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Review

Temporins, anti-infective peptides with expanding properties

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Abstract. Antimicrobial peptides are effector molecules of the innate immune response of all pluricellular organisms, providing them with first-line defence against pathogens. Amphibian skin secretions represent one of the richest natural sources for such peptide antibiotics, and temporins, a large family of antimicrobial peptides from frog skin, are among the smallest ones found in nature to date. Their functional role and modes of action have been described, along with their interesting and unique properties. These properties make temporins good molecules for an in-depth understanding of host defence peptides in general. Furthermore, they are attractive templates for the future design of new therapeutics against infectious diseases with new modes of action, urgently needed due to the increasing resistance of microorganisms to the available drugs.

Keywords. Frog skin secretion, antimicrobial peptide, temporin, infectious disease, membrane-active peptide, lipid-peptide interaction, innate immunity.

Introduction

All living multicellular organisms are constantly exposed to multiple harmful microbes and, therefore, the capacity to overcome infections is essential for their survival. Several host defence mechanisms have been engendered during evolution, including the generation of gene-encoded antimicrobial peptides (AMPs). AMPs are produced in bacteria and fungi [1–3] as well as in higher eukaryotes (plants, animals and humans) [4–9], where they represent key components of the innate immune system. The latter is a fast-acting weapon against invading pathogens that functions before the adaptive immune response (in vertebrates) is activated [10–13].

In mammals, AMPs are commonly present in granulocytic leukocytes, in the skin and in the mucous membranes of the respiratory, genitourinary and gastrointestinal tracts [8, 14–16], where their compromised expression has been recently proposed to trigger inflammatory diseases [17].

During the last decades, the widespread use and abuse of conventional antibiotics has led to the emergence of multi-drug-resistant bacterial and fungal strains. This has become a severe health problem prompting the search for alternative antimicrobials with new modes of action. In addition to their ability to kill rapidly a broad spectrum of microorganisms and to limit the induction of microbial resistance, AMPs from higher eukaryotes also act as intercellular signalling molecules and coordinate the innate and adaptive host defence responses, influencing processes like cytokine release, cell proliferation, angiogenesis, wound healing and chemotaxis [18–20]. Therefore, they are considered as potential compounds for future therapeutics [21] and hundreds have already been isolated from different biological resources (see an updated list at http://www.bbcm.univ.trieste.it/~tossi/pag1.htm) or designed de novo and synthesized.

Moreover, the finding that some AMPs and their derivatives are potent antitumour agents with low toxicity against non-malignant cells increases the interest in such

molecules as appropriate templates for the development of novel chemotherapeutics [22].

In contrast to classical antibiotics, animal and plant AMPs are ribosomally made as pre-propeptides, are processed to the active form and promptly mobilized after pathogen attack [23, 24]. Despite substantial variations in their chain length and structure, most of these peptides share two major and fundamental features for their mode of action: (i) a net positive charge (due to the presence of basic amino acids) and (ii) a potential to adopt amphipathic α helix and/or β sheet structures (i.e. structures with separate hydrophobic and hydrophilic domains) upon their interaction with the phospholipid membrane of the target cell, which is highly negatively charged in bacteria. Mode-of-action studies showed that many AMPs physically permeate and destroy the cell membrane, causing damage hard to fix, rather than acting via a receptor-mediated mechanism [25]. This makes it difficult for the bacteria to develop resistance, which would require a change in membrane composition, a very expensive solution for the majority of microbes [5]. Conversely, commonly used drugs operate on specific intracellular targets and do not modify the bacterial morphology, making it easy for the microorganisms to become resistant to those drugs [25]. Among the several sources for natural peptide antibiotics, amphibian skin is the richest [26, 27], especially that of frogs of the genus *Rana,* which has a worldwide distribution with approximately 250 different species [28].

