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Current Review

Antimicrobial Peptides

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Plants and animals have to survive in a world laden with pathogenic bacteria and fungi. Yet, perhaps the most interesting aspect of this ecosystem is not the expected staggering losses from *susceptibility* to infection as much as the *survivability* displayed by the diverse organisms in spite of infection. This resilience may be attributed to the presence of a repertoire of host defense mechanisms. In animals (including humans), defense may be mediated by events such as immune response, complement activation, phagocytosis, and release of small molecular weight antimicrobial peptides (Elsbach 1990). However, in insects, amphibians, and other lower organisms—although comparable immune responses are less well characterized—it is clear that small molecular weight peptides play a major role in warding off infection (Kimbrell 1991).

Plants, like animals, display apparent protective mechanisms. Responses to disease initiation are multifarious and include the production of proteins that alter the properties of the extracellular matrix of the host (i.e., extensins and glycine-rich proteins) as well as proteins that are directly involved with antimicrobial activity such as the glucanases and chitinases, protease inhibitors, and enzymes associated with phytoalexin biosynthesis (Bowles 1990; Broglie and Broglie 1993). In addition, other types of antifungal proteins from plants have also been described (Vigers *et al.* 1991; Terras *et al.* 1992, 1993; Molina *et al.* 1993a). Furthermore, as elaborated later in the text, potent antimicrobial peptides have been isolated and characterized from several plant species. Although the precise function of these molecules within the plant is a matter of debate, it is tempting to speculate that defense against microbial and/or insect predators may be a major function.

Among crop plants, fungal, bacterial, and viral diseases contribute significantly to the overall loss in yield. A major goal for plant biotechnology is the genetic engineering of disease and insect resistant varieties. For example, genetically engineered protection against viruses in transgenic plants (see review by Fitchen and Beachy 1993) and the resistance to insects of transgenic tobacco expressing the toxin from *Bacillus thuringiensis* (Barton *et al.* 1987) clearly indicate the potential of introducing foreign "defense" genes into plants. Antimicrobial peptides with broad spectrum activity provide a rich source of genes for transformation of plants for disease resistance. Antimicrobial activity has predominantly been measured against bacterial pathogens of pharmaceutical significance and very little effort has been directed toward studies on the efficacy against plant-pathogenic bacteria or fungi. It is hoped that this review, which highlights some recent strides in the structure-activity relationships of antimicrobial peptides, will provide a fresh perspective for researchers involved in the control of plant diseases.

In the broadest sense, the term "antimicrobial" can be used to define any compound that displays the ability to destroy or inhibit the growth of bacterial and fungal cells. For the purposes of this review, attention will be focused on peptides that are all approximately 50 amino acids in length and share the common function of *in vitro* antimicrobial activity. They come in a remarkable array of molecular architecture, composition, and size and may be classified arbitrarily into linear peptides, disulfide-linked peptides, lantibiotics, and designed synthetic peptides (Table 1).

Linear peptides.

Cecropins are a family of homologous antibacterial peptides of 35–37 residues derived from the Cecropia moth, *Hyalophora cecropia* (reviewed in Boman *et al.* 1991) for which the genes have been isolated. Included in the general family of cecropins are the homologous bactericidal peptides from other insect species and mammals (reviewed in Boman *et al.* 1991). The solution conformation of cecropin A (Holak *et al.* 1988) suggests that the antibacterial activity is linked to a molecular architecture consisting of a cationic *N*-terminal amphipathic region and a hydrophobic C-terminal amphipathic half separated by a hinge. The preeminent role of the structure of cecropin in relation to its biological activity has been confirmed through the design and synthesis of several analogs (Fink *et al.* 1989). The cecropins have no effect on eukaryotic cells (Steiner *et al.* 1981), but affect prokaryotic cells through the formation of time-variant and voltage-dependent ion channels (Christensen *et al.* 1988). The retention of full biological activity of the synthetic D-isomer of cecropin B precludes a receptor-mediated mechanism of membrane perturbation (Wade *et al.* 1990). Jaynes (1990) has reported on the superior biological activity of Shiva-1, a cecropinlike synthetic peptide exhibiting ~46% homology to the natural molecule. Furthermore, the potent antimicrobial activity of cecropin B against peach- pathogenic bacteria at concentrations that do not affect the plant cells (Mills and Hammerschlag 1993) indicates the potential usefulness of lytic peptides in disease resistance strategies in agriculture (Destefano-Beltran *et al.* 1993).

