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We are now facing an era of antibiotic resistance in our hospitals and communities. Whereas, not long ago, the arsenal of antibiotics appeared sufficient to cope with bacterial diseases, we are now heading towards (and arguably already in) a crisis situation with antibiotic resistance. As a result, a mounting effort is underway to identify and develop novel antibiotic therapies to help deal with current and emerging resistant bacteria. However, while improved variants of traditional antibiotics have been developed for years, often to address problems with resistance but ultimately leading to modifications of the same resistance mechanisms as observed with the parent compounds, the development of fundamentally new compounds has lagged behind. Among the very few novel therapies in the pipeline, the recently developed cationic antimicrobial peptides have emerged from their origin as nature's antibiotics [1] to become promising developmental and clinical candidates. Cationic antimicrobial peptides have been isolated from virtually all living organisms, ranging from the simpler (bacteria) to the more complex (animals, including humans, and plants). Currently, over 1000 natural antimicrobial peptides have been identified with many more having been synthetically created and demonstrated to have broad antimicrobial activities that can include the direct inhibition of bacteria, viruses and fungi, as well as a broad range of activities as immune modulators [2] . This class of antibiotic offers some hope in the search for novel therapeutic approaches. Indeed, some related peptide antibiotics containing modified amino acids (polymyxin B, gramicidin S and the lantibiotic nisin) have already been used in microbial control in both medical and food spoilage applications. However, while there is a wealth of knowledge regarding antimicrobial peptides, there are still many aspects that are poorly understood and/or controversial. The aim of this review is to examine what is known about cationic antimicrobial peptides with an emphasis on their mechanism of action against bacteria. The authors refer the reader to other reviews for more information on other areas of antimicrobial peptide biology, including their immunomodulatory functions [3] , structural aspects [4] , therapeutic applications [5] and the mechanisms of bacterial resistance toward such peptides [6,7] .

Cationic antimicrobial peptides: a basic definition

The term 'cationic antimicrobial peptides' should be reserved for discussion of peptides that have, as their primary biological function, the killing of microorganisms; when discussing other properties associated with these molecules (e.g., chemoattractant, immunomodulatory, wound healing and angiogenic), or when these alternative biological functions are the predominant modes of action *in vivo* , the term 'host defence peptides' is preferred [8] . All natural cationic antimicrobial peptides share many common features, including small size (generally 12-50 amino acids long), cationicity (with an overall charge ranging from +2 to +9) and hydrophobicity [4] . They are usually gene-encoded peptides that are expressed either constitutively or inducibly (through signals received from infectious or inflammatory agents [2]) and are produced by all living eukaryotes and prokaryotes as part of their innate immune defenses (e.g., human epithelia are in part protected by cationic peptides of the defensin and cathelicidin classes [9]). Some cationic antimicrobial peptides (e.g., lactoferricin) are proteolytic digestion products of larger proteins [10] . While bacterial antimicrobial peptides (referred to as bacteriocins) are a general defense mechanism, eukaryotes tend to produce antimicrobial peptides in many different cell types (e.g., [beta]-defensins in the skin and mucosa [11]). The known cationic antimicrobial peptides are described in two large databases [101,102] . Owing to their small size and conserved overall physical properties, combined with their substantial natural variability of sequence and

length, it is now relatively easy to design and create synthetic antimicrobial peptides based on known peptides or subsequences [12,13] . While such peptides tend to group into structural families, they have limited sequence homologies, even within a given family, which may account for their continued evolutionary success. Cationic antimicrobial peptides (including synthetic ones) are generally classified into four groups: [alpha]-helical, [beta]-sheet, extended structures rich in certain amino acids and loop peptides, of which the two former are by far the more common groups [4] . While variability exists at both primary and secondary structure levels, they share a common structural arrangement at the tertiary level [14] in that they form amphiphilic molecules with their charged/polar and hydrophobic residues segregating into patches (or faces) upon interaction with a membrane.

The broad antimicrobial properties of cationic peptides, their advantageous physical properties and their evolutionary success makes them ideal for further development of their potential therapeutic applications. However, despite the wealth of knowledge already gained in identifying such peptides, there are still many areas that remain unclear or controversial, including their mechanisms of action.