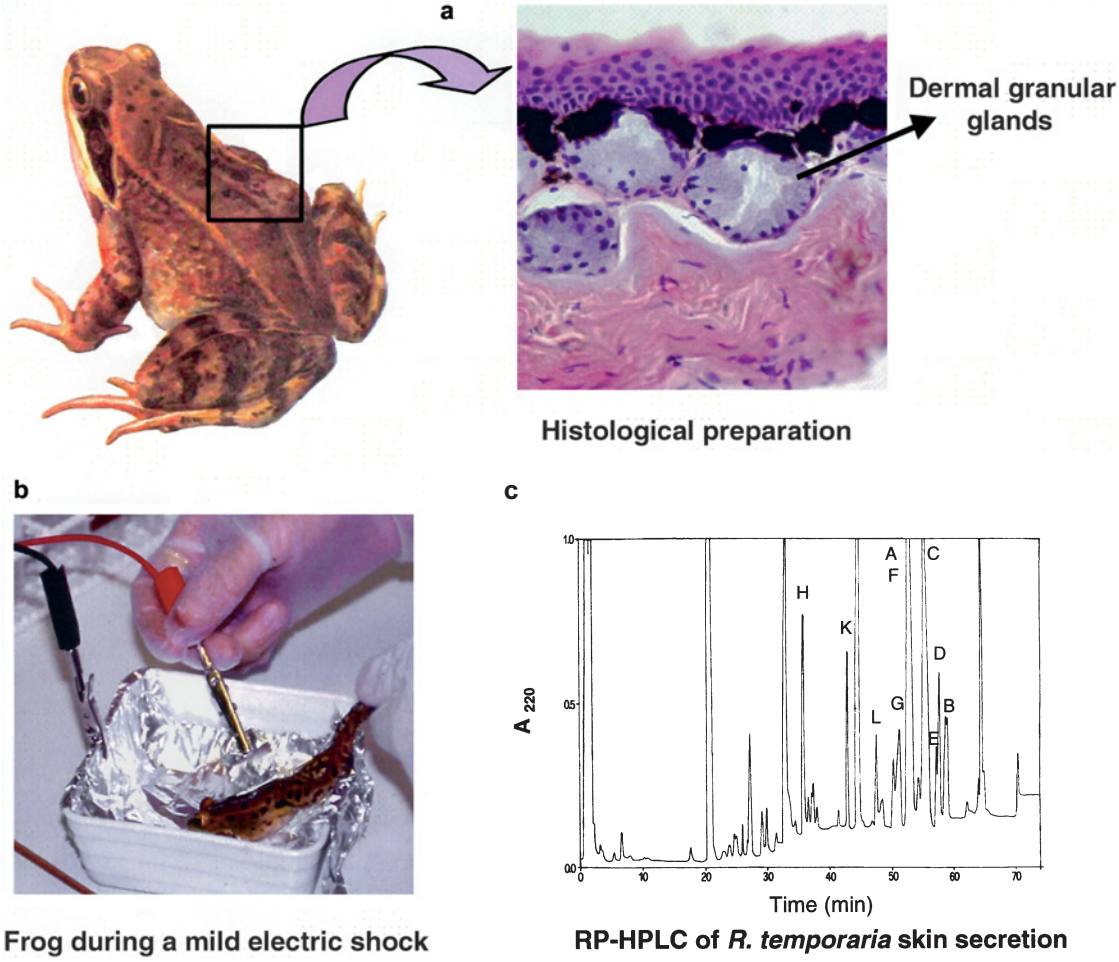

Figure 1. Steps involved in the isolation and characterization of frog skin AMPs. (*a*) Frog skin AMPs are produced by dermal serous glands, which are mainly located in the dorsal region of the animal and are controlled by sympathetic axons. The cytoplasm of the cells is rich in granules and the lumen is reduced to a small empty cavity. Contraction of myocytes surrounding the glands causes a synchronous discharge of their content with a holocrine mechanism. (*b*) After giving the animal a mild electrical stimulation (10 V, with pulse duration of 5 s), by gently streaking the dorsal skin surface with an electrode, the skin secretion is washed off with diluted acetic acid and fractionated by reverse-phase high-performance liquid chromatography (RP-HPLC) [34]. (*c*) Peaks containing peptides with antimicrobial activity, detected by using the inhibition zone assay [100], are then analysed to determine the peptide amino acid sequence and secondary structure (CD spectroscopy). The positions of temporins are indicated by the corresponding letters on the RP-HPLC profile.

