Evolution of Antimicrobial Peptides: A View from the Cystine Chapel

Robert I. Lehrer

Abstract An animal's environment contains smaller entities that may attack it and cause illness or death. The immune system evolved to protect against such threats. It has two branches, one innate and the other adaptive. The former relies on field-tested molecules that have been selected over eons. Since they are gene-encoded, these innate molecules are deployed with little or no delay. The adaptive immune system consists of molecular and cellular machinery that produces custom-tailored molecules. Its handiwork is relatively slow, and many clients in need of its products would be lost if their innate systems did not also exist. This chapter focuses on cysteine-containing antimicrobial peptides that contain one or more internal disulfide bonds. Special emphasis is placed on the evolution of two superfamilies of defensins: small, usually cationic and amphipathic host defense molecules with three or four intramolecular disulfide bonds. The ancient roots of both defensin groups predate the advent of adaptive immunity by hundreds of millions of years. One superfamily includes the α-, β-, γ-, and θ-defensins of vertebrates, and the "big defensins" found in cephalochordates, mollusks, and crustaceans. The other superfamily of defensins is expressed in arthropods, mollusks, and fungi and may have arisen much earlier. Like defensins, the evolution of other families of cysteine-containing AMPs can be traced to the predawn of vertebrate existence. Collectively and individually, antimicrobial peptides provide a broad range of protective effects. Yet, despite their essential contributions to animal existence, and perhaps because specificity ranks higher than efficacy in the view of most immunologists, AMPs have often been undervalued. Ironically, it is precisely because AMPs lack specificity that these broadly efficacious molecules have been conserved and refined for more than one billion years.

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Abbreviation

AMP(s) Antimicrobial peptide(s)

1 Introduction

Antimicrobial peptides (AMPs) are central components of the innate network of gene-encoded proteins and peptides that protects animals from microbial, viral, or cellular intruders. Because they are gene encoded, some AMPs are pre-deployed at barrier sites, including the skin or at places that are vulnerable to invasion in the respiratory, gastrointestinal, and genitourinary tracts. Other AMPs provide reinforcements that are delivered rapidly by mobile convoys of neutrophils or produced locally in response to various molecular alarm signals.

Adaptive immune responses are more specific, but their precision comes at considerable cost because it requires the relatively slow clonal expansion of effector cells. Under optimal in vitro growth conditions, pathogenic bacteria may double every 20–30 min. In theory, a single exponentially growing bacterium with a 30-min generation time could produce over 10^{14} ($>2^{48}$) progeny in 24 h *if* its environment met its nutritional needs and removed wastes and growth-limiting signals. Unfettered microbial growth does not occur in vivo, in large part because innate defenses such as barriers, fever, phagocytosis, nutrient and iron limitation, and antimicrobial peptides (AMPs) prevent it. Invertebrates cannot mount adaptive immune responses, yet they are the most numerous animals and species on earth. Some invertebrates have life spans that exceed 100 years, including certain marine tubeworms (Bergquist et al. 2000), bivalve mollusks, red sea urchins, and deep sea corals (Ebert 2008).

Hans G. Boman (1924–2008), a pioneer in the field of animal AMPs, divided AMPs into five structural groups: (i) linear, mostly helical, peptides without cysteines, with or without a hinge; (ii) linear peptides without cysteine and with a high proportion of certain residues; (iii) peptides with one disulfide bond; (iv) peptides with two or more S–S bonds giving mainly or only β-sheet structures; and (v) antibacterial peptides derived from larger polypeptides with other known functions (Boman 1995). Rather than attempting to review the evolution of all five, we will focus on groups (iii) and (iv): AMPs that contain cysteine and form one or more disulfide bonds.

Piecing together AMP evolution and assembling a jigsaw puzzle are similar exercises but have some notable differences. Both can be time consuming and vexing. However, when the jigsaw puzzle comes in a box with a picture of the completed puzzle on its cover, and it contains all of the pieces without extraneous ones mixed in, success can be anticipated—or at least recognized. In contrast, our AMP puzzle has no cover picture, its pieces are scattered with many still missing, and similar pieces from extraneous puzzles are mixed in. Limiting our scope to

AMPs that contain cysteine is helpful, because cysteines carry information relevant to secondary and tertiary structure, and their placement and pairing motifs can provide recognizable hallmarks. However, just as an art historian would examine the ears and fingernails of subjects in a painting when attempting to identify its creator (Roskill 1989), we will also consider ancillary features such as the structure and layout of AMP precursors and genes and the presence of short "signature" sequence motifs. In appraising real estate, location is of primary importance. Similarly, expression in a "professional phagocyte," especially a granulocyte or a cell whose name includes the word "killer" (e.g., NK-cells), will also be noted.

Multigenerational human families may contain "black sheep." Similarly, some relatives of AMPs may deal in drugs (endorphins), deliver lethal weapons (toxins), engage in molecular texting (signaling), or do molecular tailoring (immunomodulation). Because such activities are peripheral to any homily about AMPs based on the Book of Genes, we will overlook them in this chapter. Now let us enter the cystine chapel.

2 AMPs with One Cysteine

Relatively few of these AMPs have been described. Distinctin, a 5.4-kDa heterodimeric AMP, was isolated from the skin of a tree frog, *Phyllomedusa distincta* (Batista et al. 2001). Each monomer had a net charge of +4, but their sequences were different. One monomer contained 22 residues and its C-terminus ended with cys-lys-ile-ile. The other monomer had 24 residues and its C-terminus ended with cys-lys-val. An intermolecular disulfide bond between these cysteines created a four-helical bundle that protected distinctin from degradation by proteases (Raimondo et al. 2005). A homodimeric AMP, di-(ILQKAVLDCLKAAGSSLSKAAITAIYNKIT), which was called dicynthaurin, was isolated from the hemocytes of a protochordate, the tunicate *Halocynthia aurantium*. Its monomers were C-terminally amidated and covalently linked by a disulfide bond (Lee et al. 2001). The hemocytes of this tunicate also contained a 3.4-kDa heterodimeric AMP called halocidin. The halocidin subunits contained eighteen (WLNALLHHGLNCAKGVLA) and fifteen (ALLHHGLNCAKGVLA) amino acids and were linked covalently by a single cystine disulfide bond (Jang et al. 2002).