In contrast to cecropin, the antibacterial activity of the structurally similar bee venom peptide, melittin (Steiner *et al.* 1981) is less exploited because of the known hemolytic and toxic properties of this molecule (Haberman 1972). However, residues essential to hemolytic function have been mapped (Blondelle and Houghten 1991) and several cecropin-melittin hybrids have been synthesized, wherein this undesirable property has been eliminated with full retention or superior antibacterial activity (Boman *et al.* 1989; Andreu *et al.* 1992; Wade *et al.* 1992). Interestingly, the honeybee (*Apis mellifera*) secretes a variety of antibacterial peptides into the hemolymph as part of the defense response (Casteels *et al.* 1993). Apidaecins, a family of heat-stable, proline-rich, non-helical antibacterial peptides of 18 residues, display marked activity against many plant-associated bacteria through a non-lytic mechanism while apparently not affecting eukaryotic cells (Casteels *et al.* 1993). More importantly, the inactivity of the D-isomer of the peptide (in contrast to cecropin all D-isomer) implicates a stereoselective recognition process for the antimicrobial function (Casteels and Tempst 1994). Abaecin is another proline-rich cationic peptide which contains 34 residues but has weaker antimicrobial activity than the apidaecins especially against Gram-negative plant pathogens (Casteels *et al.* 1990). It is not known whether this peptide also displays stereoselective recognition. Furthermore, the non-lytic mechanism associated with the apidaecins makes them attractive candidates in transgenic plant studies.

Just as the insect species, the amphibian species represent a rich source of antimicrobial peptides. Perhaps the earliest report was that of Csordas and Michl (1970) who isolated a 24-residue magaininlike hemolytic antibiotic peptide (bombinin) with an amidated C terminus from the skin of the European toad, *Bombina variegata*. More recently, Gibson *et al.* (1991) have reported on the isolation of amidated, non-hemolytic, prokaryote-specific bombininlike antimicrobial peptides from skin secretions of the Asian toad, *Bombina orientalis*. Also, skin secretions of the frog *Xenopus laevis* contain the antimicrobial peptide magainin (Zasloff 1987) and a family of similarly sized antimicrobial peptides collectively referred to as the magainin family (review by Bevins and Zasloff 1990). Moore *et al.* (1991) have also isolated these peptides from the stomach of *Xenopus laevis* and, in addition, have purified a novel magaininlike antimicrobial peptide termed PGQ. The 23-residue-long magainins 1 and 2 have been shown to be effective against a variety of bacteria, fungi, and protozoa (Zasloff 1987; T. Rood, personal communication) but to be ineffective against eukaryotic membranes including erythrocytes. Although the precise mechanism of antimicrobial action of magainin is as yet undefined, the ability to adopt an amphipathic α-helical structure in the presence of phospholipid bilayers is deemed to play an important role (Bechinger *et al.* 1992; Ludtke *et al.* 1994). Furthermore, analogs of magainin with improved antibacterial activity have been made through substitutions and chain extension to increase the α-helicity and cationic nature (Chen *et al.* 1988; Besalle *et al.* 1992) as well as through deletions of amino acids (Cuervo *et al.* 1988). Another example of an amphibian antimicrobial peptide is that of dermaseptin isolated from the skin secretion of the South American arboreal frog *Phyllomedusa sauvagii* (Mor *et al.* 1991). This 34-residue, non-hemolytic peptide is unrelated to the magainins and exhibits potent antifungal activity against opportunistic human pathogens, such as the filamentous fungi *Aspergillus fumigatus* and *Arthroderma simii* (Mor *et al.* 1991). The amino-terminal amphipathic α-helical domain comprising 18 residues is responsible for the antimicrobial activity (Mor and Nicolas 1994).