Frogs represent a suitable model system to study the *in vivo* role of AMPs in vertebrates [29], and previous reports have highlighted the functional importance of such molecules even in the control of the animals' natural flora [30]. Their synthesis is transcriptionally regulated by the NF-κB/IκBα machinery [31] and is modulated by microorganisms [32]. The AMPs are stored in granules of holocrine-type dermal glands, and are released into the skin secretion, as a reaction to stress or injury [26] (see scheme in Fig. 1). In addition to their large spectrum of antibacterial and antifungal activities, several amphibian AMPs can inhibit cell-mediated HIV capture and infection [33].

This review focuses on temporins, a large family of AMPs isolated from frog skin, some of which possess attractive and unique properties that make them good candidates to increase our knowledge about peptide-mediated innate immunity, and promising templates for the future design of new anti-infective agents.

Table 1. Primary structures and related net charge, at neutral pH, of 40 temporins and temporin-like peptides.

Peptide	Source	Sequence	Net charge	Reference
		1 10		
Consensus sequence		**FLPLIASLLS KLL**		
Temporin A	° *R. temporaria*	FLPLIGRVLS GIL-NH_2	+2	34
Temporin B	*R. temporaria*	LLPIVGNLLK SLL-NH_2	+2	34
Temporin C	*R. temporaria*	LLPILGNLLN GLL-NH_2	+1	34
Temporin D	*R. temporaria*	LLPIVGNLLN SLL-NH_2	+1	34
Temporin E	*R. temporaria*	VLPIIGNLLN SLL-NH_2	+1	34
Temporin F	*R. temporaria*	FLPLIGKVLS GIL-NH_2	+2	34
Temporin G	*R. temporaria*	FFPVIGRILN GIL-NH_2	+2	34
Temporin H	*R. temporaria*	LSP---NLLK SLL-NH_2	+2	34
Temporin K	° *R. temporaria*	LLP---NLLK SLL-NH_2	+2	34
Temporin L	*R. temporaria*	FVQWFSKFLG RIL-NH_2	+3	34
Temporin-1ARa	* *R. areolata*	FLPIVGRLIS GLL-NH_2	+2	91
Temporin-1AUa	* *R. aurora aurora*	FLPIIGQLLS GLL-NH_2	+1	54
Temporin-1BYa	* *R. boylii*	FLPIIAKVLS GLL-NH_2	+2	92
Temporin-1Ca	* *R. clamitans*	FLPFLAKILT GVL-NH_2	+2	93
Temporin-1Cb	*R. clamitans*	FLPLFASLIG KLL-NH_2	+2	93
Temporin-1Cc	*R. clamitans*	FLPFLASLLT KVL-NH_2	+2	93
Temporin-1Cd	*R. clamitans*	FLPFLASLLS KVL-NH_2	+2	93
Temporin-1Ce	*R. clamitans*	FLPFLATLLS KVL-NH_2	+2	93
Temporin-1Ec	° *R. esculenta*	FLPVIAGLLS KLF-NH_2	+2	94
Temporin-1Ga	* *R. grylio*	SILPTIVSFL SKVF-NH_2	+2	71
Temporin-1Gb	*R. grylio*	SILPTIVSFL SKFL-NH_2	+2	71
Temporin-1Gc	*R. grylio*	SILPTIVSFL TKFL-NH_2	+2	71
Temporin-1Gd	*R. grylio*	FILPLIASFL SKFL-NH_2	+2	71
Temporin-1Ja	° *R. japonica*	ILPLVGNLLN DLL-NH_2	0	53
Temporin-1La	* *R. luteiventris*	VLPLISMALG KLL-NH_2	+2	95
Temporin-1Lb	*R. luteiventris*	NFLGTLINLA KKIM-NH_2	+3	95
Temporin-1Lc	*R. luteiventris*	FLPILINLIH KGLL-NH_2	+3	95
Temporin-1Oa	° *R. ornativentris*	FLPLLASLFS RLL-NH_2	+2	56
Temporin-1Ob	*R. ornativentris*	FLPLIGKILG TIL-NH_2	+2	56
Temporin-1Oc	*R. ornativentris*	FLPLLASLFS RLF-NH_2	+2	56
Temporin-1Od	*R. ornativentris*	FLPLLASLFS GLF-NH_2	+1	56
Temporin-1P	* *R. pipiens*	FLPIVGKLLS GLL-NH_2	+2	95
Temporin-1PLa	* *R. palustris*	FLPLVGKILS GLI-NH_2	+2	96
Temporin-1PRa	° *R. pirica*	ILPILGNLLN GLL-NH_2	+1	55
Temporin-1PRb	*R. pirica*	ILPILGNLLN SLL-NH_2	+1	55
Temporin-1TGa	° *R. tagoi*	FLPILGKLLS GIL-NH_2	+2	97
Temporin-1Va	* *R. virgatipes*	FLSSIGKILG NLL-NH_2	+2	98
Temporin-1Vb	*R. virgatipes*	FLSIIAKVLG SLF-NH_2	+2	98
Temporin-1Vc	*R. virgatipes*	FLPLVTMLLG KLF-NH_2	+2	98
Ranatuerin-6	* *R. catesbeiana*	FISAIASMLG KFL-NH_2	+2	99