3 AMPs with Two Cysteines

3.1 Frog Skin Peptides

Frogs of the widely distributed *Rana* genus express many AMPs that contain two cysteines and one intramolecular disulfide bond. These AMPs, which evidently arose via gene duplication events, are stored in specialized skin structures called

granular glands or poison glands (Conlon et al. 2004). Typically the peptides are hydrophobic and cationic and form an amphipathic α-helix in membrane-mimetic solvents. Their names (e.g., brevinins, esculentins, gaegurins, ranalexins) often derive from the genus or species name of the frog. Granular glands contain additional bioactive peptides with other functions (Chen et al. 2006). Recently, J. Michael Conlon, a prolific contributor to this literature, voiced skepticism about the contribution of these peptides to ranid host defense by pointing out that some anurans have skin that does not synthesize AMPs and that many frog skin AMPs show low potency in vitro (Conlon 2011a, b). Furthermore, although some frog skin AMPs inhibit the chytrid fungus, *Batrachochytrium dendrobatidis*, which is widely held responsible for worldwide anuran population declines, the ability of these AMPs to protect frogs is not clearly correlated with resistance to fatal chytridiomycosis in the wild (Conlon 2011a, b). We can consider only a few frog skin peptides here.

Ranalexin is a 20 amino acid peptide from the bullfrog *Rana catesbeiana* (Clark et al. 1994). The two cysteines in its sequence (FLGGLIKIVPAMICAVTKKC) form a disulfide bond that creates a heptapeptide loop containing two positively charged lysine residues. The ranalexin propiece has a net charge of −5, and its C-terminal AMP domain has a net charge of +3. A truncated ranalexin analog that lacked the carboxyl-terminal cysteine had markedly reduced antimicrobial activity, suggesting that the 7-membered loop contributed to this function (Clark et al. 1994). In addition to AMPs such as dermaseptins (Amiche and Galanth 2011), the skin of frogs in the genus *Phyllomedusa* expresses various hormones and neuropeptides (Vouille et al. 1997). The signal sequences of these very different molecules are encoded by nucleotides homologous to those in the first coding exon of dermaseptin genes (Vouille et al. 1997). The mammalian cathelicidin gene family (discussed in Sect. 4) also contains unrelated exons that are linked to exons encoding identical or substantially similar signal peptides and propieces.

Some frog skin AMPs exhibit potent trypsin-inhibitory activity, which is imparted by a loop region (Yan et al. 2011). Skin secretions from the Chinese Bamboo odorous frog, *Huia versabilis*, contain an octadecapeptide (SVIGCWTKSIPPRPCFVK-amide) that potently inhibits trypsin but lacks antimicrobial activity. Its 11-member loop resembles those in Bowman-Birk peptide protease inhibitors (Li et al. 2007). Based on their similar precursor structures, frog skin peptides with antimicrobial and/or trypsin-inhibitory activity probably evolved from a common ancestor. An ability to inhibit proteases is useful, since microbial proteases can promote virulence by degrading the tissues and antimicrobial molecules of the infected host (Orth et al. 2010; Dubin 2002).

Basir and Conlon purified 26 peptides from skin secretions of *Rana palustris*, the North American pickerel frog. Half of the peptides contained two cysteines, and half were cysteine-free. Six of the former had 12-residue loops, two had 11-residue loops, and five had 7-residue loops (Basir and Conlon 2003). As not a single one of these peptides inhibited the growth of *Escherichia coli* or *Staphylococcus aureus*, their functions are not yet known (Rinaldi 2002). Comparing signal sequence motifs in AMP precursors from higher (neobatrachian) and archaic (archaeobatrachian) frogs

suggested that convergent evolution of AMP genes took place in at least three different lineages (Koenig and Bininda-Emonds 2011). It is not known if transdermal absorption of frog skin AMPs occurs allowing them to afford systemic protection, as well as protecting the skin.

3.2 Bactenecin Dodecapeptides

Two mammalian AMPs, whose 12 residues include a pair of cysteines, were purified from the neutrophils of cattle, *Bos taurus* (Romeo et al. 1988), and sheep, *Ovis aries* (Huttner et al. 1998). The precursors of both contained a conserved, 114-residue targeting domain called "cathelin" (Romeo et al. 1988; Storici et al. 1992; Bagella et al. 1995). Cathelin is an abbreviation of "cathepsin L inhibitor" (Ritonja et al. 1989), and any AMP whose precursor has a cathelin domain is classified as a cathelicidin (Tomasinsig and Zanetti 2005; Zanetti 2004, 2005; Zanetti et al. 1995). The sequences of bovine (RLCRIVVIRVCR) and ovine (RICRIIFLRVCR) dodecapeptides are almost identical. Cathelin is discussed in Sect. 4.1, and other cathelicidins are described in Sects. 4.5 and 4.7.

3.3 Arenicin

Certain invertebrates have leukocytes (hemocytes) that contain AMPs with a single disulfide bond. Coelomic cells of the marine polychaete worm, *Arenicola marina*, contain a pair of 21-residue AMPs (Ovchinnikova et al. 2004) named arenicins 1 and 2. Each has a net charge of +6 and both kill bacteria and fungi. Their sequences, RWC(V/I)YAYVRVRGVLVRYRRCW, are almost identical, and both have a disulfide bond linking Cys3 to Cys20 (Ovchinnikova et al. 2004). In aqueous solution, the arenicins have a β-hairpin structure formed by antiparallel β-strands with a right-handed twist (Ovchinnikova et al. 2007). Arenicin analogs lacking the disulfide bond show reduced activity against a polymyxin B-resistant *Proteus mirabilis* (Andra et al. 2009).