A distinct class of antimicrobial peptides are the histatins (Oppenheim *et al.* 1988; Oppenheim 1989). Twelve peptides varying in length from 7 to 38 residues have been isolated from human parotid and submandibular secretions. These histidine-rich proteins display antimicrobial activity against a variety of infectious microorganisms in the oral cavity and potent fungistatic effects on the yeast *Candida albicans*. It is believed that the mechanism of antimicrobial action is related to the ability of histatin to undergo a membrane-induced helical conformation followed by membrane perturbation (Raj *et al.* 1994).

The cytoplasmic granules of mammalian polymorphonuclear leukocytes contain a variety of antimicrobial peptides

Table 1. Categories of antimicrobial peptides

Category	Examples (references)
Linear peptides	**mammalian and insect *Cecropin family*/*Cecropin analogs*** (review by Boman *et al.* 1991); **hybrid peptides of *Cecropin and Melittin*** (Boman *et al.* 1989; Andreu *et al.* 1992; Wade *et al.* 1992); *Melittin and Melittin analogs* (Blondelle and Houghten 1991); *Apidaecins and Abaecin* (Casteels *et al.* 1990, 1993; Casteels and Tempst 1994); ***Magainin family and Magainin analogs*** (review by Bevins and Zasloff 1990; Chen *et al.* 1988; Bessalle *et al.* 1992); ***Bombinin*** (Csordas and Michl 1970); ***Bombinin-like peptides*** (Gibson *et al.* 1991); ***Dermaseptin*** (Mor *et al.* 1991; More and Nicolas 1994); ***PGQ*** (Moore *et al.* 1991); ***PR-39*** (Agerberth *et al.* 1991); ***Indolicidin*** (Selsted *et al.* 1992); ***Histatins*** (Oppenheim *et al.* 1988, 1989); ***peptide derived from pig myeloid cells*** (Zanetti *et al.* 1994); ***peptides from human neutrophil cathepsin G*** (Bangalore *et al.* 1990; Shafer *et al.* 1991); ***Antimicrobial peptides from bovine neutrophils*** (Frank *et al.* 1990); Seminalplasmin (Sitaram and Nagaraj 1993); ***Antimicrobial domain from Lactoferrin*** (Bellamy *et al.* 1992,1993); ***Drosocin*** (Bulet *et al.* 1993)
Disulfide-linked peptides	**mammalian *Defensins*** (Ganz *et al.* 1992; Lehrer *et al.* 1993; Selsted *et al.* 1993); **insect *Defensins*** (Matsuyama and Natori 1988; Lambert *et al.* 1989; Fujiwara *et al.* 1990; Bulet *et al.* 1991; Hoffman and Hetru 1992); ***Thionins*** (Bohlmann and Apel 1991; Bohlmann 1994); ***Tachyplesin*** (Nakamura *et al.* 1988; Miyata *et al.* 1989); ***Maize Basic Peptide I*** (Duvick *et al.* 1992); ***Tracheal antimicrobial peptide*** (Diamond *et al.* 1991); ***Antimicrobial peptides from seeds of amaranth*** (Broekart *et al.* 1992); ***Antimicrobial peptides from seeds of Mirabilis jalapa*** (Cammue *et al.* 1992); ***Ranalexin*** (Clark *et al.* 1994); ***Brevenin*** (Morikawa *et al.* 1992; Simmaco *et al.* 1993).
Lantibiotics	***Subtilin, Nisin, Epidermin*** (review by Hansen 1993; Schnell *et al.* 1988; Buchman *et al.* 1988; Banerjee and Hansen 1988; Kuipers *et al.* 1992; Liu and Hansen 1992); ***Lactacin 481*** (Piard *et al.* 1993)
Designed synthetic antimicrobial synthetic	***Basic amphipathic peptides*** (Lee *et al.* 1986 1989; Agawa *et al.*, antimicrobial peptides 1991; Blondelle and Houghten 1992; Bessalle *et al.* 1993; Zhong *et al.* 1994)

