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Minireview

The dermaseptin precursors: a protein family with a common preproregion and a variable C-terminal antimicrobial domain

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Abstract Preprodermaseptins are a group of antimicrobial peptide precursors found in the skin of a variety of frog species. Precursors of this family have very similar N-terminal preprosequences followed by markedly different C-terminal domains that correspond to mature antimicrobial peptides. Some of these peptides are 24–34 amino acids long and form well-behaved amphipathic α-helices, others are disulfide-linked peptides of 20–46 residues, still others, highly hydrophobic, are the smallest antimicrobial peptides known so far being only 10–13 residues in length. All these peptides are broad-spectrum microbicides that kill many bacteria, protozoa, yeasts and fungi by destroying or permeating the microbial membrane. In frogs belonging to the genus *Phyllomedusinae*, preprodermaseptins encoded peptides also include dermorphins and deltorphins, D-amino acid-containing heptapeptides which are very potent and specific agonists of the μ- or δ-opioid receptors. The remarkable similarity between preproregions of precursors that give rise to peptides with very different primary structures, conformations and activities suggests that the corresponding genes originate from a common ancestor. The high conservation of the precursor prepropart indicates that this region must have an important function.

Key words: Antimicrobial peptide; Opioid peptide; Frog skin

1. Introduction

The production of gene-encoded antimicrobial peptides as an immune strategy is widely used in nature and has been conserved in evolution. Small-sized antimicrobial peptides, named bacteriocins, have been produced by a number of Gram-positive and Gram-negative bacteria for million of years for containing the proliferation of microorganisms that are closely related or confined within the same ecological niche, thus helping the producing microbe to compete for limited resources [1–6]. In vertebrates, cationic antimicrobial peptides, 10–46 residues long, are secreted by specialized epithelial cells to form a first line of host defense against noxious microorganisms and to control bacterial proliferation in the respiratory tract, the gastrointestinal and genital systems and the skin [7–9]. Whereas many antibiotics, or secondary metabolites, disable microorganisms over a period of days by inhibiting critical enzymes, most of the antimicrobial peptides isolated from vertebrates kill microorganisms rapidly by destroying or permeating the microbial membrane and impairing ability to carry out anabolic processes [7–9]. This makes them promising candidates for inclusion in new treatments of nosocomial infections and multi-drug-resistant infections [10].

Since the discovery in 1969 of bombinin in the skin of the frog *Bombina* [11], the first gene-encoded peptide antibiotic originating from the mucosal epithelium of a vertebrate animal amphibian skin has become an important source of new antimicrobial agents [7–9]. Frog skin secretions are a rich source of biologically active peptides which are very similar to mammalian peptides which interact with receptors of the central and peripheral nervous system and the gastrointestinal tract [12–14]. There are many examples, including tyrotropin-releasing hormone, angiotensins, caeruleins/cholecystokinins, tachykinins, bradykinins and bombesin/gastrin-releasing peptide [14], as well as pancreatic polypeptide/peptide tyrosine-tyrosine/neuropeptide tyrosine [15] and calcitonin gene-related peptide [16]. The frog dermatous glands also produce huge amounts of cytolytic and antimicrobial peptides that defend the naked skin against the microbe-laden external environment and assist in wound repair. As a rule, a given amphibian species produces a unique repertoire of small-sized peptide antibiotics that have overlapping structural features, but often target specific microorganisms. It is thought that the simultaneous presence of closely related antimicrobial molecules acting in synergy provides frogs with a better shield against a wider range of harmful invaders, bacterial, fungal and protozoan.

Amphibians belonging to different families, genera and species store distinct set(s) of antimicrobial peptides with a differing chain length, charge, hydrophobicity and spectrum of action. These peptides are often produced in such enormous quantities that it is possible to isolate enough material from a single animal to determine its amino acid sequence and establish its antimicrobial spectrum.