4 AMPs with Four Cysteines

4.1 Cathelin

Although it is not an AMP and may not even inhibit cathepsin L (Zhu et al. 2008), were an "Oscar" to be given for best supporting role in an AMP production, cathelin would almost certainly win. Its first known appearance in a supporting role took

place in *Myxine glutinosa*, the Atlantic hagfish—a primitive fish without jaws, vertebrae, or the usual accoutrements of adaptive immunity, i.e., discrete thymus tissue and immunoglobulin genes (Basanez et al. 2002; Bajoghli et al. 2011). Cathelin domains typically contain 99–114 residues (Zanetti 2005), including four conserved cysteines that form two intramolecular disulfide bonds (Zhu 2008a). These cysteines are also conserved in cystatins, an even older superfamily of cysteine protease inhibitors. Shunyi Zhu reviewed the relationships between cystatins and cathelin and concluded that the emergence of cathelicidins may have taken place after the gain of a 3′ intron in a duplicated copy of an ancestral cystatin (Zhu 2008a, c). Cathelicidins developed into a multigene family in Cetartiodactyla, a clade that includes whales, dolphins, and even-toed ungulates. Their expansion resulted from gene duplications and changes in the structure of antimicrobial domains secondary to exon shuffling, gene duplication, and post-duplication sequence remodeling (Zhu and Gao 2009).

4.2 Cathelicidins and Vitamin D

The specific granules of human neutrophils contain hCAP18, the 18-kDa, cathelin-containing precursor of LL-37, an α-helical AMP (Agerberth et al. 1995; Cowland et al. 1995). The roles of LL-37 in inflammation and immunity are described in chapter "LL-37: An Immunomodulatory Antimicrobial Host Defence Peptide". Since LL-37 lacks cysteine, its evolution would not be described in this chapter except for an event that brought its expression under the control of vitamin D (Gombart et al. 2005). This resulted from the insertion of a vitamin D response element into its promoter by a primate-specific, short interspersed element (SINE) (Gombart et al. 2009). α-Defensins (Ogata et al. 1992; Miyakawa et al. 1996) and LL-37 both exert in vitro activity against *M. tuberculosis*, and 1α,25 dihydroxy-vitamin D enhances the ability of human macrophages to inhibit intracellular growth of the tubercle bacillus in an LL-37 dependent manner (Sonawane et al. 2011). These findings have re-awakened interest in vitamin D therapy for tuberculosis (Selvaraj 2011). Ironically, good empirical medical practice in the nineteenth century included giving cod-liver oil to patients with tuberculosis (Williams 1849) and exposing them to sunlight (Solis-Cohen 1901)—both excellent ways to provide vitamin D. Mice also have a single 37-residue, α-helical cathelicidin peptide called "CRAMP" (Gallo et al. 1997). However, because the murine gene lacks a vitamin D response element, giving this vitamin does not induce or enhance CRAMP production (Gombart et al. 2009).

4.3 LEAP-2

The liver produces a pair of cysteine-containing molecules called *l*iver-*e*xpressed *a*ntimicrobial *p*eptides (LEAPs)-1 and -2. LEAP-1, which is better known as hepcidin, contains eight cysteines and is discussed in Sect. 6.1. LEAP-2 (net charge, +4)

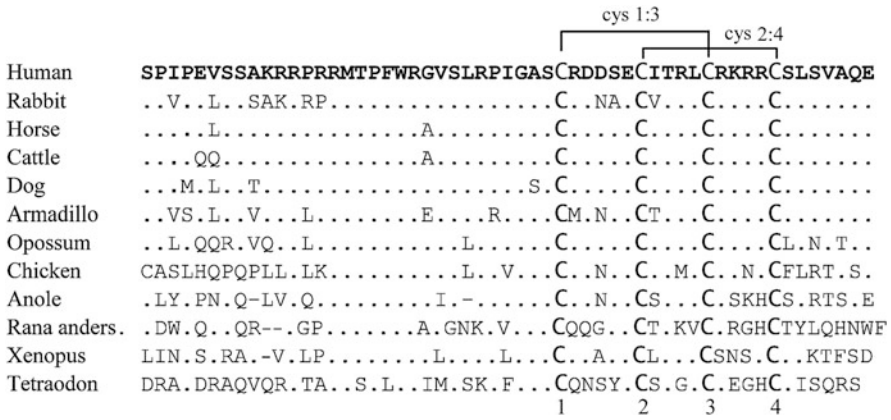

Fig. 1 Liver-expressed antimicrobial peptide-2 (LEAP-2). The sequence of human LEAP-2 is shown in its entirety. Residues that are identical to those in human LEAP-2 in the other peptides are represented by *dots*. Cysteine residues are numbered at the *bottom*, and their connectivity is shown at the *top*. *Tetraodon nigroviridis* is the green puffer fish, *Rana andersoni* is a Vietnamese frog, *Xenopus* is an African clawed frog, and the green anole, *Anolis carolinensis*, is an arboreal lizard

was isolated from human blood plasma ultrafiltrates and has homologs in all vertebrate classes (Fig. 1). The sequence of LEAP-2 is unusually well conserved in mammals and marsupials, suggesting that an endogenous binding partner may exist. The 77-residue precursor of LEAP-2 is synthesized mainly in the liver, an organ unique to vertebrates and a counterpart to the fat body of insects (Arrese and Soulages 2010). The largest native LEAP-2 molecules in plasma contain 40 amino acid residues and are accompanied by shorter forms with N-terminal and C-terminal truncations (Krause et al. 2003). LEAP-2 has features associated with classic peptide hormones (Krause et al. 2003). The likelihood that it has important functions other than antimicrobial activity is reinforced by studies showing that its disulfide bonds are not required for its antimicrobial effects but are essential to maintain the shape of its central core, which contains a short 3_{10}-helix from Asp_{20} to Glu_{22}, a type I β-turn from residues Cys_{23} to Arg_{26}, and a β-hairpin from Cys_{28} to Cys_{33} with a type I' β-turn (Henriques et al. 2010).