that play a major role in host defense. Best characterized of these is the defensin family (discussed in a later section). Other linear peptides have also been described. Bac7 (59 residues) and Bac5 (42 residues) belong to the bactenecin family of cationic antimicrobial peptides isolated from bovine neutrophils (Frank *et al.* 1990). They are characterized by an unusually high proportion of prolines (>45%) and arginines (>23%) which render them unlikely to adopt an α-helical conformation in the manner of the other peptides described earlier. Nevertheless, the ability of these peptides to permeabilize *E. coli* inner and outer membranes indicates that they may adopt some form of amphipathic structure on membrane surfaces as suggested by Frank *et al.* (1990). A proline/ arginine rich antimicrobial peptide that is similar in composition to the bactenecins is the peptide PR-39, isolated from the pig intestine (Agerberth *et al.* 1991). These peptides show some overall similarity to the proline-rich peptides apidaecin and abaecin that was discussed earlier. Indolicidin, another potent cationic antimicrobial peptide with an amidated C terminus, isolated from the cytoplasmic granules of bovine neutrophils, contains only 13 residues, including five tryptophans and three prolines (Selsted *et al.* 1992). No information is available at the present time concerning the structure-activity relationship of this unusual peptide. Lactoferrin is a well-known antimicrobial protein of ~75 kDa that is released from activated neutrophils in an inflammatory response and is also found in mammalian milk. Bactericidal domains of lactoferrin (~ 25 residues) exhibiting severalfold higher activity on a molar basis than the parent molecule have been synthesized (Bellamy *et al.* 1992). One of these, lactoferricin B, derived from the N terminal region of bovine lactoferrin, has been found to be particularly potent against *C. albicans* (Bellamy *et al.* 1993). Perhaps the shortest antimicrobial peptides to date are those derived from human neutrophil lysosomal Cathepsin G, a serine protease with a distinct bactericidal function (Bangalore *et al.* 1990; Shafer *et al.* 1991). These peptides with the sequence Ile-Ile-Gly-Gly-Arg and His-Pro-Gln-Tyr-Asn-Gln-Arg are broad-spectrum antibiotics that do not fit the classical cecropin or magainin type of amphipathic structure for antimicrobial activity. Another example of an antimicrobial peptide which does not appear to fall clearly within the definition of an α-helix or a β-sheet conformation is that derived from pig myeloid cells (Zanetti *et al.* 1994).

Another example of a nonhemolytic broad spectrum antimicrobial peptide is the 47-residue seminalplasmin from bovine seminal plasma (Sitaram and Nagaraj 1993). In contrast, an internal 13-residue antibacterial fragment derived from seminalplasmin is more hydrophobic than the parent molecule and also displays strong hemolytic activity (Sitaram and Nagaraj 1993). Interestingly, while some of the naturally occurring antimicrobial peptides described in this review are carboxamidated through a posttranslational modification (i.e., bombilin and tachyplesin), Bulet *et al.* (1993) have isolated a 19-residue, proline-rich, cationic antibacterial peptide drosocin from *Drosophila* that carries an *O*-glycosylated substitution at a threonine residue that appears to be critical for biological activity.

Disulfide-linked peptides.

In contrast to the linear forms, the disulfide-linked antimicrobials are structurally rather more complex, with the internal disulfide bonds dictating the conformation for biological activity. This is illustrated in the defensin family of peptides. Typically, mammalian Defensins isolated from humans, rabbits, guinea pigs, and rats are highly cationic and consist of 29–35 residues with conserved cysteine residues (review by Lehrer *et al.* 1993). They exhibit cytotoxicity against many types of mammalian cells and microbicidal activity against a variety of Gram-negative and gram-positive bacteria, human pathogenic fungi such as *C. albicans* and *Aspergillus fumigatus*, mycobacteria, and spirochetes (Lehrer *et al.* 1993). Synthetic rabbit defensin NP-1 has been shown to have potent fungicidal effects that are indistinguishable from that of natural NP-1 against maize pathogens (Rao *et al.* 1992). X-ray structural analysis of human Defensin (HNP-3) reveals a rich β-sheet structure and a dimeric assembly that confers an amphipathic conformation that is implicated in the permeabilization of cell membranes (Hill *et al.* 1991). Defensinlike antibacterial peptides (insect defensins) with conserved cysteine residues such as royalisin (Fujiwara *et al.* 1990), phormicin (Lambert *et al.* 1989), and sapecin (Matsuyama and Natori 1988; Yamada and Natori 1993) have also been isolated from a variety of insect species (see reviews by Kimbrell 1991; Hoffmann and Hetru 1992). Yamada and Natori (1994) have shown that a synthetic hendecapeptide (11 residues) derived from an α-helical region of sapecin B while displaying no hemolytic activity has superior antimicrobial activity to that of the parent peptide including activity against *C. albicans* and other fungi.. The fact that this peptide can release entrapped glucose from acidic liposomes suggests that the microbicidal function may involve a lytic mechanism. A distinct family of defensinlike peptides, but with a different disulfide array, has also been isolated from bovine neutrophils (Selsted *et al.* 1993). Termed β-defensins, these 38–42 residue cationic peptides are mainly antibacterial. The 38-residue tracheal antimicrobial peptide (TAP) isolated from mammalian tracheal mucosa (Diamond *et al.* 1991) shows homology to the β-defensins. However, TAP also displays strong activity against *C. albicans*.

