
Function of Genetic Material: Progressive Insight into Antimicrobial Peptides and their Transcriptional Regulation

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1 Introduction

Antimicrobial peptides (AMPs) are small proteins that display growth inhibitory effects on a multitude of organisms. They are constituents of the innate defence mechanism of multicellular organisms (Zasloff 2002), in which they are essential factors in repelling pathogen attack. Interestingly, the host range of AMPs is largely restricted to a specific group of pathogens. Furthermore, they often possess high antimicrobial potential. Due to these two aspects, AMPs are considered to be “nature’s antibiotics” (Wang and Wang 2004), representing attractive alternatives to chemical antimicrobial agents presently in use (Reddy et al. 2004a). However, before research can take full advantage of AMP characteristics, more detailed information has to be accumulated concerning their expression and regulation, respectively.

During the last few years, dramatic progress has been made in understanding how expression of AMP-encoding genes is induced and regulated. It appears that many organisms, such as *Arabidopsis thaliana*, possess highly sophisticated defence-related signalling pathways (Nimchuk et al. 2003). These enable the host to discriminate between different qualities of pathogen infection and other stress-evoking factors, and to react accordingly.

In this review, the interested reader is given an overview on the recent progress achieved in understanding AMP expression and regulation of AMP-encoding genes. For further leading in-depth information, the reader is referred to a selection of comprehensive review articles from Lemaitre (2004), Zhao (2005) and Zipfel and Felix (2005).

2 What are antimicrobial peptides?

Antimicrobial peptides, comprising of antibacterial or antifungal activity, have been identified in many species, ranging from bacteria and fungi to insects, mammals and plants. In evolutionary terms, they are thought to be ancient

constituents of the innate immune system. Although prokaryotes are devoid of any kind of immune response, some of them are also known to produce peptides with antimicrobial potential (Cheigh and Pyun 2005).

Generally, bacterial AMPs provide their host with a selective advantage. This advantage may imply that prokaryotic strains are able to successfully defend their assigned ecological niches against putative competitors (Bhatti et al. 2004).

Regardless of their origin, AMPs are gene-encoded, low-molecular weight proteins, generally consisting of fewer than 100 amino acid residues (Ganz 2005). Strikingly, there is little sequence or structural similarity to perceive among them. Although they exhibit a rather wide range of variance, it is impressive to note that all AMPs seem to operate via the same fundamental mode of action. This involves the permeabilisation of microbial membranes, concomitantly resulting in growth arrest of putative pathogens or rival strains. An underlying prerequisite for the membrane perturbing effect is certainly the cationic net charge of AMPs, which is a common characteristic for this outstanding group of peptides. Furthermore, AMPs generally exhibit an amphipathic configuration (De Smet and Contreras 2005), which is also assumed to be involved in the process of membrane permeabilisation.

The characteristic event of membrane permeation consists of the electrostatic interaction between AMPs and membranes. It has been suggested that positively charged peptides can displace charge-neutralising cations, which were found to localise on membrane surfaces. Upon removal, AMPs can bind to negatively charged membrane constituents, such as lipopolysaccharides. Alternatively, AMPs can neutralise the membrane charge within the affected area, subsequently resulting in the permeabilisation of membranes (Bowdish et al. 2005).

2.1 Function

Antimicrobial peptides form the first line of innate host defence in multicellular organisms. In contrast to the adaptive immune system, which may take days or weeks until it successfully responds to invasive attack, the innate immune system provides a rapid means to combat pathogen infection right from the start (Clark and Kupper 2005).

Presumably, all metazoans have evolved an inborn defence mechanism. Distinguishing features of this innate immunity comprises of pattern recognition receptors (PRR), which usually exhibit a broad range of specificity. PRR are able to recognize many related molecular structures, referred to as

pathogen-associated molecular patterns (PAMPs) (Nurnberger et al. 2004; Zipfel and Felix 2005). PAMPs generally show little variance. They typically consist of polysaccharides and polynucleotides, exclusively present in the invading pathogen. No memory of prior exposure to a certain pathogen is required for PRR expression, which explains the rapidity with which the innate immune response is able to respond to invasive attack.

Some organisms, such as plants and lower animals, do not possess an adaptive immune response and are therefore utterly depend on their innate immune system. However, although devoid of an acquired immunity, these organisms are highly successful in protecting themselves against life-threatening invaders. This circumstance clearly speaks in favour of the efficiency of the innate immune system, which has stood the test of time for million of years.

Remarkably, the effective range of AMPs is not exclusively restricted to the innate immune system. These peptides were also found to trigger and to interact with the adaptive immune response (Oppenheim et al. 2003). In metabolic terms, this is a greatly economic means of responding to pathogen attack. The host merely invests energy into the expression of basic defence machinery.

General elicitors, collectively termed now as pathogen-associated molecular patterns (PAMPs; Fig. 1), interact with host receptors, such as pattern recognition receptors (PRRs) in plants or Toll-like receptors (TLRs) in animals. The receptors, in turn, translate the signal into cellular reactions that result in the activation of plant defence reactions (Fliegmann et al. 2004).

The correlation between environmental stimulus and defence gene expression may be diverse. However, AMPs are a potent means of providing their host with a selectional advantage, be it either the protection against pathogen invasion or the defence of an ecological niche against a putative competitor.