4.4 Lactoferricins

It was shown in 1946 that transferrin, which was then called siderophilin, imparted candidastatic properties to serum (Schade and Caroline 1946). Structurally related proteins called lactoferrins (lactotransferrins) and conalbumins (ovotransferrins) were later discovered. These glycoproteins contain 670–690 amino acid residues, show 50–70 % sequence identity, and bind reversibly and with high affinity to iron (Baker et al. 2002). Lactoferrin is expressed widely, and large amounts are present

Fig. 2 Antimicrobial peptides with two disulfides. The top two peptides were isolated from the leukocytes of horseshoe crabs: tachyplesin from *Tachypleus tridentatus* and polyphemusin from *Limulus polyphemus*. Gomesin was purified from the leukocytes of a tarantula spider, *Acanthoscurria gomesiana*, and androctonin was purified from the leukocytes of the scorpion, *Androctonus australis*

in the secondary (specific) cytoplasmic granules of neutrophils, and in glandular secretions, including milk and tears. The N-terminal domain of lactoferrin is highly cationic and peptides released from it by proteases are called lactoferricins. Many lactoferricins are bactericidal in vitro, including lactoferricin B (Bellamy et al. 1992) from bovine lactoferrin (Gifford et al. 2005; Tomita et al. 1994). The sequence of lactoferricin B (net charge, +7) is FKCRRWEWRMKKLGAP-SITCVRRAF, and its two cysteines form a disulfide bond. Although this bond is not required for antimicrobial activity (Hoek et al. 1997), it allowed lactoferricins to enter this cystine chapel as the sole representative of Boman's group V, AMPs derived from larger proteins with other functions.

4.5 Porcine Protegrins

Eleven different cathelicidins are expressed in porcine neutrophils. They include PR-39, a proline (P) and arginine (R)-rich peptide with 39 amino acid residues, two prophenins (PFs 1 and 2) with many proline (P) and phenylalanine (F) residues, five protegrins (PGs) including the three shown in Fig. 2, and three linear peptides (PMAPs) with 23, 36, and 37 residues. The porcine cathelicidin genes are clustered on chromosome 13 and contain four exons and three introns (Zhao et al. 1995; Sang and Blecha 2009). Exons 1–3 encode the signal peptide and the cathelin domain. Exon 4 primarily encodes the various mature AMPs, which range from the 16–18-residue protegrins to the 78-residue prophenins. The proline-rich porcine cathelicidins have type II poly-L-proline helical structures, PMAPs-23,-36, and -37 have largely α-helical structures, and protegrins have β-hairpin configurations.

4.6 *Protegrin Analogs in Invertebrates*

AMPs that resemble protegrins in their size, structure, and potency exist in several invertebrates. They include the tachyplesins and polyphemusins of horseshoe crabs (Miyata et al. 1989); gomesin, an 18-residue AMP from hemocytes of the spider, *Acanthoscurria gomesiana* (Silva et al. 2000); and androctonin, a 25-residue peptide from leukocytes of the scorpion, *Androctonus australis* (Ehret-Sabatier et al. 1996). Leukocytes of the spider crab, *Hyas araneus*, contain a chimeric, proline-arginine-rich AMP whose C-terminal residues include four cysteines that form two disulfide bonds (Stensvag et al. 2008). There is no evidence indicating a common ancestry of protegrins and any of the invertebrate AMPs shown in Fig. 2. Structural and antimicrobial properties of protegrins (Kokryakov et al. 1993; Harwig et al. 1996; Steinberg et al. 1997; Aumelas et al. 1996), tachyplesins (Matsuzaki et al. 1993; Tamamura et al. 1993; Ohta et al. 1992; Nakamura et al. 1988), and gomesin (Fazio et al. 2006) are described elsewhere.

4.7 *Bovine Cathelicidins*

In addition to 13 β-defensins (Selsted et al. 1993), cattle neutrophils contain six cathelicidin AMPs (Scocchi et al. 1997), of which only the cyclic dodecapeptide described in Sect. 3.2 contains cysteine. The other bovine cathelicidins include Bac5, which is composed largely of X-P-P-Y repeats; PR59, a proline and arginine-rich peptide; indolicidin (LPWKWPWWPWRRG), a 13-residue tryptophan-rich peptide; and BMAP28 and BMAP34, which are α-helical. Bovine neutrophils store their cathelicidins as inactive propeptides in large cytoplasmic granules that are more numerous than and compositionally distinct from the azurophil and specific granules of human neutrophils (Zanetti et al. 1990; Gennaro et al. 1983). Phagocytic and soluble stimuli trigger concomitant proteolytic activation and secretion of these cathelicidins.

5 AMPs with Six Cysteines

5.1 *Introducing Defensins*

Many of the AMPs described in this section are called defensins. They are small (2–5 kDa) antimicrobial and/or antiviral peptides whose six or eight conserved cysteines form three or four intramolecular disulfide bonds. They have a largely β-sheet structure that may include an N-terminal α-helical domain, whose presence results in a cysteine-stabilized alpha-beta (CSαβ) structure. Animal defensins comprise two large superfamilies. The first superfamily considered below contains five subfamilies, four expressed in vertebrates and one expressed in invertebrates.

The vertebrate peptides are called alpha (α), beta (β), gamma (γ), and theta (θ) defensins and are descendants of invertebrate AMPs called "big defensins." The word "defensin" has itself evolved and expanded since its introduction to describe three peptides from human neutrophils that are now classified as α-defensins (Ganz et al. 1985; Selsted et al. 1985). β-defensins, the oldest subfamily expressed in vertebrates, occur in fish (Zou et al. 2007), reptiles (Alibardi et al. 2012), birds (van Dijk et al. 2008), and mammals (Scheetz et al. 2002). Defensins have not yet been described in any amphibian. β-defensins have given rise to two identifiable offspring, α-defensins and γ-defensins. Their parentage of α-defensins makes β-defensins the grandparents of primate θ-defensins. Section 5.6 explains our conclusion that β-defensin genes are descendants of an exon expressed in the "big defensin" family of invertebrate AMPs.

5.2 α- and β-Defensins

Using hidden Markov model profile searching, Lynn and Bradley found α-defensins in the genomes of basal mammals, including elephants, lesser hedgehogs, and armadillos (Lynn and Bradley 2007). Their identification of an α-defensin gene in the short-tailed opossum suggests that α-defensins evolved before placental mammals and marsupials diverged, some 130 million years ago (Lynn and Bradley 2007). Although α-defensins are expressed in mice, rats, guinea pigs, hamsters, rabbits, elephants, and primates, the horse is the only Laurasiatherian—a clade that includes whales, most hoofed mammals, carnivores, and others (Hou et al. 2009)—now known to express α-defensins (Bruhn et al. 2009).