Antibacterial mechanism of action of cationic peptides

Most bacteria are potentially susceptible to cationic antimicrobial peptides, with the exception of certain recalcitrant species, such as *Burkholderia* and usually *Serratia* and *Proteus* spp. [8] . While there are many reports on such peptides in the literature, there are, in fact, only a small percentage that have been studied in depth. The mechanism of action of cationic antimicrobial peptides against bacteria has been studied intensively for only a few select peptides. Controversy regarding their mechanisms of action is based on the complicated nature of their action. Indeed, it has been suggested that cationic antimicrobial peptides are 'dirty drugs' [6] in that they potentially have many targets owing to their amphiphilic nature and cationic charge. Compared with conventional antibiotics, such as the [beta]-lactams, which have a clean mechanism of action with a single target site, this makes these peptides harder to study but conversely more intriguing. While the dogma for many years was that the sole mechanism of action for cationic antimicrobial peptides against bacteria was to interact with and destroy the membrane permeability barrier of bacterial cells, it has recently become apparent that alternative and/or additional nonmembrane targets exist. This gives rise to two functional classes of cationic antimicrobial peptides: membrane disruptive and membrane nondisruptive [4] , although the distinction may be a bit blurred as peptides that attack membranes in one species could conceivably have nondisruptive actions in another species. Regardless of which class the peptide falls into, all must interact with the membrane (sometimes two) whether or not it is disrupted, if only to reach its final internal target destination [2] .

Owing to the structural differences in the two main classes of bacteria, the pathway to the cytoplasmic membrane traveled by antimicrobial peptides will differ. However, the initial interaction of a cationic antimicrobial peptide with a bacterial cell is through similar mechanisms. Positively charged antimicrobial peptides are attracted to the polyanionic outer surfaces of a cell (due to cell wall-associated teichoic and lipoteichoic acids in Gram-positive and lipopolysaccharides [LPS] in Gram-negative bacteria). The interaction of cationic antimicrobial peptides with Gram-negative cells is better understood and explained here. Following the initial electrostatic attraction between cationic antimicrobial peptides and the outer leaflet of a Gram-negative cell, the cationic peptides initiate their own passage across the outer membrane through a 'self-promoted uptake' mechanism [15] . These peptides achieve this because they have a higher affinity for negatively charged LPS than native divalent cations, such as Mg^{2+} and Ca^{2+} [2] and, as such, are able to cause areas of instability in the outer membrane allowing subsequent translocation of the cationic antimicrobial peptides across the outer membrane bilayer. Examples of each type of cationic antimicrobial peptide action are summarized in Table 1 & Figure 1.

Membrane-disruptive cationic antimicrobial peptides

Interactions with the cytoplasmic membrane begin when the cationic antimicrobial peptides associate with the phospholipids. As long as the peptide:lipid ratio is low, the cationic antimicrobial peptides remain associated, parallel to the plane of the membrane inserted at the interface of the hydrophilic lipid headgroups and the hydrophobic fatty acyl chains [16] . However, as the peptide:lipid ratio increases, these peptides are able to aggregate and/or reorient in the membrane and disrupt membrane

integrity as described through one of four proposed models: barrel-stave, aggregate, carpet and toroidal pore (Figure 1). While each of these models have been implicated with selected cationic antimicrobial peptides, no single model is more accurate than another, although the aggregate model explains how cationic antimicrobial peptides can affect killing through both membrane permeation and internal target attack.

Barrel-stave model

The barrel-stave model is sometimes known as helical bundle (e.g., noncationic peptide alamethicin [16] and possibly gramicidin S [17]). Following their association to the cytoplasmic membrane and at a critical threshold peptide:lipid ratio, the peptides reorientate themselves perpendicular to and are proposed to span the lipid bilayer. Their orientation is such that the hydrophobic side chains face towards the hydrophobic core of the membrane and the polar side chains are proposed to face inwards creating a hydrophilic pore that spans the width of the membrane. The creation of the pore could then lead to the leakage of cellular contents. However, this model does not explain how pore formation can occur in most cases, since the channels that form are often quite irregular in size and duration, and mildly cation selective (whereas, if many cationic headgroups were oriented into the lumen of a narrow channel, it would be expected to be anion specific) [17] .

