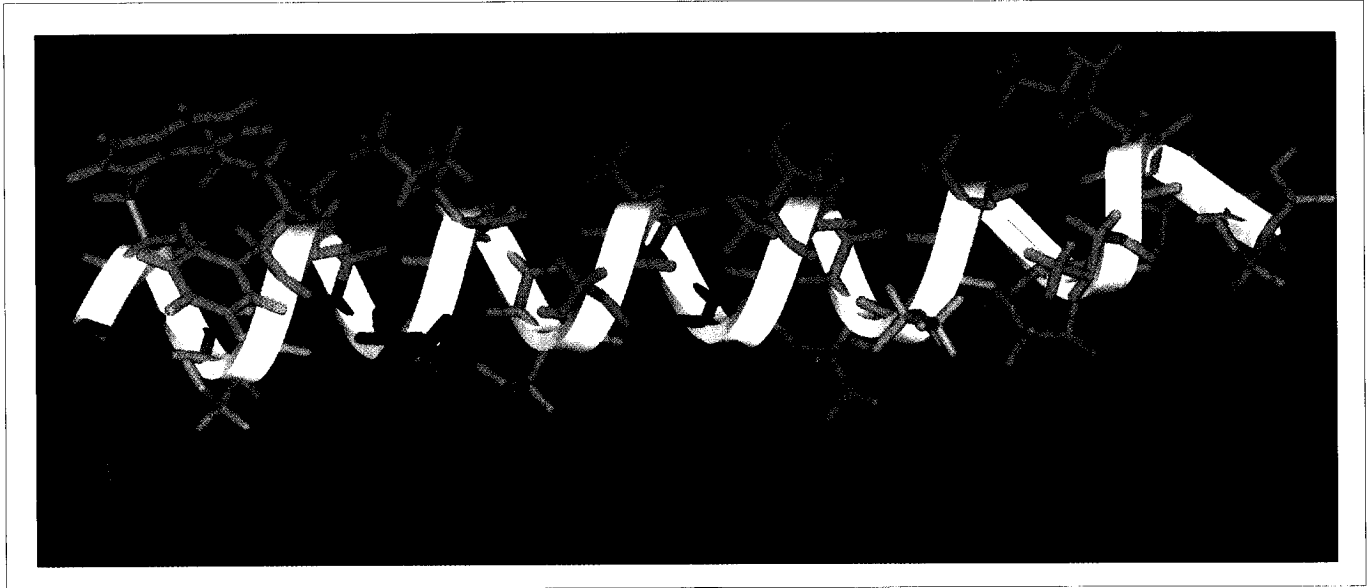
Secondary structure of the human Defensin-1 peptide, a member of one of the most common classes of cationic antimicrobial peptides. The yellow ribbons represent the three regions of  $\beta$ -sheet structure, the green tube is the random structure and the larger white tubes represent the  $\beta$ -turns. Only the four arginine side chains are shown, in red, for clarity, with the disulphide bridges shown in bronze.

stimulation via the autonomic nervous system. Production of HBD-2 is induced by inflammation, and so is likely to take place after infection and injury.

Cattle leukocytes contain over a dozen different  $\beta$  defensins, and additional bovine  $\beta$  defensins were found in cattle tongue and tracheal epithelium. Synthesis of bovine lingual and tracheal  $\beta$  defensins increases after injury or exposure to lipopolysaccharide, and responds to humoral mediators (cytokines) such as tumor necrosis factor  $\alpha$ <sup>18</sup>. Local induction of various endogenous antibiotic peptides is likely to prove a common response that protects mucosal and epithelial surfaces after injury or infection.

#### Plant and insect defensins

The antimicrobial and cytotoxic properties of 5 kDa cationic, cysteine-rich plant peptides, now called  $\alpha$  and  $\beta$  thionins, were recognized over 50 years ago<sup>19</sup>. Thionins are compact, L-shaped molecules, their long arm formed by two disulphide-linked  $\alpha$ -helices and their short arm containing two antiparallel  $\beta$  sheets<sup>20</sup>.