In 2004, the genome of chickens (*Gallus gallus*) was reported to contain 13 avian β-defensin genes (Xiao et al. 2004), including the three initially purified from chicken neutrophils and called gallinacins (Harwig et al. 1994). Xiao et al. divided these peptides into two subgroups, based on their sites of expression. β-defensins 1–7 were expressed mainly in the respiratory tract and bone marrow, and β-defensins 8–13 were expressed primarily in the urogenital tract and liver. Chicken β-defensin genes were clustered, consistent with evolution via the duplication and diversification of an ancestral gene.

Studies of human and rodent α-defensin (DEFA) and β-defensin (DEFB) genes also show clustering and reduplication. The five human α-defensin genes, which include two (DEFA1 and DEFA3) that are themselves reduplicated, all reside on human chromosome 8p23 within 450 kb of DEFB1, the gene for human β-defensin-1 (Linzmeier et al. 1999). Based on the relative placements of the DEFA and DEFB1 genes, it was proposed that myeloid α-defensin genes (DEFA1, DEFA3, and DEFA4) evolved from DEFA genes encoding HD5&6, α-defensins expressed by human small intestinal Paneth cells (Linzmeier et al. 1999). Gene copy number polymorphism and strain-dependent variability in mouse DEFA genes (Linzmeier and Ganz 2005; Amid et al. 2009) make the defensin segment of their respective genomes very formidable puzzles to complete.

5.3 θ-Defensins

Theta-defensin genes arose in Old World monkeys via the mutation of a pre-existing α-defensin gene (Nguyen et al. 2003). Mature θ-defensin peptides contain only 18 residues, which include the requisite six cysteines and three disulfide bonds. A peptide-bond connects their amino- and carboxy-terminal residues, making θ-defensins the only known peptides of animal origin with a cyclic backbone. θ-defensin peptides have been isolated from the leukocytes or bone marrow of rhesus macaques (Leonova et al. 2001; Tang et al. 1999) and baboons (Garcia et al. 2008; Stegemann et al. 2010). However, they are absent from human leukocytes and are unlikely to exist in the leukocytes of chimpanzees, bonobos, and gorillas (Nguyen et al. 2003). Like humans, these apes have θ-defensin (DEFT) pseudogenes that contain a stop codon mutation within the signal sequence domain. The human DEFT gene is transcribed; however, the resulting mRNA is not translated because of the premature stop codon.

Family reunions can be confusing, so we will summarize what has already been said about defensin evolution. So far, we have introduced three generations of vertebrate defensins. The grandparents (β-defensins) have existed for ~250 million years. Their α-defensin offspring arose ~125 million years ago, and their θ-defensin grandchildren were born around 35–50 million years ago (Nguyen et al. 2003). θ-defensins are not further described in this chapter. Interested readers can consult other publications to learn about their unusual mode of assembly (Tang et al. 1999), antiviral properties (Venkataraman et al. 2009; Cole et al. 2002; Wang et al. 2003), and antimicrobial activities (Tongaonkar et al. 2011; Welkos et al. 2011; Tran et al. 2002).

5.4 Gamma (γ)-Defensins

γ-Defensins, which are also called "ovodefensins" (Gong et al. 2010), have so far been found only in the eggs of reptiles and birds. Although humans tend to view eggs primarily from a culinary perspective, they are incubators that contain sufficient nutrients and minerals to support embryonic growth and development and provide physical and chemical barriers to prevent infection. The chemical barriers in egg white include lysozyme, ovotransferrin, and perhaps ovalbumin itself, since this member of the serpin family contains multiple oligopeptide antimicrobial domains (Pellegrini et al. 2004). They also include defensins, such as the *Caretta caretta* γ-defensin shown in Fig. 3, which was purified from the egg white of a marine sea turtle. The *Caretta* peptide is cationic (net charge, +6), has six cysteines and three intramolecular disulfide bonds, exerts strong antibacterial activity against *Escherichia coli* and *Salmonella typhimurium*, and has impressive antiviral properties (Chattopadhyay et al. 2006). A similar peptide exists in eggs of an Indian tortoise, *Geomyda trijuga trijuga* (Chakrabarti et al. 1988). Based on its properties and structure, the *Caretta* peptide qualifies to be called a defensin, but to which

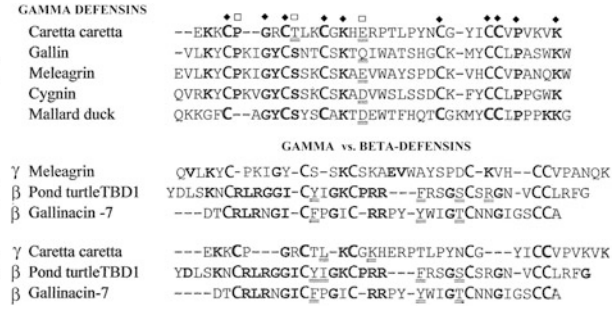

Fig. 3 Gamma defensins. Series I shows sequences of five γ-defensins, isolated from the white of various eggs. *Caretta caretta* is a turtle. Gallin, meleagrin, and cygnin came respectively from the eggs of chickens (*Gallus gallus*), turkeys (Meleagris gallopavo), and black swans (*Cygnus atratus*). Series II and III compares the sequences of meleagrin and the Caretta caretta peptide to b-defensins obtained from the leukocytes of a turtle and the chicken

subfamily should it be assigned? Its cysteines were reported to pair in a 1–6; 2–5; 3–4 manner (Chattopadhyay et al. 2006). This differs from the cysteine pairing in a β-defensin purified from leukocytes of the European pond turtle, *Emys orbicularis*, which manifests the cys 2–4 disulfide bond found in α- and β-defensins. The backbone fold of the *Caretta* peptide differs from the fold of α- and β-defensins (Chattopadhyay et al. 2006). For these reasons, we agree that the Caretta peptide is a charter member of the γ-defensin (ovodefensin) subfamily.