The microbicidal skin peptides of the anura frogs are grouped into three broad families on the basis of their sequence/tri-dimensionnal structure characteristics [7–9]: (a) linear amphipathic helix forming peptides such as the magainins and related peptides from the mesobatrachia African clawed frog *Xenopus laevis*, the bombinins from the archeobatrachia *Bombina* sp. and the dermaseptins from the neobatrachia South American arboreal frogs *Phyllomedusa*. These peptides manifest broad-spectrum antimicrobial activity against bacteria, yeast, protozoa and fungi but have no or little harmful effect on human cells. (b) Four different groups of related peptides isolated from various species of the Ranidae family (neobatrachia), namely the brevinins-1, -2 and the esculentins-1 and -2. Different from the peptides mentioned above, they

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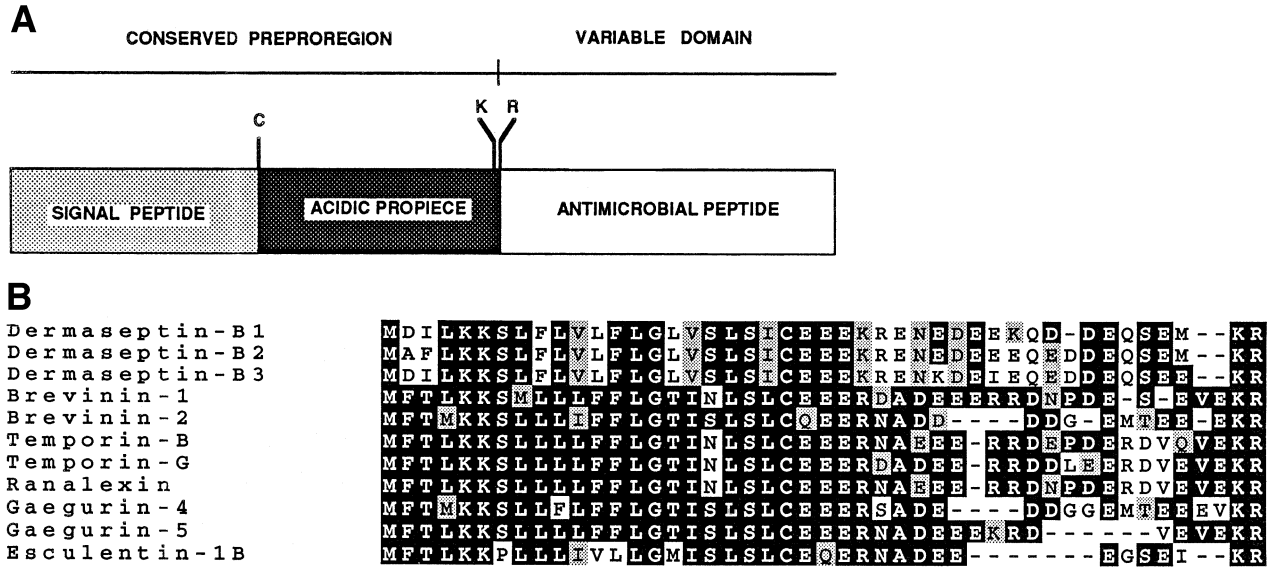

Fig. 1. (A) Schematic representation of precursors of the preprodermaseptin family. (B) Alignment of the amino acid sequences of the preproregions of representative members of the preprodermaseptins B, preprobrevinins-1 and -2, preproesculentins-1 and preprotemporins, including the signal peptide (residues 1–22), the acidic propiece and the processing site Lys-Arg. Identical (black background) and similar (shaded background) residues are highlighted. Gaps (-) have been introduced to maximize sequence similarities. The arrow indicates the putative cleavage site of the signal peptide.

all contain two cysteine residues at the C-terminus which are linked in a disulfide bridge. (c) The temporins, isolated from *Rana temporari*, are the smallest antimicrobial peptides known so far being only 10–13 residues in length.

2. The preprodermaseptins, a novel family of antimicrobial peptide precursors

A rapidly expanding group of antimicrobial peptide precursors with unique features has recently been identified in frog skin [17–24]. Precursors belonging to this family, tentatively designated preprodermaseptins, show an N-terminal preprosequence of approximately 50 residues which is highly conserved both intra- and inter-species (82% intraspecies amino acid identity in *P. bicolor*) and markedly diverse C-terminal sequences corresponding to antimicrobial peptides (Fig. 1). The conserved preproregion includes a 22 residue signal peptide and a 16–25 residue acidic propiece which is terminated by a typical prohormone processing signal Lys-Arg. Also, the 5′-untranslated regions in the corresponding mRNAs are ~50% identical at least over the first 20 bp immediately upstream of the ATG initiation codon, but sequences diverged beyond that point. The similarity is not quite as high in the 3′-untranslated region of the mRNAs. The presence of a conserved 5′-region in the mRNAs of this group of precursors allowed for the amplification of the cDNA of novel putative congeners in which the conserved preproregion is followed by peptide progenitor sequences with structural features consistent with antimicrobial activity [25].