Toroidal pore model

In the toroidal pore model (also known as the wormhole model; e.g., magainin 2 [18]), peptides bind to the membrane and cause the lipids to fold inwards and form a channel lined by lipid headgroups and associated peptides at the membrane interface, providing a continuous channel between the inner and outer leaflets. The peptides remain largely associated with the lipid headgroups throughout the process, unlike the barrel-stave model where peptides are only initially associated with the headgroups before interacting with the lipid tails. The resulting pore leads to cell leakage [19] .

Carpet model

Unlike the other models, in the carpet model (e.g., cecropin [20]) the cationic antimicrobial peptides do not insert into the membrane but rather remain associated with the interfacial region of the outer leaflet of the cytoplasmic membrane. At a critical point, the mass of peptides forms a 'carpet' that is able to induce weaknesses within the bilayer by destroying membrane electrostatics, causing the membrane to collapse into a micellar configuration. Cell death would eventuate from loss of cytoplasmic contents. A prediction of this model is that there would be a critical concentration at which the membrane would collapse, although this does not always seem to agree with the experimental evidence [17] .

Aggregate model

The aggregate model is similar to the detergent model (e.g., polyphemusin and indolicidin) [21] . After binding to the membrane interface, at sufficient concentrations the peptides reorient themselves, enabling micelle-like complexes to form with the lipids and span the lipid bilayer in a peptide-lipid complex [17] . These transmembrane random aggregates of lipids, cationic peptides and water can form a channel for ion leakage causing cell death through leakage of cytoplasmic contents, or can spontaneously disintegrate leading to the translocation of the cationic peptides into the cytoplasm, where they can affect killing internally. This model offers an explanation for how cationic antimicrobial peptides can disrupt membrane integrity and/or allow for peptide translocation across the membrane to the cytoplasm [8] .

Membrane-nondisruptive cationic antimicrobial peptides

As stated earlier, the dogma for the mechanism of action of cationic antimicrobial peptides was through the interaction with, and disruption of, the cytoplasmic membrane permeability barrier. One reason for this could relate to how these studies were conducted. It is quite apparent that high levels of peptide (well above MICs and their equivalent peptide:lipid ratios) [22] have been used in many studies providing, perhaps, a false indication of mechanism of action, masking any potential intracellular effect [23] . Indeed, there are indications that virtually any antimicrobial peptide if tested at high enough levels (i.e., higher than physiologically relevant concentrations) can cause membrane permeation [22] . For example, at the MIC a pleurocidin analog

was able to rapidly inhibit RNA and protein synthesis without any effect on the integrity of the membrane permeability barrier, consistent with intracellular uptake and action [23] . Conversely, at ten-times the MIC, cells became depolarized (i.e., protons and ions equilibrated across the membrane within minutes) indicating that, at these high concentrations, the peptide disrupted membranes.

Recent evidence suggests that a number of cationic antimicrobial peptides are able to act on internal targets, either as their major mechanism of action following their translocation across the membrane or as an additive effect, combined with (often incomplete) membrane disruption. Indeed, various studies have identified a variety of possible internal targets consistent with the concept that cationic antimicrobial peptides are dirty drugs [6] . We proposed previously that cationic antimicrobial peptides have a 'multitarget' mechanism of action [4] , whereby they are able to interact with multiple anionic targets (including nucleic acids to affect RNA or DNA synthesis, cellular enzymes, the process of cell division or membranes). Certainly, many examples of cationic antimicrobial peptides that have more than one alternative nonmembranous target site within a bacterial cell (e.g., indolicidin) now exist. This could explain why it has been so hard to find and/or construct resistant mutants to many peptides in bacteria. In this article, we provide a few of the better-characterized examples (see later and Table 1); however, we consider that such a mode of action should be investigated for many peptides when considering their mechanisms of action. For example, although cecropin and magainin 2 are usually considered to be examples of membrane-disruptive peptides, the former has been shown to influence the transcription of many *Escherichia coli* genes at around the MIC [24] , suggesting a possible internal action, while the latter has been demonstrated in one study to translocate into cells [25] . We have also found that LL-37 is able to influence the expression of many *Pseudomonas aeruginosa* genes at concentrations substantially below the MIC (Overhage J, Hancock REW, Unpublished Data), despite many papers indicating that this peptide acts through membrane disruption.