The consensus sequence was derived by D. Wade (http://www.ijc.com/abstracts/TOC2002). Basic and acidic residues are indicated by red and blue letters, respectively. Gaps (–) were inserted to maximize identities. The symbols * and ° indicate frogs of Northern America or Eurasian origin, respectively.

Temporin biosynthesis and characteristics

Temporins were initially identified in 1996 in the skin of the European red frog *Rana temporaria* (Table 1) [34]. Later on, they were also discovered in frogs of Northern America and Eurasian origin (Table 1) as well as in the venom of wasps [35, 36]. Similar to other peptides from amphibia [37–39] that have antimicrobial or various pharmacological effects, temporins are synthesized as large precursors containing a single copy of the mature peptide at the C terminus and a highly conserved region comprising a 22-residue signal peptide and an acidic intervening sequence [34]. The signal peptides are highly similar to those present in the precursors of other AMPs from the *Rana* genus [37, 40], as well as in the precursors of the opioid (dermorphins and deltorphins) and antifungal (dermaseptins) peptides occurring in the skin of frogs of the subfamily Phyllomedusinae [41]. Thus, these molecules likely arose through duplication from a common ancestor gene followed by local hypermutations. In contrast to the other families of Ranidae peptides (e.g. brevinins, ranalexins, ranatuerins and esculentins) [26, 28], temporins lack the 'Rana box' motif (i.e. a C-terminal cyclic heptapeptide domain stabilized by a single disulphide bridge), and are amidated at their C terminus, as a result of a post-translational modification [42]. The temporin family includes more than 40 members with properties that render them interesting molecules for in-depth investigation of their biological function and mode of action. These properties include the following: (i) temporins are among the smallest amphipathic α-helical AMPs found in nature (10–14 amino acids); (ii) their net positive charge at a neutral pH is low, ranging from 0 to +3 (Table 1); (iii) some of them act efficiently and rapidly against a wide range of pathogens (bacteria, viruses, fungi, yeasts and protozoa) and are not toxic to normal mammalian cells; (iv) their mode of action includes perturbation of the cytoplasmic membrane but in a different way from that proposed for the majority of cationic α-helical AMPs [43, 44]; (v) some temporins display immunomodulatory effects [45]; (vi) they preserve biological function in serum [46] and (vii) they have an *in vivo* efficacy in preventing prosthetic graft infections [47, 48] and against sepsis [unpublished data].