At present, 47 chemically different antimicrobial peptides corresponding to the C-terminal variable sequences of precursors belonging to the preprodermaseptin family have been fully characterized in six different frog species (Table 1). The mature antimicrobial peptides have all been given individual names and grouped to form seven distinct peptide subfamilies on the basis of their origins and/or structural characteristics. They include the dermaseptins B [19,26,27] and phylloxin [28], linear α-helical peptides from *Phyllomedusa* ssp., brevinins and esculentins, disulfide-bridged peptides from *Rana* ssp. [20–23,29–31], and the temporins, 10–13 residues long peptides from *R. temporaria* [24]. Studies on the biosynthesis and intracellular processing of dermaseptins B from *P. bicolor* indicate that they are synthesized in the multi-nucleated cells of the granular glands of the skin as prepropeptides which are processed by the removal of the signal peptide to the proform and then stored in the large granules of the glands. Glands may release their content onto the skin surface by a holocrine mechanism involving rupture of the plasma membrane and extrusion of the granules through a duct opening to the surface.

The remarkable similarity between preproregions of precursors that encode structurally diverse end products in distantly related frog species suggests that the corresponding genes originate from a common ancestor. Genes encoding dermaseptins B from *P. bicolor* have been cloned. They show a two exon coding structure, the first containing codons for the conserved 22 residue signal peptide and the first three glutamic residues of the acidic propiece and the second exon coding the remainder of the conserved acidic propiece plus the processing signal Lys-Arg and a dermaseptin B progenitor sequence [32,33]. The small intron that interrupts the two coding exons exhibits strong sequence conservation in all the dermaseptin genes, suggesting that gene family expansion may have occurred recently or that specific gene conversion events have occurred to ensure overall sequence conservation. Since the conserved preproregion is in the same gene as the mature peptide, it cannot have been added on by post-transcriptional events.

3. Structure and activity of the antimicrobial peptides

Although differing widely in length and amino acid se-

quence (Table 1), all these peptides are polycationic, amphipathic and membranotropic. Dermaseptins B and phylloxin form a well-behaved α-helix in an anisotropic environment, such as a membrane interface, containing sharply demarcated polar and non-polar surfaces. These peptides are cidal against a very broad spectrum of microorganisms, including bacteria, fungi, protozoa and yeast, but differ widely in their potency for killing the various agents [19,28,33]. It is noteworthy that the antimicrobial potencies are essentially independent of the bacterial envelope structure. The toxicity of dermaseptins B is a direct consequence of their ability to collapse the electrochemical transmembrane potential of the target cells [33]. Although the precise mechanism by which dermaseptins B act and the molecular basis for their selective cytotoxicity are poorly understood, evidence suggests that these peptides permeate the target cells via a no-pore carpet-like mechanism or the formation of a transmembrane bundle of helices [34–38].

The brevinins and the esculentins are characterized by the presence of two cysteine residues in positions one and seven counted from the C-terminus, which are linked in a disulfide bridge. The structure of some of these peptides has been analyzed by CD spectroscopy, NMR spectroscopy and molecular modeling [39–42]. Type-1 brevinins have a poorly structured hydrophobic N-terminal region, a proline hinge region in the middle and a C-terminal amphipathic loop delineated