Cationic antimicrobial peptides that act on nucleic acids

Many cationic antimicrobial peptides have been shown to interact with nucleic acids (DNA and RNA). One of the better-studied peptides that interacts with DNA is buforin II, a 21-amino acid LysC endoproteinase derivative of the 39-amino acid buforin I, which was isolated from the Asian toad *Bufo bufo gargarizans* [26] . It was found that buforin II was able to translocate across lipid bilayers of liposomes without inducing lipid flip-flop [24] , while fluoroisothiocyanate (FITC)-labeled buforin II was found to penetrate the *E. coli* cytoplasmic membrane and accumulate inside, even below its MIC [27] and this, together with the observation that it did not cause membrane disruption or cell lysis, indicated that it probably had an internal target. Interestingly, buforin forms a disrupted [alpha]-helix with a proline hinge (a very common motif among antimicrobial peptides) and when this proline residue was removed the peptide could no longer enter cells, instead localizing on the cell surface and permeabilizing the cell membrane, indicating that small changes in structure can influence the mechanism dramatically [28] . It was demonstrated through gel retardation studies that buforin II was, in fact, able to bind to DNA [27] . This is perhaps not surprising given that buforin I shares 37/39 amino acids of homology with the N-terminal region of the DNA-binding nuclear protein histone H2A from *Xenopus* [26] . Indeed, several histones have been demonstrated to have antimicrobial activity of their own [29] , while other cationic antimicrobial peptides, such as hipposin [30] and parasin I [31] , also share homology with the N-terminal region of the histone H2A, although their mechanisms of action have not yet been well studied.

Indolicidin is a 13-amino acid tryptophan-rich (39%) [32] peptide from bovine neutrophils and a member of the cathlecidin group (the name relates to the sequence homology shared between the N-terminal pre-pro region of the peptide and that of a protease inhibitor cathelin [33]). Although we originally concluded that its mechanism of action was through membrane permeabilization but not cell lysis [34] , this erroneous conclusion related to the rather high concentrations used in these studies and many subsequent studies have suggested that despite its tendency to cause partial membrane disruption, it has an alternative mechanism of action. One study found that when indolicidin was added, filamentation of *E. coli* cells occurred (indicative of inhibition of cell division), which correlated with a lack of incorporation of thymidine into the cell, suggesting that it was interacting with DNA [35] . Further studies demonstrated that indolicidin was able to bind to DNA through gel-retardation studies [32] . In another study, it was shown that indolicidin could interfere with the human DNA-binding enzyme

topoisomerase 1, which is involved in relaxation of supercoiled DNA [36] . This interference occurred without unwinding occurring, indicating that indolicidin could interact with other DNA-associated enzymes. Although indolicidin contains a PWWP motif (which is found in DNA-binding proteins such as DNA methyltransferase proteins), it was found through N-terminal truncation of indolicidin (leaving PWWP present) that the resulting peptide lost its DNA-binding ability [36] . Therefore, this suggests that activity is not dependent on this motif and, while these studies were carried out in a mammalian system, they could be indicative of how indolicidin interacts with prokaryotic DNA. Other cationic antimicrobial peptides shown to inhibit bacterial growth through interactions with nucleic acids were a pleurocidin derivative, frog dermaseptin and the indolicidin variant CP10A [37] .

Cationic antimicrobial peptides that act on protein synthesis

PR39, a 39-amino acid cecropin (originally isolated from pig intestines [38] and later found in pig neutrophils [39]) was shown to inhibit protein synthesis in *E. coli* . As a result of a cessation of protein synthesis, DNA synthesis was also halted through a possible degradation of DNA replication enzymes [40] . Studies with an indolicidin variant CP10A (containing three proline-to-alanine substitutions) found that this peptide was able to inhibit protein synthesis in *Staphylococcus aureus* through the disruption of histidine incorporation at twofold the MIC [37] . In addition, other macromolecular synthetic pathways were also affected by CP10A. A pleurocidin (from winter salmon [41]) derivative was also shown to effect protein synthesis, inhibiting histidine incorporation in *E. coli* at the MIC [23] . Other cationic antimicrobial peptides that have been demonstrated to act on protein synthesis include the [alpha]-helical peptides dermaseptin and pleurocidin, and the [beta]-sheet peptide human defensin human neutrophil peptide-1 [2] . Note that some of these peptides also inhibited synthesis of other macromolecules.