A potent disulfide-linked antimicrobial peptide that is smaller than the defensins is tachyplesin. Isolated from the horseshoe crab *Tachypleus tridentatus,* this hemolytic, cationic, carboxamidated peptide is only 17 residues long, contains two disulfide bonds, and exhibits activity against *C. albicans* (Nakamura *et al.* 1988). However, as in the case of defensin NP-1, the antimicrobial function appears to be related to the amphipathic structure that arises from the distribution of bulky hydrophobic groups at the "head" of the antiparallel β-pleated sheet structure linked by a turn to a hydrophilic "tail" comprising six cationic residues derived from the N and C terminii of the protein (Kawano *et al.* 1990; Ohta *et al.* 1992; Katsu *et al.* 1993).

As mentioned above, the amphibian skin tissue is a source of several linear antimicrobial peptides. Interestingly, three different *Rana* species produce highly homologous cationic antimicrobial peptides that contain a single disulfide bond at the C terminus of the protein. These include the 20-residue ranalexin from the bullfrog *Rana catasbeiana* (Clark *et al.* 1994), the 24-residue brevinin-1 and 33-residue brevinin-2 from the Japanese frog *Rana brevipoda porsa* (Morikawa *et al.* 1992) and the corresponding peptides from the European frog *Rana esculenta* (Simmacco *et al.* 1993) designated as

brevinin-1E and brevinin-2E, respectively. Esculentin, is an unrelated (by sequence homology) 46-residue antimicrobial peptide that occurs in the European species, but shares the unique disulfide bond at the C terminus. A molecular model of ranalexin indicates a remarkable structural similarity with the well-known membrane active antibiotic polymixin, a cyclic peptide (Clark *et al.* 1994). The similarity encompasses an amphipathic structure comprising a "head" region containing a cationic ring structure and a "tail" of hydrophobic residues arranged as an α-helix. A single disulfide-bridged antibacterial peptide, Bactenecin, also occurs in the cytoplasmic granules of bovine neutrophils (Romeo *et al.* 1988).

Disulfide-containing antimicrobial peptides have also been isolated from several plant species. Best characterized of these are probably the thionins, a family of ~5 kDa (37–45 residues) cationic peptides distributed in the tissues of many cereals such as barley and wheat (review by Bohlmann and Apel 1991). The *in vitro* toxicity of thionins against bacteria, yeast, fungi, and animal cells has been documented by several researchers (review by Bohlman 1994; Molina *et al.* 1993b), and it is believed that they form part of the defense arsenal of the plants (Bohlmann 1994). As in the case of many linear peptides, the biological activity appears to be associated with a membranolytic function that arises from an amphipathic structure stabilized by four disulfide bonds (Teeter *et al.* 1990).