Table 1
Antimicrobial peptides of the preprodermaseptin family

Dermaseptin B		
Dermaseptin B1	AMWKDVLKKIGTVALH——AGKAALGAVADTISQ[a]	[27]
Dermaseptin B2	GLWSKI-KEVGKEAAKAAAKAAGKAALGAVSEAV-[a]	[26,27]
Dermaseptin B3	ALWKNMLKGIGK————LAGQAALGAVKTLVGA	[19]
Dermaseptin B4	ALWKDILKNVGK————AAGKAVLNTVTDMVNQ[a]	[19]
Dermaseptin B5	GLWNKI-KEAAK————SAGKAALGFVNEMV-[a]	[19]
Dermaseptin B6	ALWKDILKN————————AGKAALNEINQLVNQ[a]	[19]
Phylloxin		
Phylloxin	GWMSKIASGIGTFLSGIGQQ[a]	[28]
Brevinin-1		
Brevinin-1	FLPVLAGIAAKVVPALF**C**KITKK**C**	[29]
Brevinin-1E	FLPLLAGLAANFLPKIF**C**KITRK**C**	[20,30]
Brevinin-1EA	FLPAIFRMAAKVVPTII**C**SITKK**C**	[20,30]
Brevinin-1EB	VIPFVASVAAEMMQHVY**C**AASRK**C**	[20,30]
Brevinin-1EC	FLPLLAGLAANFFPKIF**C**KITRK**C**	[20,30]
Ranalexin	FFGGLIKIVPAMIPKIF**C**KITRK**C**	[21]
Gaegurin-5	FLGALFKVASKVLPSVF**C**AITKK**C**	[23]
Gaegurin-6	FLPLLAGLAANFLPTII**C**KISYK**C**	[23]
Brevinin-2		
Brevinin-2	G-LLDSLKGFAATAGKGVLQSLLSTAS**C**KLAKT**C**	[29]
Brevinin-2E	G-IMDTLKNLAKTAGKGALQSLLNKAS**C**KLSGQ**C**	[20,30]
Brevinin-2EA	G-ILDTLKLNAISAAKGAAQGLVNKAS**C**KLSGQ**C**	[20,30]
Brevinin-2EB	G-ILDTLKNLAKTAGKGALQGLVKMAS**C**KLSGQ**C**	[20,30]
Brevinin-2EC	GILLDKLKNFAKTAGKGVLQSLLNTAS**C**KLSGQ**C**	[20,30]
Brevinin-2ED	G-ILDSLKNLAKNAG——QILLNKAS**C**KLSGQ**C**	[20,30]
Brevinin-2EE	G-IFDKLKNFAK——GVAQSLLNKAS**C**KLSGQ**C**	[20,30]
Brevinin-2EF	G-IMDTLKNLAKTAGKGALQSLVKMAS**C**KLSGQ**C**	[20,30]
Gaegurin-1	S-LFSLIKAGAKFLGKNLLKQGACYAA**C**KASKQ**C**	[23]
Gaegurin-2	G-IMSIVKDVAKNAAKEAAKGALSTLS**C**KLAKT**C**	[23]
Gaegurin-3	G-IMSIVKDVAKTAAKEAAKGALSTLS**C**KLAKT**C**	[23]
Rugosin A	G-LLNTFKDWAISIAKGAGKGVLTTLS**C**KLDKS**C**	[31]
Rugosin B	S-LFSLIKAGAKFLGKNLLKQGAQYAA**C**KVSKE**C**	[31]
Esculentin-1		
Esculentin-1	GIFSKLGRKKIKNLLISGLKNVGKEVGMDVVRTGIDIAG**C**KIKGE**C**	[30]
Esculentin-1A	GIFSKLAGKKIKNLLISGLKNVGKEVGMDVVRTGIDIAG**C**KIKGE**C**	[20]
Esculentin-1B	GIFSKLAGKKLKNLLISGLKNVGKEVGMDVVRTGIDIAG**C**KIKGE**C**	[20]
Esculentin-2		
Esculentin-2A	GILSLVKGVAKLAGKGLAKEGGKFGLELIA**C**KIAKQ**C**	[20]
Esculentin-2B	GIFSLVKGAAKLAGKGLAKEGGKFGLELIA**C**KIAKQ**C**	[20]
Gaegurin-4	GILDTLKQFAKGVGKDLVKGAAQGVLSTVS**C**KLAKT**C**	[23]
Rugosin C	GILDSFKQFAKGVGKDLIKGAAQGVLSTMS**C**KLAKT**C**	[31]
Temporin		
Temporin A	FLPLIGRVLSGIL[a]	[24]
Temporin B	LLPIVGNLLKSLL[a]	[24]
Temporin C	LLPILGNLLNGLL[a]	[24]
Temporin D	LLPIVGNLLNSLL[a]	[24]
Temporin E	VLPIIGNLLNSLL[a]	[24]
Temporin F	FLPLIGKVLSGIL[a]	[24]
Temporin G	FFPVIGRILNGIL[a]	[24]
Temporin H	LSPNLLKSLL[a]	[24]
Temporin K	LLPNLLKSLL[a]	[24]
Temporin L	FVQWFSKFLGRIL[a]	[24]

Peptides are grouped to form seven subfamilies (bold letters) on the basis of their structural characteristics. Gaps (–) have been introduced to maximize sequences similarity.
[a]Amidated C-terminus.