**Figure 3**

Secondary structure of a typical  $\alpha$ -helical antimicrobial peptide (CP-26, a cecropin–melittin hybrid, R. E. W. Hancock, unpublished). Such a structure is formed only after interaction with the membrane, and the peptide is in random structure in free solution. The helix–bend–helix shown is common; helices are indicated by the yellow spiral, which is slightly kinked on the right side of centre (the bend). Side chains are coloured as follows: lysine residues, red; other hydrophilic residues, bronze; hydrophobic residues, green. The perspective on this model shows the amphipathic nature of the  $\alpha$  helix.

Thionin homologs are ubiquitous among plants, suggesting their great antiquity. The more-recently characterized plant defensins, also called  $\gamma$ -thionins, are 5 kDa molecules with 45–54 amino acid residues that have been found in the seeds of many plants, both mono- and dicotyledonous. Plant defensins have four intramolecular disulphide bridges, which stabilize a triple-stranded antiparallel  $\beta$ -sheet structure that also contains an  $\alpha$ -helical element<sup>21</sup>.

Drosomycin, an inducible antifungal peptide of the fruit fly, has 44 residues, including eight cysteines that form four intramolecular disulphide bridges paired identically to those found in plant defensins. Remarkably, the primary sequence of drosomycin is 38% homologous to Rs-AFP1, a plant defensin from radish seeds<sup>22</sup>. NMR studies have revealed that insect defensins and  $\gamma$ -thionin (defensin) from barley or wheat endosperm have similar three-dimensional structures<sup>23</sup>, strongly suggesting that plant and insect defensins belong to a peptide superfamily that originated before plants and animals diverged<sup>24</sup>. Like drosomycin, plant defensins show considerable antifungal activity but little antibacterial activity.

The tissues of plants also contain other antimicrobial peptides whose structures contain two or three disulphide bridges. One group shows homology to a cysteine–glycine-rich domain found in many plant lectins that bind chitin, a polysaccharide found in fungal cell walls and insect exoskeletons<sup>24</sup>.

#### *Protegrins and tachyplesins*

Although pigs and horseshoe crabs occupy very different environments, the blood cells of both contain 2 kDa antimicrobial peptides composed of 16–18 amino acid residues that form an antiparallel  $\beta$  sheet

stabilized by two disulphide bridges<sup>25–27</sup>. Both protegrins and tachyplesins display activity against fungi, bacteria and certain viruses, including extracellular HIV-1. Both retain antimicrobial efficacy in solutions with physiological concentrations of sodium chloride and divalent cations<sup>11</sup>.

#### *Proline-rich peptides: extended helices*

Various proline-rich antimicrobial peptides have been recovered from the leukocytes of different mammals<sup>10</sup>, from a marine invertebrate<sup>28</sup> and from the haemolymph of many insects<sup>28,29</sup>. The proline-rich apidaecins, originally isolated from honeybees and wasps, are specific for Gram-negative bacteria, which they recognize in a stereoselective manner<sup>30</sup>. The smallest of these peptides, indolicidin (a tridecameric cathelicidin from bovine neutrophils), is 13 amino acids in size and has five tryptophan and three proline residues<sup>31</sup>. Like some other proline-rich peptides, it has a circular dichroism spectrum typical of a poly-L-proline type-II helix (essentially an extended helix)<sup>31,32</sup>. Certain proline-rich peptides have broad antimicrobial specificity, including fungi.

#### *Other peptides*

A number of other antimicrobial peptides exist, often with poorly described structures. The bacteriocins of Gram-positive bacteria include certain cationic antibacterial peptides such as nisin<sup>33</sup> (which has a partly  $\alpha$ -helical structure), but also include many narrower-spectrum peptides with neutral or even negative overall charges. Bacteriocins like nisin, which have unusual amino acids, have the group name lantibiotics. Other peptides form loop structures owing to a disulphide bridge (in the peptide bactenecin from cattle and sheep)

or a covalent bond (in the bacterial peptide gramicidin S)<sup>1</sup>. In addition, antimicrobial peptides have been found *in vivo* and made *in vitro* that arise from the digestion of larger proteins such as bactericidal/permeability-increasing protein (BPI) or lactoferrin<sup>34</sup>.