There were three main reasons for suggesting the term γ-defensins: consistency, orderliness, and whimsy. Gamma (γ) is consistent with the α, β, and θ nomenclature used to classify other vertebrate defensin subfamilies. Gamma is also orderly, since γ follows α and β in the Greek alphabet. Finally, gamma is somewhat whimsical since it follows α and β in αβγo, a Greek word for egg. For readers who prefer their eggs and peptides prepared Latin style, ovodefensin is a suitable alternative—at least until a γ-defensin is found outside the confines of an egg. Figure 3 shows additional avian γ-defensins, including gallin (Gong et al. 2010), expressed in the chicken oviduct; meleagrin (Odani et al. 1989) from the turkey, *Meleagris gallopavo*; cygnin (Simpson and Morgan 1983) from the black swan, *Cygnus atratus*; and BPS1 (Naknukool et al. 2008) from the mallard duck, *Anas platyrhynchos*. The cysteine-connectivity of these avian γ-defensins remains to be determined, so the figure shows the disulfide pairing of the *Caretta* peptide. Another mallard duck peptide, BPS2, has an identical sequence to that of cygnin, and related peptides exist in the zebra finch (Gong et al. 2010). To allow comparison, Fig. 3 also shows three β-defensins: TBD1, from turtle leukocytes; gallinacin-7, from chicken leukocytes; and HBD-126, a human epididymal β-defensin.

5.5 NK-Lysin and Granulysin

Certain AMPs that contain six cysteines have not evolved from a defensin lineage. This is exemplified by the AMP family that includes porcine NK-lysin (Andersson et al. 1995), human granulysin (Krensky 2000), and amoebapore (Bruhn et al. 2003) from the pathogenic protozoan, *Entamoeba histolytica*. NK-lysin, purified from porcine small intestinal tissue, was the first member of this group to be characterized (Andersson et al. 1995, 1996). It contained 78 residues, was cationic, had six cysteines and three disulfides, and had impressive cytolytic and antimicrobial activity, including an ability to kill *Mycobacterium tuberculosis* (Andreu et al. 1999). However, its four α-helix bundle structure (Dandekar and Leippe 1997) differs substantially from the β-sheet or CSαβ-structures of defensins, and its family allegiances are elsewhere. NK-lysin, granulysin, and amoebapore belong to the SAPLIP (*sap*osin-*li*ke *p*rotein) family, whose evolutionary history is described elsewhere (Bruhn 2005; Rorman et al. 1992; Zhai and Saier 2000; Leippe and Herbst 2004).

5.6 Big Defensins

For help in tracing the origins of vertebrate β-defensins, we could have no better guide than Aphrodite, who—according to Greek mythology—arose from the sea in an oyster shell. However, it would be the oyster and not the Goddess who could lead us back to our destination. Oysters and other mollusks express many varieties of AMPs, including several that are called defensins. Because the giant Pacific oyster, *Crassostrea gigas*, is an important commercial species, it has become a subject of detailed scientific study. Like primates, mollusks express three families of defensins, including "big defensins"—the likely ancestor of β-defensins.

Big defensins were discovered in the hemocytes of a horseshoe crab, *Tachypleus tridentatus* (Saito et al. 1995; Iwanaga et al. 1998). This big defensin contained 79 amino acids, organized in two tandem domains, each of which could exert antimicrobial activity by itself. The N-terminal domain was amphipathic, cysteine-free, and non-cationic. However, the C-terminal domain contained 37 amino acids, was cationic, and its six cysteines paired in exactly the same manner (cys1–5, 2–4, 3–6) as they pair in vertebrate β-defensins. Recent studies indicate that the C-terminal domain of the *C. gigas* big defensin is encoded by a separate exon (Rosa et al. 2011).

Figure 4 aligns the sequences of ten big defensins and β-defensins from fish, reptiles, birds, and mammals. Considering that β-defensins often show only 30–40 % homology with other β-defensins, the homology between big defensins and β-defensins is impressive. Because a peptide with six cysteine residues could join them pairwise in 15 different ways, their identical pairing in big defensins and β-defensins is not trivial. Additional support for the ancestry of big defensins to β-defensins comes from the recently reported presence of big defensins in the amphioxus, *Branchiostoma* (Teng et al. 2012). The existence of big defensins

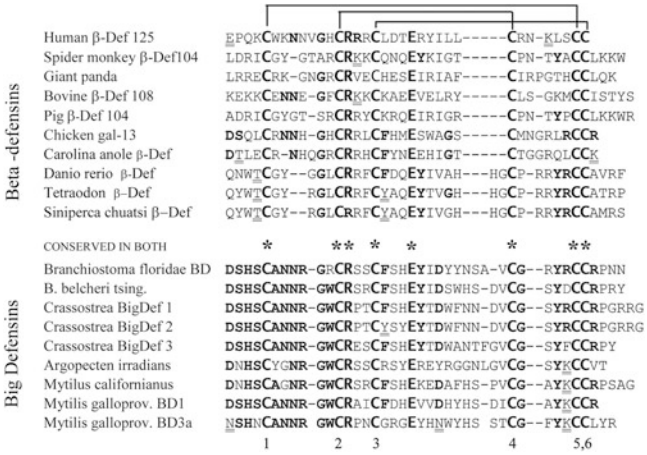

Fig. 4 Big defensins and beta-defensins. The upper ten sequences are of β-defensins from three fish (*Siniperca chuatsi*, *Tetraodon nigriviridis*, *Danio rerio*), a reptile (*Anolis californianus*), the chicken, (*Gallus gallus*), and five mammals. Residues (6 cys, 1 arg, 1 glu) shown in a larger font t are conserved in {beta}-defensins and big defensins. The nine big defensin sequences include 7 from mollusks and two from lancelets (*Branchiostoma floridae* and *Branchiostoma belcheri*). *Crassostrea* is an oyster, *Argopecten* (the bay scallop) is a saltwater clam, and *Mytilus* species are mussels. Identical residues are *bolded*, and conservative substitutions are *double underlined*. The cysteines are numbered at the *bottom*, and their connectivity is shown at the *top*. *Dashes* represent gaps that were introduced to maximize the alignment. *Stars* indicate residues that are highly conserved in both big defensins and β-defensins

in both protostomes (horseshoe crabs and mollusks) and deuterostomes (*Branchiostoma*) is noteworthy, especially since these phyla diverged ~670 million years ago (Ayala et al. 1998).