The targets of temporins and their structure-function relationship

Why should frogs produce a large number of similar molecules? The answer to this question is still not clear. However, it could relate to several functions that these peptides might have *in vivo,* as discussed below. Temporins are mainly active on Gram-positive bacteria, including clinically isolated methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* and *E. faecalis*, with minimal inhibitory concentrations ranging from 2.5 to 20 μM [49, 50]. Some members are also lethal to Gram-negative bacteria and fungi, such as *Batrachochytrium dendrobatidis*, which is associated with a global amphibian decline [51], and *Candida albicans* [34]. Furthermore, a very broad spectrum of activity was observed with temporin L (net charge +3), against both Gram-positive and Gram-negative bacteria, yeasts, human erythrocytes and cancer cells [52]. Overall, in most cases, there is a direct correlation between the antibacterial potency and the net positive charge of temporins. For example, natural isoforms (see Table 1) carrying a net charge of zero (temporin-1Ja) or +1 (temporins-C, D, E, 1AUa, 1PRa, 1PRb) are practically devoid of bactericidal effects [34, 53–55]. However, there are exceptions, like temporin-1Od, which inhibits the growth of *S. aureus,* although it does not contain positively charged amino acids, except the free N-terminal amino group [56]. The length of temporins is also a crucial factor for antimicrobial efficacy. In fact, the two 10-residue homologues (temporins H and K) do not kill bacteria. Nevertheless, temporin-H acts synergistically against Gram-negative strains when combined with classical antibiotics [57].

Besides their antibacterial and antifungal activities, temporins have additional anti-infectious properties. For example, temporin A reduces within 10 min the infectivity of the channel catfish virus and the non-enveloped frog virus-3, over an extensive temperature range [58]. Moreover, recent studies revealed that temporins A and B have a strong antiparasitic action on protozoa of the *Leishmania* genus (the aetiological agent of severe worldwide infectious diseases in vertebrates, including humans). Apart from temporins, only a few AMPs have so far displayed anti-protozoa activity, and reports on their mode of action are scarce [59]. For *Leishmania*, these include dermaseptins [60], the frog skin polypeptide YY [61], indolicidin isolated from granules of bovine neutrophils [62], gomesin, from the tarantula spider *Achantoscurria gomesiana* [63], and the cecropin-melittin hybrid peptides [64, 65]. In contrast with temporins, which are highly active towards both the insect (promastigote) and the mammalian intracellular stage (amastigote) of the parasite, the other mentioned AMPs exhibited considerably lower efficacy against amastigotes compared with promastigotes; however, the molecular basis for these differences is not yet clear. An appealing and therapeutically advantageous peculiarity of temporins is that they do not harm macrophages (the host cell for amastigotes) at doses that are lethal to the intracellular parasites [46]. Unlike indolicidin or dermaseptins, most temporins do not display a significant haemolytic activity and preserve their function at physiological salt concentration and in 33% human serum [46]. Furthermore, temporin A was found not to affect the viability and proliferation of cultivated ker-

atinocytes [66], and its topical application provides prophylaxis against graft infections from staphylococci, in a subcutaneous rat pouch model [48].

Structure-function analysis with synthetic analogues of temporin A has indicated that several positions along the peptide sequence are essential determinants for antimicrobial activity [49, 67]. On the other hand, the effectiveness and target specificity of temporins are governed not only by their physical-chemical properties (sequence, charge distribution, amphipathicity and helicity), but also by the type of cell wall of the target microorganism, its envelope and metabolism. Indeed, although temporin A is more active than temporin B against many bacteria [34], a reversal in their potency is directed towards some species of the microbial flora of the frog [57]. Therefore, in order to protect frogs from a vast spectrum of invading pathogens and from opportunistic infections due to the animal's microflora [29, 30], it is reasonable that nature equipped a single specimen with many peptide homologues.