Every organism encounters pathogen invasion most of the time, which implies that parasites try to enter their host with the aim to feed and to propagate at its expense. Pathogen invasion can constitute a considerable metabolic burden for the affected organism, significantly reducing its fitness and chances for survival. In order to successfully counteract parasite intrusion, the host mounts an immune response. This includes the production of AMPs, which provides it with a selectional advantage over non-producing strains. The defence of a certain habitat or ecological niche by means of AMP activity particularly holds true for prokaryotes. Both *Lactococcus lactis* subsp. *lactis* and *Listeria monocytogenes* are lactic acid bacteria sharing the same habitats. *L. lactis* produces an AMP referred to as nisin. This peptide has proven to effectively inhibit the growth of *L. monocytogenes* (Bhatti et al. 2004), thereby keeping the putative rival for nutrients successfully at bay.

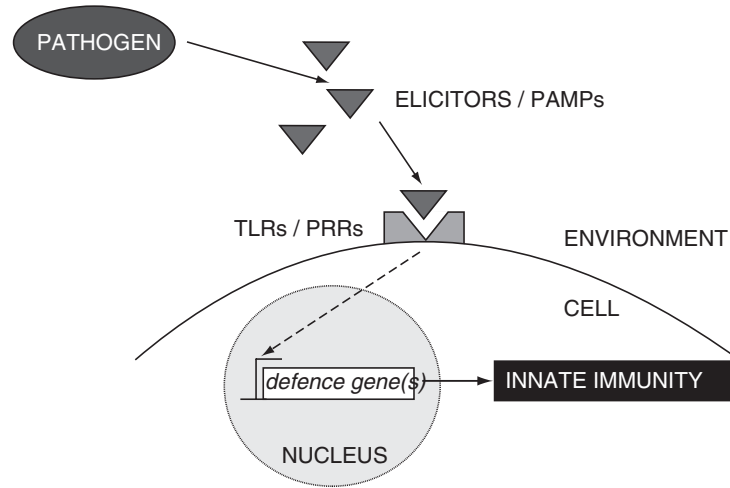


Fig. 1. Schematic drawing outlining the general recognition of elicitors or pathogen-associated molecular patterns (PAMPs). Defined receptors, such as the pattern recognition factors (PRRs) in plants or the Toll-like receptors (TLRs) in animals, perceive these pathogen-derived signals, consequently resulting in the transcription of specifically associated defence genes

2.2 Mechanism of action

The molecular activity of AMPs can generally be ascribed to an electrostatic relation between positively charged peptides and negatively charged microbial membranes (Park and Hahm 2005). For many AMPs described so far, this interaction appears to be highly unspecific, since it does not seem to operate via a receptor-mediated mechanism. Nevertheless, the consequences for an invading pathogen under the influence of AMP activity may be dramatic: peptide–membrane interactions can result in the permeabilization of membranes, loss of membrane potential and the discharge of cytosolic metabolites (Tossi and Sandri 2002).

Outstanding characteristics such as size, amino acid composition, amphipathicity and cationic charge facilitate the attachment and insertion of AMPs into membrane bilayers. Three models have been put forward to explain the formation of pores: the barrel-stave, the carpet and the toroidal model. The publications by Park and Hahm (2005) and Zemel (2003) provide detailed information on these model mechanisms. In any event, however helpful the character of these models may be, they provide only scant insight into how peptide damage and killing of microorganisms truly occurs.

For some AMPs, the interaction with specific membrane-associated targets or receptors has recently been described (Thevissen et al. 2004).

It was shown that some antifungal peptides interact with fungal glucosyl-ceramides. Interestingly, the analysed AMPs originated from different eukaryotic kingdoms, which leads to the conclusion that they once must have evolved from a single precursor molecule (Thevissen et al. 2004).

Several other observations suggest that the translocation of AMPs across membranes can influence septum formation in ascomycetes. Furthermore, the synthesis of cell walls, nucleic acids and proteins may also be affected, as well as the activity of certain enzymes (Olmo et al. 2001; Park and Hahm 2005).

2.3 Classification

Due to the vast variety of peptides displaying antimicrobial potential, the classification of AMPs into different categories is not an easy task. Neither a taxonomic nor a functional classification seems to be sufficiently rigorous, therefore some rather broad structural characteristics have been applied for the categorisation of these peptides. A descriptive summary on important AMP subfamilies is given by Marshall and Arenas (<http://www.ejbiotechnology.info/content/vol6/issue3/full/1>).

In plants, eight distinct classes of AMPs have been identified so far (Garcia-Olmedo et al. 1998; Lay and Anderson 2005). In humans, three major groups of AMPs are characterised (De Smet and Contreras 2005), whereas the innate immune system of insects is said to consist of seven important families of peptides (Royet et al. 2005). Nonetheless, it is worth mentioning that the definite classification of many individual AMPs still remains a controversial issue.

There are presently two extensive AMP databases freely available on the world-wide-web (<http://www.bbcm.univ.trieste.it/~tossi/pag1.htm> and <http://aps.unmc.edu/AP/main.php>).

3 Structure and gene regulation of antimicrobial peptides

The great attractiveness of AMPs for medicine or applied biotechnology can be attributed to their biological origin, high sustainability, broad diversity and appealing range of specificity. In order to best exploit these peptides, detailed knowledge has to be gathered concerning their tertiary structure, their mode of action and the regulation of AMP-encoding genes.

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