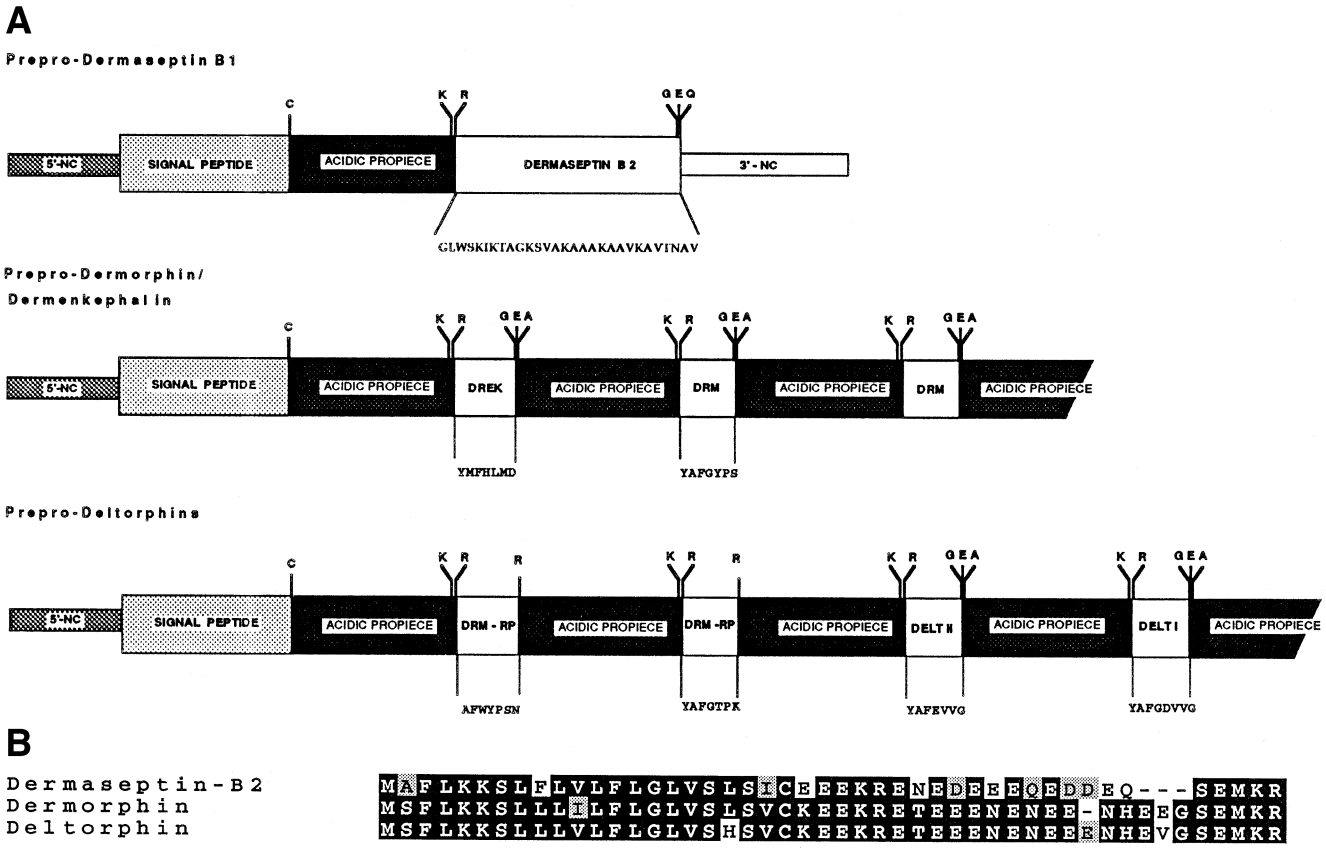

Fig. 2. (A) Block diagrams of preprodermaseptin B2 cDNA, preprodermorphin (clone D-1/2) [44] and preprodeltorphin (clone AD 2) [45] cDNAs. Regions of great similarity among all three cDNAs are labelled with the same boxed patterns as follows: 5′ NC, 5′-non-coding region (dark dotted box); signal peptide (hatched box); acidic spacer peptide (black box); C, cysteine; KR, Lys-Arg dipeptide; GEQ/A, Gly-Glu-Gln/Ala tripeptide. Open boxes denote sequences with low or no homology among the four precursors: 3′ NC, 3′-non-coding region; DRM, dermorphin; DREK, dermenkephalin; DRM-RP, dermorphin-related peptide Tyr-Ala-Phe-Trp-Tyr-Pro-Asn; DELT 1, deltorphin I. Only partial sequences of he cDNAs encoding preprodermorphin and preprodeltorphins are shown since the number of repeats varies and is clone-dependent. In D-1/2, the unit corresponding to DRM-GEA-acidic spacer-KR is repeated four times. In AD 2, the unit corresponding to DELT-GEA-acidic spacer-KR is repeated three times. (B) Maximized multiple sequence alignments of the amino acid sequences of the preproregions of preprodermaseptin B2, preprodermorphin and preprodeltorphins, including the signal peptide (residues 1–22), the acidic propiece and the processing site Lys-Arg. Identical (black background) and similar (shaded background) residues are highlighted. Gaps (–) have been introduced to maximize sequence similarities.

by the disulfide bridge. The disulfide bridge might stabilize the induction of a helical structure spanning residues 8–17 in a lipid membrane environment. Esculentins and brevinins have a distinct activity spectrum whereas peptides within each subfamily do not show significant differences in their cell lytic activities. Most of these peptides also exhibit hemolytic activity. Temporins have a length of 10–13 residues and a limited antimicrobial spectrum [24]. Although temporins show sequence similarity to hemolytic peptides isolated from Vespa venom [43], they do not lyse erythrocytes.

4. Preprodermaseptin-encoded peptides also include opioids

The conserved preproregion of the dermaseptin precursors has striking similarities to plurifunctional precursor proteins preprodermorphin [44] and preprodeltorphins [45]. Dermorphin, Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH2 [46], deltorphin A, Tyr-D-Met-Phe-His-Leu-Met-Asp-NH2 [47–49], and the deltorphins B and C, Tyr-D-Ala-Phe-Xaa-Val-Val-Gly-NH2 (where Xaa is either Asp or Glu) [50], are opioid heptapeptide amides isolated from *Phyllomedusa* ssp. skin which have been shown to be highly potent