Cationic antimicrobial peptides that act on translation/protein folding

As with eurkaryotic cells, bacterial cells possess chaperones that assist in the folding of proteins and peptides into their correct conformations. Recently, it was found through the use of immunoaffinity purification and mass spectrometry that a 70-kDa bacterial chaperone, DnaK, was the target for the insectproduced cationic antimicrobial peptides pyrrhocoricin, drosocin and apidaecin (Table 1) [42] . This study demonstrated that these peptides were specific for *E. coli* DnaK but did not interact with another bacterial chaperone, GroEL, or the human chaperone Hsp70. Further studies revealed that pyrrhocoricin and drosocin affected the ATPase activity of DnaK, which was probably caused by these peptides binding to DnaK and preventing its multihelical lid from functioning [43] .

Cationic antimicrobial peptides that disrupt cell wall synthesis/cell division/septum formation

In addition to affecting protein synthesis, PR39 and an equally active truncated variant, PR26, were shown to have other mechanisms of action [39] . It was suggested in these studies, in contrast to other work, that these peptides were not internalized by bacterial cells. Instead, using *Salmonella typhi* as a model, these two peptides were able to affect cell septum formation (leading to filamenteous cells) by interacting with membrane-associated, cell septum-forming molecules. This mechanism of action has also been postulated for microcin 25 activity on *E. coli* [44] and filaments have also been observed in the action of indolicidin on *E. coli* [35] . Conversely, the bactenecin derivative Bac2A, acting on *Staphylococcus epidermidis* , caused the induction of division septum defects [37] .

The bacteriocins are a special category of cationic antimicrobial peptides that are produced solely by bacteria. Lantibiotics are a specific class of bacteriocin that are highly posttranscriptionally modified antimicrobial peptides containing unusual amino acids, such as the thioether lanthionine. The best characterized lantibiotic, nisin, produced by the bacterium *Lactococcus lactis* subsp. *lactis* has been shown to have multiple mechanisms of action. As with many other cationic antimicrobial peptides, it can cause inhibition through pore formation [45] . However, it also has alternative mechanisms, such as binding to the peptidoglycan precursor carrier lipid II, inhibiting the synthesis and regeneration of the cell wall and, consequently, cell division. Indeed, the efficiency of pore formation by nisin improved greatly when lipid II was present in liposomes [46,47] , leading to the proposal that lipid II is a docking molecule for nisin [48] . This mechanism has also been demonstrated for other

lantibiotics, including gallidermin and lactacin 3147, but not epilancin K7 or Pep5 [46] .

A lipid II-independent mode of action has also been postulated for nisin. It, and another lantibiotic, Pep5 (produced by *S. epidermidis*), were involved in the activation of cell wall lytic enzymes [49] . Their inhibitory effect occurs following their ability to outcompete these enzymes for binding to teichoic acids and lipoteichoic acids at which sites amidases are stored as an inactive form. The subsequent premature release of the lytic enzymes leads to a weakened cell wall and death caused by cell lysis.

A recent report found yet another mechanism of cell wall inhibition by certain lantibiotics [50] . In this case, some lantibiotics, such as (nisin structural analog) mutacin 1140 from *Streptococcus mutans* , are too small to effectively form transmembrane pores, yet are still effective antimicrobials [51] . Their mechanism was suggested to involve binding to, and sequestering, lipid II at the cell division septum, effectively blocking the ability of target bacteria to synthesize the cell wall.

Synergistic activity of cationic antimicrobial peptides

Synergy occurs when two antimicrobials work in concert to reduce the individual concentration of each molecule required to kill a microorganism. It is possible that this has arisen naturally in many higher organisms, since virtually all of these produce more than one peptide at a particular site (e.g., skin of humans [11]). However, despite many studies investigating synergism between cationic antimicrobial peptides, only those that used standardized techniques, such as the checkerboard titration technique [52] , can be considered to truly indicate synergism [53] . Nevertheless, a few antimicrobial peptides have been demonstrated to show synergy, for example, protegrin 1 (from pigs) with indolicidin, bactenecin or LL37 [53] . In this same study, indolicidin and bactenecin (which naturally coexist in cows) were also somewhat synergistic. Another study demonstrated that the different cationic peptides produced as part of the innate defense can work in synergy. Two out of three gallinacins (gallinacins 7 and 9), cationic peptides produced in chicken (*Gallus gallus domesticus*), demonstrated enhanced activity against *Salmonella enteriditis* [54] .