Role of temporins in host defence

The *in vivo* role of each temporin has not yet been elucidated. We cannot rule out a possible synergism between several temporins or between temporins and other molecules of a different nature, as well as additional biological functions that enhance host defence indirectly. The latter function was described previously, in the case of dermaseptins [68]. In support of this, temporins possess other interesting non-antimicrobial functions: (i) similar to the mammalian AMPs, β-defensins and cathelicidins [69, 70], temporin A has chemotactic effects on human phagocytes, presumably mediated by cell surface receptor(s) [45]. This important link between the innate and adaptive immune system (by recruiting immune cells to the sites of infection) adds a beneficial characteristic to temporins as candidates for drug development; (ii) temporins from *R. grylio* induce relaxation of vascular tissue from the rat thoracic aorta [71]; (iii) temporins B and L modulate the hydrolytic activity of secretory phospholipase A_2 from bee venom and human lachrymal fluid, thus improving the efficacy of the immune response to infections [72]; (iv) temporin L binds purified lypopolysaccharide (LPS or endotoxin) *in vitro* and prevents lethality in rat models of septic shock [unpublished results]. Neutralization of LPS, released from Gram-negative bacteria during infection, may help to eliminate, during or after antimicrobial therapy, the risk of sepsis, a serious symptom which can easily result in mortality.

How do temporins function on intact cells?

To date, mode-of-action studies were conducted mainly with temporins purified from *R. temporaria*. The selectivity of many cationic AMPs towards bacteria is generally attributed to their higher affinity to the bacterial membranes, which are composed predominantly of the negatively charged phosphatidylglycerol (PG) and cardiolipin [73], compared with those of eukaryotic cells, which are rich in sterols and zwitterionic phospholipids [74]. The peptides initially bind electrostatically to the anionic membrane, which stabilizes their amphipathic α-helical structure, required for the subsequent membrane perturbation [74, 75]. However, before reaching the cytoplasmic membrane, the peptides need to traverse the cell wall that, in Gram-negative strains, is surrounded by a second membrane, consisting primarily of anionic LPS [76, 77]. This outer membrane is a barrier against temporins since it probably interferes with both the electrostatic attraction and uptake process of the peptide. In support of this, *Escherichia coli* strains with the progressively decreased LPS-polysaccharide chain are concomitantly more sensitive to these peptides [34, 52].

Recent studies with temporins revealed that the permeation of the cytoplasmic membrane is not necessarily the lethal step. In particular, the activity of temporin L on *E. coli* was investigated by using a triple-colour staining method to simultaneously visualize the effects of the peptide on the viability and membrane integrity of individual cells [78]. The data indicated that the membrane structure can be altered even at peptide concentrations markedly lower than those required to inhibit bacterial growth (Fig. 2). Surprisingly, and in contrast with many other membrane-active AMPs [43, 44, 79–81], temporin L does not lyse bacteria and does not cause membrane blebbing but, rather, causes the cells to have a ghost-like appearance (Fig. 2, right inset). Overall, all temporins endowed with bactericidal activity modify the permeability of the microbial membrane, allowing the passage of small and large molecules (e.g. the enzyme β-galactosidase), in a rapid and dose-dependent manner [52, 57], which leads to cell death [78].

As stated above, temporins are only mildly cationic. This might explain their ability to also target and damage the cytoplasmic membrane of *Leishmania* protozoa (Fig. 3) despite its different lipid composition compared with that of bacteria (it contains only traces of PG and 30% of sterols, i.e. ergosterol [46, 82]) and the presence of a glycocalyx layer mainly consisting of the highly anionic lipophosphoglycan (LPG) [83]. However, contrary to the bacterial LPS, the LPG glycocalyx of *Leishmania* is not a barrier for temporins and electrostatic interactions do not contribute substantially to the activity of the peptide on the parasites [46]. Nevertheless, it is still not known whether temporins can enter cells and, therefore, we cannot exclude the possibility that these peptides act *in vivo* in a more complex way, e.g. inhibiting metabolic functions by binding DNA, or altering enzyme activity [84, 85]. Besides, since no apoptotic mechanism could be de-