at and exquisitely selective for the μ- and δ-opioid receptors, respectively [51,52]. These peptides are synthesized as a part of two larger precursor proteins containing multiple copies of the heptapeptide bioactive sequences (Fig. 2). Precursors of this family are composed of a signal peptide of 22 residues, followed by an acidic propiece of 25–26 residues which terminates with two consecutive basic amino acids Lys-Arg and an opioid peptide progenitor sequence. Whereas preprodermaseptins contain a single copy of the acidic propiece and an antimicrobial peptide assembled in tandem, preprodermorphin and preprodeltorphins are made of five highly homologous repeats of this tandem module. A comparison of the cDNA and deduced amino acid sequences of the dermaseptin and opioid precursors revealed that the signal peptides (72% identical at the amino acid level) and the acidic propeptides (~50% identical at the amino acid level) are highly similar. This similarity also extends into the 5′-untranslated regions of the respective mRNAs.

Dermorphin and the deltorphins are thus unexpected additions to the structurally and functionally diverse peptide subfamilies associated with the rapidly evolving C-terminal domains of the dermaseptin family of precursors.

5. Conclusion

The preprodermaseptin family counts numerous members which are strikingly diverse in length, primary structure, conformation, biological activity and mode of action. Several mechanisms may contribute to the generation of precursors containing conserved preproregions associated with multiple forms of mature active peptides. If the dermaseptin B genes are representative of other preprodermaseptin-like genes, this organization would favor recombination mechanisms, or exon shuffling, involving association of diverse coding exons 2 with the first highly conserved coding exon. The duplication and recombinational events that promoted the association of such a homologous exon with sequences encoding a variety of end products in various amphibian species remain to be elucidated but are likely to have occurred at the very early stages of evolution.

Although the overall organization of eukaryotic signal peptides is evolutionarily conserved, no sequence similarity exists [53]. The evolutionary pressure which results in the conservation of the signal peptide in preprodermaseptins suggests that this topogenic sequence may also help to ensure the correct proteolytic maturation of the precursor or targeting the mature peptide to the secretory granules.

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