The combined effect of two antimicrobials working on different target sites of bacteria might allow for more effective killing. For example, in Gram-negative bacteria, this can occur when peptides, through their barrier-disruptive effects, coincide with self-promoted uptake across the outer membrane, and can assist the concomitant uptake of conventional antibiotics, such as [beta]-lactams, macrolides, quinolones or lysozyme [55] . For example, tachyplesin III (from the horseshoe crab) demonstrated strong inhibitory activity against multidrug-resistant *P. aeruginosa* strains when combined with [beta]-lactam antibiotics or colistin [56] . A recent study found that pre-incubation of a pyrrhocidin variant A3-APO (which, similar to its parent peptide, is thought to act against *E. coli* chaperone DnaK) at a subinhibitory concentration with TEM-1-([beta]-lactam-resistant)expressing *E. coli* cells followed by the addition of amoxicillin restored a susceptible phenotype to the bacteria [57] . This susceptibility was not seen when A3-APO was absent or added simultaneously with amoxicillin. These data show an effect of delayed synergy (as opposed to simultaneous synergy) in which it is thought that the A3-APO is blocking protein synthesis from occurring within the cell to a level that renders the bacterial cell susceptible to amoxicillin due to a lack of the production of the [beta]-lactamase (TEM-1).

Another advantage of cationic antimicrobial peptides used in combination with conventional antibiotics is that they may also have antiendotoxic capabilities, thus, having the potential to reduce sepsis that can be caused by endotoxin from Gram-negative bacteria (also known as LPS). By contrast, sepsis is actually promoted by many conventional antibiotics. Peptides such as LL-37, are able to potently reverse the ability of LPS to stimulate the production by human cells of proinflammatory cytokines, such as TNF-[alpha], through a diverse series of mechanisms [58] .

In some cases, synergy can occur between peptides and enzymes. Lysozyme, a cell wall-degrading enzyme that is present in most animals and is normally unable to cross the outer membrane of Gram-negative bacteria, shows synergy with some peptides [53] . Phospholipases are host-derived enzymes that catalyze the hydrolysis of sn-2-ester bond in phospholipids [59] . While secretory phospholipase 2 itself has been demonstrated to be antimicrobial [60] , a recent study demonstrated that the cationic antimicrobial peptides indolicidin, magainin 2 and others were able to enhance its hydrolytic activity against liposomes

[61] .

Designer cationic antimicrobial peptides

Along with the 1000 or so cationic antimicrobial peptides already identified from various organisms, new research now looks to capitalize on nature's efforts using various techniques to create libraries of novel antimicrobial peptides [8] . Conveniently, such peptides are fairly simple to work with, owing to their physical attributes. Basic approaches have looked at substitutions, insertions and deletions of amino acids. Other approaches have looked at creating new peptides through 'cutting and pasting' two peptides together (e.g., CEME that is created with the N-terminal domain of cecropin and C-terminal domain from melittin, and P-Der that contains the first seven amino acids of dermaseptin fused with the 20 C-terminal amino acids of pleurocidin [62]). Newer approaches have focused on powerful design and screening techniques. Two notable systems include the synthetic combinatorial libraries, where semi-random synthesis of peptides are created and screened for inhibitory activity [63] and amino acid substitution analysis of a base (parent) cationic antimicrobial peptide (e.g., Bac2A) using high-throughput synthesis on cellulose membranes, followed by real-time screening through the measurement of activity of an incorporated luciferase enzyme cassette in a model bacterium (in this case, *P. aeruginosa*) [12] . One recent report employed a novel approach in cationic antimicrobial peptide design looking at antimicrobial activity of cationic antimicrobial peptides designed according to the linguistic properties (patterns) of amino acids [64] .

Expert commentary & five-year view

Despite the past 20 years demonstrating great advances in the field of cationic antimicrobial peptides, they have offered only limited new information in the understanding of their mechanism(s) of action. Although many new peptides are being isolated or created synthetically, there still remains some uncertainty as to how they actually work. While thinking was initially focused towards a single mechanism of action, that is, pore formation, recent research with several cationic antimicrobial peptides has led to the proposal of a 'multitarget' mechanism of action [4] and the concept of these peptides as dirty drugs [6] to explain the many different methods such peptides can use to kill a bacteria. With this in mind, researchers must now broaden their focus when studying the mechanisms of action so as not to exclude any possibilities.