### Role in natural resistance to infection

The potent antimicrobial properties of various antimicrobial peptides and their strategic location in phagocytes and at interfaces between the organism and its environment provide indirect evidence that antimicrobial peptides participate in resistance to infection. This inference is strengthened by the rapid induction of certain antimicrobial peptides after plants or animals are infected or injured. Because host-defence mechanisms are multiple and include elements in addition to antimicrobial peptides, it is difficult to demonstrate the contribution of any one peptide or mechanism. Nevertheless, transgenic plants that express new antimicrobial peptides have been reported to show enhanced resistance to fungi<sup>35</sup>, and fruit flies bearing mutations in the Toll-signaling pathway that rendered them unable to induce drosomycin synthesis showed dramatically reduced survival after fungal infection<sup>36</sup>. Compelling experimental evidence recently linked salt inhibition of epithelial antimicrobial peptides to the impaired pulmonary defenses against *Pseudomonas aeruginosa* characteristic of patients with cystic fibrosis<sup>37,38</sup>. To this can be added the ability of certain cationic peptides to provide significant protection in a galactosamine-sensitized-mouse model of lethal endotoxic shock<sup>39,40</sup>, and the *in vivo* effectiveness of  $\alpha$ -helical peptides<sup>40</sup>, protegrins<sup>41</sup> and indolicidin<sup>42</sup> in various experimental infections.

### Mode of action

The mode of antimicrobial action of the cationic antimicrobial peptides, including the well-studied peptides melittin, magainin, gramicidin, cecropin and defensins<sup>12,31,37</sup>, has been studied in much detail. The main site of action of the peptides is the cytoplasmic membranes – the peptides tend to assemble to form channels<sup>8,9,31</sup>. [An alternative hypothesis states that the peptides cluster at the membrane surface and cause a cooperative permeabilization of the cytoplasmic membrane (the so-called ‘carpet effect’)<sup>43</sup>.] Oriented-circular-dichroism studies in model lipid systems indicate that sublytic magainin concentrations decrease the membrane thickness by associating with bilayer head groups. At higher peptide:lipid ratios, a substantial fraction of magainin orients perpendicularly to the membrane, and pores can be detected by neutron in-plane scattering<sup>9</sup>. It has been suggested that these pores formed by a toroidal (‘wormhole’) mechanism, in which the membrane phospholipids bend backwards upon themselves after magainin monomers have expanded the bilayer’s head-group region. The selectivity for microbial cells compared to host cells is thought to result from the unique high content of anionic lipids on the surface of the bacterial cytoplasmic membrane, the high electrical-potential gradient

across this membrane (oriented internal-negative) and the lack of cholesterol in bacterial membranes (see Ref. 36 for a review). In Gram-negative bacteria, the peptides initially interact with lipopolysaccharide, a highly anionic outer-membrane glycolipid, and then disrupt the membrane locally (via a process termed self-promoted uptake) to access the cytoplasmic membrane. This unique mechanism of action explains many of the clinically desirable properties of antimicrobial peptides<sup>1</sup>.

### Preclinical and clinical antimicrobial studies

Antimicrobial peptides tend to be involved as a local response to infection. Thus, it has been assumed that they may be limited to the treatment of topical infections. Indeed, the first clinical trials have been directed towards topical infections<sup>1</sup>. Magainin Pharmaceuticals have taken their  $\alpha$ -helical magainin variant peptide MSI-78 into phase-III clinical trials with 926 patients treated in two studies of efficacy against polymicrobial foot-ulcer infections in diabetics. It was recently announced (<http://www.pslgroup.com/dg/2168e.htm>) that these trials demonstrated equivalency to orally administered ofloxacin, but with less side effects. Two other companies have taken antimicrobial peptides into phase-I clinical trials. IntraBiotics are utilizing their protegrin-derived peptide IB-367 against oral polymicrobial ulcers (oral mucositis) in cancer patients, and Applied Microbiology have initiated a trial in collaboration with Astra, testing the efficacy of the bacterial lantibiotic peptide nisin against *Helicobacter pylori* stomach ulcers (<http://www.businesswire.com/cnn/ambi.htm>). Interestingly, only anecdotal evidence has been presented to demonstrate their efficacy in animal models of topical infections but such studies of efficacy and safety in animal models must presumably have been performed to permit these peptides to be allowed to proceed to clinical trials. A study of topical application of nisin on the teat skin of cows<sup>44</sup> demonstrated a 4-log reduction of *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* after exposure for only 1 min to the peptide, with no side effects. In addition, cecropin-melittin hybrid peptides ranging in size from 12 to 18 residues have been shown to have topical activity against *Pseudomonas aeruginosa* eye infections of rabbits<sup>45</sup>.