Duvick *et al.* (1992) have isolated several small, cationic antimicrobial peptides from maize kernels. One such peptide, which is distinct from the thionins, is MBP-1. This is a 33-residue α-helical peptide that is arginine rich, contains two disulfide bonds, exhibits activity against a representative set of maize fungal pathogens, and also shows antibacterial activity. Similarly sized peptides have been characterized by Broekaert *et al.* (1992) from the seeds of *Amaranthus caudatus*. These peptides termed *Ac*-AMP1 (29 residues) and *Ac*-AMP2 (30 residues) are identical except that the latter has one extra amino acid. The peptides are highly basic and contain three disulfide bonds. They exhibit antibacterial activity against Gram-positive bacteria and are toxic to at least six species of plant-pathogenic fungi. Interestingly, the homology these peptides exhibit to the cysteine/glycine rich domains of many chitin-binding proteins, coupled with their ability to bind to chitin, suggests that the mechanism of antimicrobial activity may be different from the examples discussed earlier. The seeds of *Mirabilis jalapa* L. are also a source of antimicrobial peptides containing three disulfide bonds (Cammue *et al.* 1992). Two peptides designated *Mj*-AMP1 (37 residues) and *Mj*-AMP2 (36 residues) have been characterized. They differ in only four amino acids and display potent activity against several plant-pathogenic fungi but antibacterial activity against only Gram-positive bacteria. Remarkably, the peptides show sequence similarity to μ-agatoxins, a class of neurotoxins from the venom of spiders, but do not share the neurotoxic properties.

Nakaya *et al.* (1990) have sequenced a small-sized (51 residues), very basic, antifungal protein from the mold *Aspergillus giganteus*. The molecule has four internal disulfide bonds and sequence comparison suggests that it may be structurally related to rat phospholipase A_2.

Lantibiotics.

Many Gram-positive bacteria, such as the *Lactobacillus* species, produce peptides or proteins that have bactericidal activity against other closely related species (Tagg *et al.* 1976). These are called bacteriocins and contain the common codon-encoded amino acids. The term lantibiotics (Schnell *et al.* 1988) is reserved for a second class of gene encoded, ribosomally synthesized, antimicrobial peptides of less than 4 kDa. In this case, the proteins subsequently undergo an enzyme dependent posttranslational modification that results in the creation of unusual amino acids such as dehydroalanine and dehydrobutyrine (from the precursors serine and threonine) which can in turn condense with neigboring cysteine residues to give the thioether amino acids lanthionine and β-methyllanthionine (see review by Hansen 1993). Nisin, subtilin, and epidermin are the most well-known members of the lantibiotic family with activities predominantly against wide-ranging Gram-positive bacteria. There does not appear to be any information concerning the antimicrobial activity of these peptides against plant pathogens. However, nisin has been used extensively in the food industry as a preservative (Hurst and Collins-Thompson 1979). The genes for nisin (Buchmann *et al.* 1988), subtilin (Banerjee and Hansen 1988), epidermin (Schnell *et al.* 1988), and lactacin 481 (Piard *et al.* 1993) have been cloned and indicate that the proteins are synthesized as preproproteins. The mechanism of action of lantibiotics is still under investigation, although there is some evidence that these compounds can form voltage-dependent multistate pores in bacterial membranes that can lead to an efflux of cellular constituents (Schuller *et al.* 1989). Protein engineering strategies have been adopted with nisin and subtilin to enhance their antimicrobial activities (Kuipers *et al.* 1992; Liu and Hansen 1992).

Designed, synthetic antimicrobial peptides.

In the body of work discussed here, a significant underlying theme that emerges in a majority of the antimicrobial peptides is their cationic nature and a predisposition toward the adoption of an amphipathic secondary structure upon interaction with a membrane surface. It is believed that the antimicrobial activity is linked to the ability of the amphipathic peptides to interact with host cell membranes, forming ion channels leading to permeability changes and consequent cell lysis (Christensen *et al.* 1988; Okada and Natori 1984; Westerhoff *et al.* 1989). The recognition of the significance of the cationic amphipathic α-helix has led to the *de novo* design of synthetic antimicrobial peptides incorporating this structural motif. Basic peptides of various chain lengths containing the repeat sequence Leu-Ala-Arg-Leu adopted an α-helical structure in the presence of liposomes, formed ion channels in planar lipid bilayer membranes and diplayed broad spectrum antimicrobial activity (Lee *et al.* 1986, 1989; Agawa *et al.* 1991; Anzai *et al.* 1991). Blondelle and Houghten (1992) synthesized model amphipathic peptides containing only leucine and lysine residues and demonstrated antibacterial activity that could be decoupled from the concomitant hemolytic activity by appropriate deletions and/or substitutions. On the other hand, Bessale *et al.* (1993) designed several model peptides ranging from 9 to 17 residues (modelins) based on a rational choice of the size of the amino acid, its hydrophobicity and basic character. Interestingly, it was found that some of the smaller peptides displayed significant antimicrobial activity even in the absence of an amphipathic helical struc-