The ultimate cationic antimicrobial peptide would be small, stable, easily and cheaply synthesized, have a broad inhibitory spectrum with multiple mechanisms of action, minimal toxicity for mammalian cells and retain a minimal tendency toward resistance development, as well as an ability to work in synergy with other antimicrobials. Many of these ideal properties have been achieved but, as yet, not all in the same cationic antimicrobial peptide, and there may be a long road ahead to achieve such an optimized peptide. While there are some cationic antimicrobial peptides in the commercial developmental pipeline [5] , especially as topical applications [65] , in the near future more will be identified and begin the long road to becoming viable products. This review has described how, out of hundreds of identified cationic antimicrobial peptides, only a few have actually been studied in any great detail and there remains substantial uncertainty about the defined mechanisms of action and what causes different peptides to act differently. With the increasing understanding that many of these peptides are not membrane disruptive, the focus on how these interact with bacteria has changed to include the possibility of internal targets and the potential for multiple internal targets (Table 1). Through the use of techniques such as sequence analysis (i.e., amino acid composition, primary sequence, size, charge, hydrophobicity and structure), the best inhibitory conformation can be determined. Computer-aided prediction methods can be used to increase our understanding permitting the possibility of *de novo* prediction of active peptide sequences. These methods will aid in the creation of large numbers of more active peptides that can then be optimized for other properties (particularly activity in animal models). Therefore, we submit that it is only a matter of time before new techniques such as those described previously, combined with bacterial methodologies including mutant library screens and microarrays, will lead to the more effective identification of new and common targets of cationic antimicrobial peptides in bacteria.

Table 1. Summary of cationic antimicrobial peptides and their mechanisms of action.

Presumed activity	Peptide	Mechanism of action	Ref.

Membrane disruptive: destroy cytoplasmic membrane integrity	Nisin	Channel former; lipid II inhibition and cell wall lytic enzyme release	[46,49]
	Alamethicin	Barrel-stave	[66]
	Magainin 2	Toroidal pore	[19]
	Cecropin	Carpet model	[20]
	Indolicidin	Aggregate model	[1,17]
Membrane nondisruptive	Buforin II	DNA synthesis	[27]
	Indolicidin	DNA synthesis, cell division	[34,35,37]
	Pleurocidin derivative P-Der	RNA, protein synthesis	[23,37]
	PR39	Protein synthesis, cell septum formation	[39,40]
	CP10A	RNA, protein synthesis	[37]
	Dermaseptin, HNP-1	Protein synthesis	[2] [23]
	Pyrrhocoricin, drosocin, apidaecin	Disruption of DnaK (chaperone) activity	[43] [42]
	Pep5	Cell wall lytic enzyme release	[49]
	Mutacin 1140	Lipid II sequestion	[50]
	Microcin 25	Cell septum formation	[44]

HNP: Human neutrophil peptide.

Key issues

* Cationic antimicrobial peptides offer great hope for the development of novel therapeutics against a range of microbial diseases.

* The large number of natural and synthetic cationic antimicrobial peptides provides a valuable resource even though only a few have been studied in any depth.

* Multiple mechanisms of actions have been defined for some antimicrobial peptides.

* The identification of intracellular targets for many antimicrobial peptides offers further therapeutic options.

* Synergy between cationic peptides and/or other antibiotics is weakly understood.

* Experimental studies into mechanisms of action of the cationic antimicrobial peptides are strongly affected by the concentration and test media used in studies.

CAPTION(S):

Figure 1. Summary of mechanisms of action for cationic antimicrobial peptides in Gram-negative bacteria.

Cationic antimicrobial peptides (CAPs) must first pass through the OM of the bacteria through a well-characterized self-promoted uptake mechanism that begins with attachment to the anionic lipopolysaccharide. Following passage through the cell wall, cationic antimicrobial peptides can attach and interact **(A)** with the IM as listed; the barrel-stave model **(B)** , toroidal pore **(C)** , aggregate model **(D)** and carpet model **(E)** . The aggregate model allows for CAP passage to the cytoplasm where different examples with CAPs have shown to bind and interfere with DNA and RNA synthesis **(F)** , protein synthesis **(G)** , protein folding **(H)** and cell wall synthesis/integrity **(I)** .

CM: Cytoplasmic membrane; IM: Inner membrane; OM: Outer membrane.

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