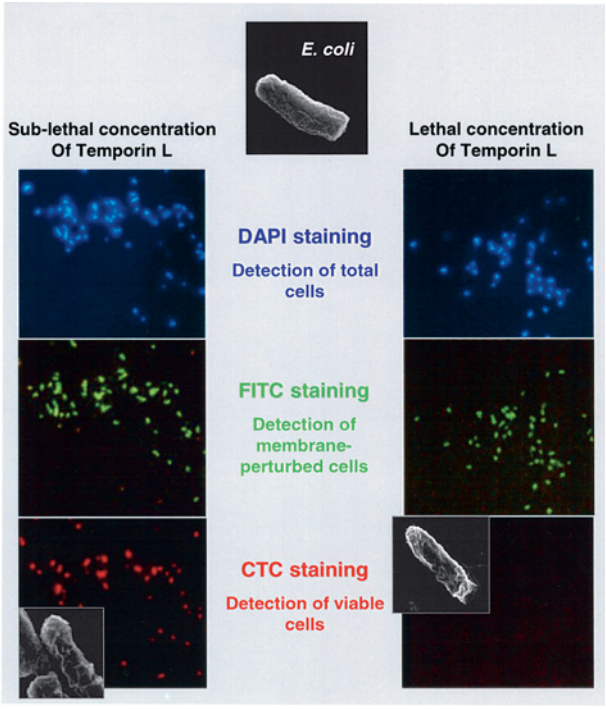

Figure 2. A triple-staining assay [78] showing that membrane permeation of *E. coli* is not the lethal event. *E. coli* cells (2×10^7) were treated with temporin L at different concentrations and incubated with (i) a blue fluorescent dye (4′,6-diamidino-2-phenylindole, DAPI) which binds to DNA and allows detection of both metabolically active and inactive cells; (ii) the green fluorescein isothiocyanate (FITC), a membrane-impermeant compound which penetrates only through a permeabilized membrane and (iii) the dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) which stains only viable bacteria. When *E. coli* cells are exposed to a low peptide concentration (2 µM, left panels), all cells (detected by DAPI) emit a bright-green fluorescence, indicating the intracellular influx of FITC, which reveals membrane perturbation. However, despite this membrane alteration, all bacteria are viable, as detected by the red fluorescence of CTC. When bacteria are treated with 50 µM peptide (right panels), a concentration that markedly increases the membrane permeability [78], all cells are dead (absence of red fluorescence). The left and right insets show the scanning electron microscopy images of cells treated with 2 and 50 µM temporin L, respectively. In the right inset, a prominent collapse of the cell structure and a deep roughening of the cell surface are observed (ghost-like shape).

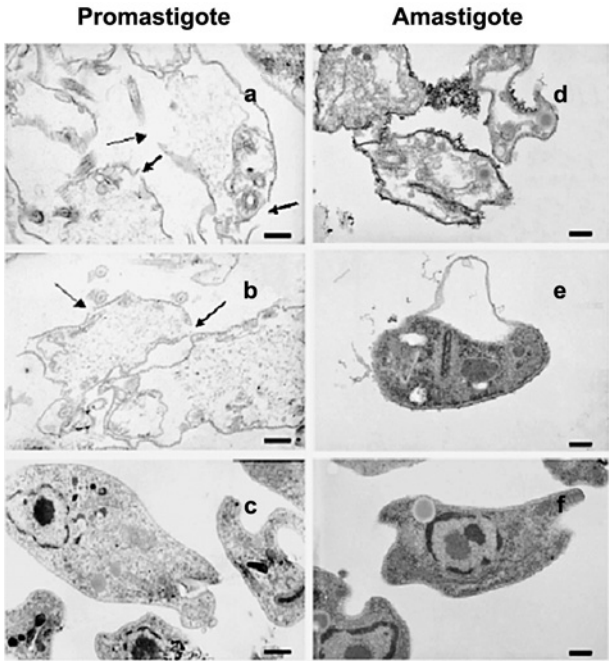

Figure 3. Transmission electron microscopy images showing the effect of temporins on *Leishmania* protozoa [46]. *Leishmania donovani* promastigotes and *L. pifanoi* amastigotes were incubated for 1 h with 20 (*a*) or 15 (*d*) µM temporin A, 20 (*b*) or 10 (*e*) µM temporin B, or in the absence of a peptide (*c* and *f*). Parasites were then fixed for 1 h with 5% (w/v) glutaraldehyde in phosphate-buffered saline, containing 2.5% (w/v) osmium tetraoxide and gradually dehydrated in ethanol and propylene oxide. Membrane disruption (indicated by arrows), membrane blebbing and breakages as well as depletion of electron-dense cytoplasmic material can be observed for the two temporins. Bar, 0.5 µm. Taken from Mangoni et al. [46].