ture. This observation, coupled with the small size that precludes their ability to span the lipid bilayer, suggests the possibility of different mechanisms for antimicrobial function of long chain versus short chain peptides (Bessale *et al.* 1993). Thus, a common approach to the design of synthetic antimicrobial peptides has involved the judicious use of hydrophobic and hydrophilic amino acids with a high α-helical propensity to generate amphipathic sequences of various lengths. Unlike these approaches, however, Zhong *et al.* (1994) analyzed known protein sequences by the hydrophobic moment algorithm (Eisenberg *et al.* 1984) and identified putative cationic amphipathic structures that were subsequently protein engineered to display broad spectrum antibacterial activity, potent activity against two maize fungal pathogens and devoid of hemolytic activity.

Applications in plant biotechnology.

One of the major goals of plant biotechnology is to develop crop plants with superior ability to resist diseases caused by fungal and bacterial pathogens and thereby increase productivity. Obvious, but challenging, approaches towards solving the problem include an understanding of the molecular basis of specificity between the pathogens and their host plants and to determine the source of resistance in resistant plants. Progress in this area has been reviewed recently (Keen *et al.* 1993; Templeton *et al.* 1994). For example, the *HM1* gene in maize encodes a protein with reductase activity that provides resistance to the fungus *Cochliobolus carbonum* by detoxifying the fungal toxin (Johal and Briggs 1992; Meeley *et al. 1992*). However, recent progress in plant transformation technologies (review by Joshi and Joshi 1991) should facilitate the expression of antimicrobial peptides to augment the disease resistance mechanisms in plants. Although several proteins such as the antibiotic resistance genes (Reiss *et al.* 1984), the herbicide resistance gene (De Block *et al.* 1987), hen egg white lysozyme (Trudel *et al.* 1992) and barley ribosome-inactivating protein (Logemann *et al.* 1992) have been succesfully expressed in plants, reports of expression of lytic peptides is rather limited. Pang *et al.* (1992) failed to see insect toxicity in extracts of tobacco plants transformed with the scorpion insectotoxin presumably because of improper folding and disulfide bond formation of the protein. However, Carmona *et al.* (1993) have reported on the enhanced resistance to bacterial pathogens of transgenic tobacco expressing barley α-thionin, a protein that contains four disulfide bonds. Montanelli and Nascari (1991) introduced the cecropin gene into potato without gross phenotypic alterations and showed antibacterial activity associated with protein extracted from fresh tissues but no unequivocal demonstration of resistance of the whole plant. Jaynes *et al.* (1993), on the other hand, expressed the modified cecropin B peptide, Shiva-1, in tobacco and reported on the enhanced resistance of transgenic plants to *Pseudomonas solanacearum*. In contrast, Hightower *et al.* (1994) report that transgenic tobacco expressing cecropin mRNA and protein are just as susceptible to infection with *Pseudomonas syringae* as the control plants. Clearly, the expression of lytic peptides impose other challenges. These include questions such as tissue-specific or constitutive expression, secretion or intracellular localization, protein stability and, especially in the case of disulfide-containing proteins, proper folding. Furthermore, given that the lytic peptides perturb biological membranes it may become necessary to express them in a biologically inactive form so as to minimize damage to host cells until pathogen invasion. Although daunting, these challenges are not insurmountable. Emerging technology in protein engineering and plant molecular biology should make it possible to "tweak" the biochemistry of plant cells through a sustained interdisciplinary approach and create transgenic plants that are phenotypically normal and healthy.

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