5.7 *Sapecin-Like Insect Defensins*

In addition to the peptides discussed above, there is a second AMP superfamily whose members are called defensins. In 1988, Matsuyama and Natori purified three AMPs (sapecins) from an embryonic cell line of *Sarcophaga peregrina*, a flesh fly. The peptides called "sapecins" were cloned the following year. The sapecin fold contained a flexible loop (residues 4–12), followed by a short helix (residues 15–23), and two extended strands that were formed by residues 24–31 and 34–40 (Hanzawa et al. 1990). In 1989, Lambert et al. isolated two antimicrobial peptides from immune blood of the dipteran *Phormia terranovae* (Lambert et al. 1989). The peptides ("phormicins") were positively charged, contained 40 residues, and had three intramolecular disulfide bridges. Their sequences differed by only a single amino acid. Because they generally resembled α-defensins, they proposed calling them "insect defensins" (Lambert et al. 1989). The sequences of sapecin, phormicin,

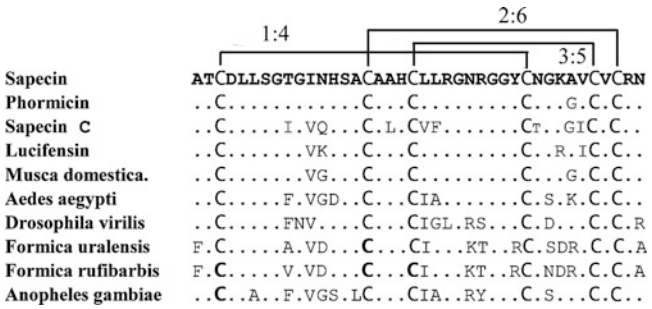

Fig. 5 Insect defensins. These sequences come from ants (*Formica* spp.), mosquitoes (*Aedes* and *Anopheles*), and flies (*Musca domestica*, the common house fly, and *Drosophila virilis*, a fruit fly). Sapecin was purified from a cell line derived from the flesh fly, *Sarcophaga peregrina*. Phormicin was isolated from larvae of the blue bottle fly *Phormia terranovae* and lucifensin from maggots of the blowfly, *Lucilia sericata*

and eight other defensins expressed by flies, mosquitoes, and ants can be seen in Fig. 5, which also shows that their cysteines pair in a 1:4, 2:6, 3:5 manner to create a cysteine-stabilized αβ-structure (Dassanayake et al. 2007). The sequences are highly conserved, even though flies and mosquitoes belong to the order Diptera, and ants belong to the order Hymenoptera. The evolution of insect defensins has been reviewed elsewhere (Dassanayake et al. 2007; Wong et al. 2007).

5.8 Older Defensins

The sequences in Fig. 6 are divided into four groups. Dashes were introduced into the group II and III sequences to align them with group IV. Group I contains defensins from a fly, a mosquito, and an ant. They resemble sequences shown in Fig. 5 except that several motifs have been boxed. Group II shows defensin sequences from more ancient invertebrates, including a dragonfly (*Aeschna cyanea*) and five arachnids: a scorpion, *Leiurus*, and four ixodid ticks. The sequences in group II show extensive homology within the group and with the molluskan defensins in group III. Residues conserved in groups II and III are indicated by their bold font and by vertical lines in the space between the groups. In addition to this conservation across the arthropod/mollusk boundary, the sequences in groups II and III are homologous to the fungal defensins in group IV. Since fungi and animals diverged from their last common ancestor more than one billion years ago, the ancestral gene for these defensins is extremely ancient. Also note that the defensins in groups II and IV contain six cysteines, and those in group III contain eight. If entry into the defensin confraternity is governed by descent, then the definition of a defensin should encompass peptides with six or eight cysteines. While this notion might furrow a few human brows, it would be logical to a tick, whose nymphal and larval forms have six legs and whose adult form has eight.

Fig. 6 Older invertebrate defensins. Four sets of sequences are shown. Set I shows three insect defensins homologous to those in Fig. 5; set II shows sequences from more primitive arthropods, including a dragonfly (*Aeschna cyanea*), four ticks (*Amblyomma maculatum*, *Argas monolakensis*, *Ixodes scapularis*, and *Ornithodorus moubata*), and a scorpion (*Leiurus quinqefasciatis hebraicum*). Set III shows defensins from the oyster, *Crassostrea gigas*, and the Mediterranean mussel, *Mytilus galloprovincialis*. Set IV shows sequences of defensins expressed by three fungi: *Pseudoplectania nigrella* (plectasin), *Ajellomyces dermatitidis*, and *Ajellomyces capsulatus*. The last two fungi may be more familiar under their older names of *Blastomyces dermatitidis* and *Histoplasma capsulatum*. Conserved residues in sets II, III, and IV appear in a *bold* font, and conservative replacements are *doubly underlined*. *Vertical lines* identify residues conserved between sets II and IV. Please note that the mollusk defensins in set III contained eight cysteines and the defensins in sets I, II, and IV contained six cysteines. In set I, the *boxed* residues show motifs that also appear in sets II–IV, perhaps coincidentally or perhaps not

5.9 For Extra Credit

Because defensins and defensin-like peptides also exist in plants (Broekaert et al. 1995; Zhang and Kato 2003; Thomma et al. 2002) and in prokaryotes (Zhu 2007), their evolutionary history might be traced even further back. Readers interested in this exercise can consult recent reports on this subject (Zhu 2007, 2008b). The AMPs of plants are described in chapter "Innate Immunity in Plants: The Role of Antimicrobial Peptides" and various antimicrobials produced by prokaryotes are described in chapter "Antimicrobial Peptides Produced by Microorganisms".