By contrast, a reasonable amount of published evidence has demonstrated efficacy against systemic infections. This includes  $\alpha$ -helical-peptide efficacy against *P. aeruginosa* peritoneal infections<sup>40</sup>,  $\beta$ -sheet-protegrin efficacy against methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE) and *P. aeruginosa* infections<sup>41</sup> and extended-helix indolicidin in liposomal formulation against *Aspergillus* fungal infections<sup>42</sup>. Other studies have demonstrated that antimicrobial peptides protect against lethal endotoxic shock in galactosamine-sensitized mice<sup>39,40</sup> and demonstrate synergy with the  $\beta$ -lactam cefpirome against *P. aeruginosa* infections<sup>46</sup>. This has indicated that antimicrobials may be able to be utilized as injectable antibiotics against serious bacterial and fungal infections that are resistant to conventional antibiotics. Perhaps

the strongest support for this is provided by results for use, in parenteral therapy (i.e. as an injectable), of the cationic protein BPI (or more correctly Neuprex, the recombinant N-terminal 21 kDa of BPI). BPI is a neutrophil-derived human protein that binds to bacterial lipopolysaccharide (also called endotoxin) and has modest antibacterial activity. Although it has just entered phase-III clinical trials against paediatric *Neisseria meningitidis* infections, early reports by Xoma of the phase-II studies have suggested a profound reduction in lethality for this previously difficult disease (<http://www.xoma.com/>). The mode of treatment in this case is via intravenous infusion. Significantly, Neuprex has been shown to be safe in more than 700 patients. The relationship of Neuprex (BPI) to cationic peptides can be seen from the fact that the antimicrobial activity of this protein is retained in relatively small cationic peptides derived from BPI. Thus, we are encouraged to believe that antimicrobial peptides have great potential to be the next breakthrough class of antimicrobials and the first truly novel class of antibiotics in 30 years.

#### Other applications

The bacteria-derived cationic antibacterial peptide nisin was first discovered in the 1920s and introduced by Aplin and Barrett as nisaplin in 1957. It is currently licensed as a food preservative in more than 50

countries<sup>33</sup>. Nisin is somewhat limited by its lack of activity against Gram-negative pathogens, but has very potent activity against Gram-positive bacteria. Interestingly, however, the application of nisin formulated with 1-propanol showed excellent activity against Gram-negative pathogens<sup>44</sup>, and other reports suggested synergy with EDTA<sup>33</sup>. It is now marketed as a teat dip to prevent mastitis. More exotic uses of other cationic peptides have included therapy of infections of farmed fish<sup>47</sup> and in the preservation of cut roses<sup>48</sup>. However, such uses are limited at present by the expense of such peptides. There are also reports of activity against enveloped viruses and cancer cells and in the promotion of repair of epithelial-cell damage.

#### Production methods and cost effectiveness

One potential limitation on the useful application of antimicrobial peptides is the cost of production. Generally speaking, natural sources are not cost effective and, with some exceptions, natural peptides are less broad in their spectrum of activity than the best synthetic peptides. One exception may be nisin, a natural product of *Lactococcus lactis*, which can be produced by fermentation.