tected on several human cancer cells upon their treatment with temporin L [52], a membrane-disturbing action seems also to be the major mechanism used by the peptide to kill neoplastic cells.

Temporins action on model membranes

Differing from many AMPs, temporins bind and permeate both zwitterionic and anionic phospholipid bilayers (mimicking the membrane composition of higher eukaryotic cells and bacteria, respectively) [86]. Very interestingly, however, despite the zwitterionic character of the outer leaflet of erythrocytes, almost all temporins are not haemolytic, the reason for which is not yet clear. Mode-of-action studies using vesicle-entrapped markers with different sizes revealed a direct correlation between the increased concentration of temporins added to the vesicles and the increased size of the markers leaked from the liposomes [87, 88]. This is in agreement with their mode of action on intact cells and suggests the formation of local holes in the lipid bilayer rather than its micellization in a detergent-like manner, a possible step in the carpet mechanism. This is further corroborated by the findings that temporins insert deeply into the membrane in a perpendicular orientation and enhance the acyl chain order of the lipids, as shown using fluorescence spectroscopy [86, 88, 89] and surface plasmon resonance (BIAcore) [unpublished results]. Since peptide channels would require a minimum length of ~23 residues to span the membrane, it would be intriguing to determine the pore architecture of temporins. Possibly, these peptides form holes in a more elaborate way, perhaps involving an N-terminal tail-to-tail dimerization, as proposed for gramicidin [90].

Final remarks

The aim of the present review was to highlight temporins, the shortest α-helical peptides isolated from amphibians, and their unique characteristics. Surprisingly, these short and mildly cationic peptides exhibit antibacterial, antifungal and antiviral activities. In addition, they are highly potent against protozoa of the *Leishmania* genus on both stages of the parasite (the insect and the mammalian forms). Altogether, temporins represent a very interesting family of innate immunity effectors that, beyond their basic scientific importance, possess attractive properties that make them exciting molecules for the future design of anti-infective and antisepsis agents with a new mode of action. These properties include (i) antimicrobial and chemotactic activities; (ii) preservation of the biological function in serum; (iii) lack of a toxic effect against mammalian normal cells at concentrations required to rapidly kill microbes; (iv) a membranolytic effect that should render it difficult for the pathogen to develop resistance; (v) synergistic action when combined with conventional antibiotics; (vi) *in vivo* efficacy as topical prophylactic agents against infections and *in vivo* antiendotoxic effects [47, 48]; (vii) a short amino acid sequence for cost-efficient chemical synthesis. Nevertheless, the *in vivo* efficacy, pharmacokinetics and toxicity of temporins have still not been fully examined, and such studies should be pursued to evaluate, in more detail, their real therapeutic value for clinical application. Furthermore, the production of fluorescently labelled peptides and synthetic analogues containing unusual amino acids will represent a helpful tool to better understand both the site(s) and the mode(s) of action of temporins.

Finally, it is worthwhile to remember that skin secretion is an inducible part of the immune system of frogs and that skin AMPs play a major role in protecting them from infections and in keeping control of those microorganisms considered to be part of the natural flora of both amphibians and humans. Moreover, the fact that biologically active peptides of the frog skin have counterparts in the mammalian gastrointestinal tract and brain has greatly contributed to raising the interest in such molecules. Therefore, a deeper knowledge of the *in vitro* and *in vivo* functions of these peptides might contribute to better understanding the regulation of innate immunity in a vertebrate and to open additional roads for novel strategies in experimental medicine.

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