For other peptides, it is important to develop cost-effective production methods, and two have been considered in depth. Peptide synthesis utilizing automated chemical procedures is quite costly compared with the

**Table 1. Use of cationic antimicrobial peptide transgenes in making disease-resistant plants and animals**

Transformed species	Peptide utilized	Class <sup>a</sup>	Peptide expression level <sup>b</sup>	Transgenic species resistant to (organism)	Ref.
Tobacco	Barley $\alpha$ -hordothionin	B	2–60 ng mg <sup>-1</sup> leaf protein	<i>Clavibacter michiganensis</i>	<sup>c</sup>
Tobacco	Barley $\beta$ -hordothionin	B	2–60 ng mg <sup>-1</sup> leaf protein	<i>Clavibacter michiganensis</i> ; not <i>Pseudomonas syringae</i>	<sup>c</sup>
Tobacco	Barley $\alpha$ -hordothionin	B	++	<i>Xanthomonas campestris</i> ; not <i>Clavibacter michiganensis</i>	<sup>c</sup>
Tobacco	Giant silk moth cecropin B	A	None detected	Not <i>B. solanacearum</i> or <i>Pseudomonas syringae</i>	53
Tobacco	Giant silk moth cecropin B	A	Not examined	<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	54
Potato	Giant silk moth cecropin B	A	None	Not <i>Erwinia</i> sp.	55
Potato	Horseshoe-crab tachyplesin I	B	+	Slight <i>Erwinia</i> resistance	56
Tobacco	Radish plant-defensin Rs-AFP2	B	0.2–2.4 $\mu$ g mg <sup>-1</sup> leaf protein	<i>Alternaria longpipes</i> (fungal)	57
Tobacco	Amaranth hevein Ac-AMP2	B	0.6–1.1 $\mu$ g mg <sup>-1</sup> leaf protein	<i>In vitro</i> activity but no <i>in planta</i> antifungal resistance	58
Tobacco	Sweet pepper knott in Mj-AMP2	B	0.9–1.4 $\mu$ g mg <sup>-1</sup> leaf protein	<i>In vitro</i> activity but no <i>in planta</i> antifungal resistance	59
Tobacco	Cecropin B analogs SB-37 and Shiva-1	A	Up to 1 $\mu$ g mg <sup>-1</sup> leaf protein	Bacterial wilt reduction ( <i>Burkholderia solanacearum</i> )	59
Tobacco	Chimeric cecropin A/B hybrid	A	Slight	None	60
Tobacco	Barley $\alpha$ -thionin	B	20 ng mg <sup>-1</sup> leaf protein	<i>Pseudomonas syringae</i> pv. <i>syringae</i>	61
Tobacco	Wheat $\alpha$ -thionin	B	?	None	61
Mouse	Cecropin B analog	A	Not reported	<i>Brucella abortus</i>	<sup>d</sup>
Tobacco	Barley lipid-transfer protein	A	+	<i>Pseudomonas syringae</i>	24

<sup>a</sup>Structural class of peptide: A =  $\alpha$  helical, B =  $\beta$  sheet.

<sup>b</sup>++, high expression level reported; +, low expression level reported; ?, expression noted but not quantified.

<sup>c</sup>D. Florack, PhD thesis, Landbouuniversiteit te Wageningen, 1994.

<sup>d</sup>S. D. Haguis et al. (1996) *Abstracts of the 96th ASM General Meeting* E-46, 274.

traditional solid-phase synthesis methods<sup>3</sup>. Intensive industrial research utilizing solution-phase chemistry has reduced these costs remarkably, but the current production cost of US\$50–100 g<sup>-1</sup> (approximately the daily dose for most antibiotics) is still considerable.

An alternative strategy is recombinant synthesis. Various procedures have been developed<sup>34</sup>, but the most broadly effective is production as fusion proteins in bacteria<sup>49</sup>. In this procedure, a fusion protein comprising (from N to C terminus) a carrier region (which may contain an affinity purification tag), an anionic segment (to stabilize the cationic peptide by binding to it and preventing both antibiotic activity of the cationic peptide segment against the host bacterium and proteolysis of this segment during recombinant production), a cleavage region (a methionine residue susceptible to cyanogen-bromide cleavage) and the cationic-peptide region. Using this procedure, we have been able to produce cationic peptides at up to 2% of the biomass of *E. coli* (R. E. W. Hancock, unpublished).

Other recombinant procedures that have been utilized include their production in insect cells using baculovirus vectors<sup>50</sup>, in fungal expression systems<sup>51</sup>, in tobacco using tobacco-mosaic-virus vectors (Geneware™ technology of Biosource Technologies) and in the milk of transgenic mice<sup>52</sup>. However, relatively few details are available regarding the commercial potential of these procedures when applied to antimicrobial peptides.

### Transgenic approaches

Because cationic antimicrobial peptides seem to be an important component of the antimicrobial defences of many species, one possible cost-effective method of improving the resistance of various species to microbial infection would be to overproduce such peptides, or improved variants thereof, using the power of transgenic technology. This approach has been utilized extensively, although with mixed results (Table 1). Such studies have mostly been performed in plants and have suggested that the appropriate peptides can be expressed both at the RNA and protein level and may lead to antibacterial and/or antifungal resistance to significant plant diseases. Although many of the published studies are in tobacco, transgenic potatoes (Table 1) and tomatoes have been produced, and the first field trials are likely to involve transgenic rapeseed.

Other researchers have utilized transgenic symbiotic bacteria that have been engineered to produce cecropin A to kill *Trypanosoma cruzi* (the agent of Chagas' disease) in the hindgut of the reduviid bug, the vector that carries this disease agent<sup>62</sup>. Another intriguing possibility is provided by preliminary reports that transgenic mice expressing a cecropin B (a silk-moth peptide) analog become resistant to *Brucella abortus* infections (Table 1). Thus, this suggests that a transgenic approach to enhancing natural resistance in food animals may have potential. Obviously, the use of this approach in farm animals, which are currently fed antibiotics as growth enhancers (reducing episodes of deleterious bacterial infections) or in aquaculture-farmed fish

species, could have a major impact by decreasing antibiotic use in animals, a practice that has been greatly criticized. These results serve to emphasize the dual potential of cationic antimicrobial peptides, because they can be used either directly as antibiotics or, through transgenic approaches, to reduce the dependency of agriculture on the use of antibiotics.

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## Overcoming apoptosis: new methods for improving protein-expression systems

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Cellular suicide, or apoptosis, is responsible for a significant proportion of cell death in many bioprocesses. With the progressive elucidation of the biochemical and genetic events leading to this form of cell death, it is now possible to implement strategies for extending the productive lifetimes of cells in culture. These strategies may include nutritional, genetic and chemical methods that enhance cell survival and performance during the critical stages of a culture process, leading to improvements in the production capacity for valuable biotechnological products.

It is now well established that cell death in multicellular organisms occurs by one of two distinct mechanisms: necrosis or apoptosis. Necrotic death involves the disruption of membrane integrity and is caused by severe physical or chemical damage to the cell. The cell swells and bursts osmotically, releasing its contents into the surrounding area (Fig. 1). In contrast to this passive death mechanism, a sophisticated biochemical and genetic network exists in most cell types that provides for cellular suicide in response to nonlethal stimuli. Programmed cell death (PCD) or apoptosis is a cascade of events controlled by endogenous cellular genes, enzymes and signalling cascades. (Although the term apoptosis was originally used to describe the morphological manifestations of this active mechanism of cell

death, it is now typically used synonymously with the phrase 'programmed cell death'.) Cells undergoing apoptosis are easily recognizable owing to the distinct physical changes that take place (Fig. 2). First, the cell sustains a significant volume loss before its chromatin and cytoplasmic organelles condense. The cell membrane begins to bulge in a process called blebbing, the condensed nucleus collapses, and finally, the blebs pinch off to form a series of membrane-enclosed bodies containing fragments of the nucleus, cytosol and cytoplasmic organelles. *In vivo*, these apoptotic bodies are phagocytosed by adjacent cells as well as by dedicated phagocytes, but, in cell culture, they eventually lose their integrity and lyse by a process called secondary necrosis.

Apoptosis is common to most higher eukaryotes, including plants, slime moulds, insects and vertebrates<sup>1</sup>. In mammals, PCD allows the emergence of digits in the hands and feet, enables the proper development of both the nervous and immune systems, limits the spread

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