5.10 The Importance of Being Nonspecific

Defensins and other AMPs had already existed for over 750 million years before the adaptive immune system of vertebrates came into existence. This is important to recall when evaluating suggestions that defensins and other AMPS function *primarily* as molecular alarms that summon the adaptive immune system into action. If AMPs were independent contractors before there was an adaptive immune system, they undoubtedly remain so now—especially given the kinetic features of innate and adaptive immunity discussed in the introduction.

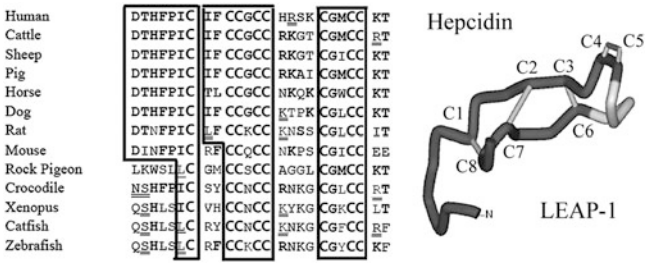

Fig. 7 Hepcidin (LEAP-1). This 25-residue iron-regulatory peptide is exceptionally well conserved among mammals but somewhat less so among the other vertebrate classes. Highly conserved motifs are *boxed*, identical conserved residues are *bolded*, and conservative substitutions are *underlined*. The disulfide pairing motif is illustrated in the structural diagram

6 AMPs with Eight or More Cysteines

6.1 Hepcidin

LEAP-1, more commonly called hepcidin, is a 25-residue cationic peptide with eight cysteines that is produced by the liver. Its sequence is unusually well conserved for an AMP, especially among mammals (Fig. 7). Although hepcidin has antimicrobial properties, its major physiological role is that of a central hormonal regulator of iron metabolism. Deficiencies of hepcidin cause disorders characterized by tissue iron overload, and excessive hepcidin is associated with anemia of inflammation, chronic renal disease, and other disorders (Ganz and Nemeth 2011).

6.2 eNAP-2 and Related WAP/FDC Peptides

eNAP-2, a 6.5-kDa AMP that is relatively rich in proline, glycine, and cysteine residues, was purified from equine neutrophils, which contain it in considerable amounts (Couto et al. 1992b). In addition to its antimicrobial activity, eNAP-2 inhibited two serine proteases of microbial origin, subtilisin A and proteinase K, by binding to the active site of these enzymes (Couto et al. 1993). This serine protease inhibition was selective in that eNAP-2 did not inactivate elastase or cathepsin G from human neutrophils or bovine pancreatic trypsin, all of which are mammalian serine proteases. Based on the sequence of its first 46 residues, eNAP-2 belongs to the large and diverse whey acidic protein/4-disulfide core (WAP/FDC) family. Other members of this family are also protease inhibitors, including secretory leukocyte protease inhibitor (SLPI), which also has antimicrobial, antiviral, and immunomodulatory properties (Hiemstra et al. 1996; Tomee et al. 1998; Williams et al. 2006; Scott et al. 2011). Figure 8 shows that homologs of

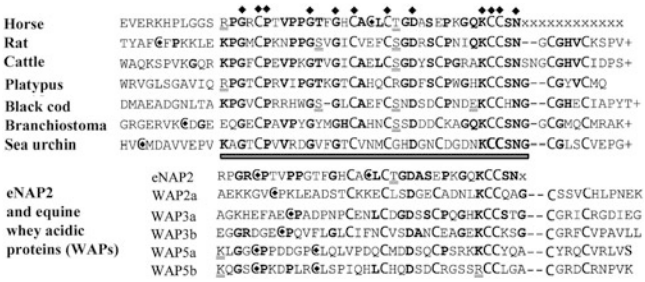

Fig. 8 eNAP-2 belongs to the "whey acidic protein/four disulfide core" (WAP/FDC) family. Two sets of sequences appear in the figure. The upper set shows eNAP-2 (first sequence) and WAP/FCD peptides from other animals. As eNAP-2 was not fully sequenced (Couto et al. 1992), its unknown residues are represented by xxx. The *long narrow rectangle* beneath the top set indicates the 35-residue region of greatest conservation. The twelve large *black diamond symbols* identify the twelve most highly conserved residues in this 35-residue region. Identical residues are in *bold* font, and conservative substitutions are *underlined*. The bottom set compare eNAP2 to other WAP/FDC peptides in the equine genome. Cysteine residues outside the highly conserved region have a small *black diamond symbol* within the letter C. Note that eNAP2 uniquely contains an additional cysteine within the highly conserved region

eNAP-2 exist not only in other vertebrates but also in the amphioxus-like cephalochordate, *Branchiostoma floridae*, and in an echinoderm, the purple sea urchin *Strongylocentrotus purpuratus*. The bottom part of this figure compares eNAP-2 to other equine WAP/FDC peptides and shows that eNAP2 is not alone in having a cysteine in other than its canonical position.

6.3 A Curious Brew

If we made a laboratory bouillabaisse with oysters, mussels, scorpions, and spiders and threw in a few primitive arachnids and insects for spice, the broth would contain multiple AMPs with six, eight, or ten cysteines. They would include MGDs (*Mytilus galloprovincialis* defensins), which have eight cysteines (Hubert et al. 1996; Mitta et al. 1999; Gerdol et al. 2011) and the cysteine-stabilized α-β (CSαβ) structure also found in scorpion toxins (Yang et al. 2000). *Mytilus* has three other AMPs that contain eight cysteines: mytilins, myticins, and mytimacins (Gerdol et al. 2011). All of these are stored as processed, active forms within leukocyte granules, able to act locally in phagosomes or systemically after entering the hemolymph. Unlike α-defensin propeptides, wherein the cationic antimicrobial domain follows an anionic propiece, the defensin domain in an MGD precursor precedes a 21-residue anionic post-piece (Mitta et al. 1999). The eight cysteine residues of MGD-1 pair in a [Cys 1–5; 2–6; 3–7; 4–8]-manner (Yang et al. 2000).

Mytimacins have 85–101 residues, including 8–10 conserved cysteines and four or five intramolecular disulfide bridges (Gerdol et al. 2011). They are homologous

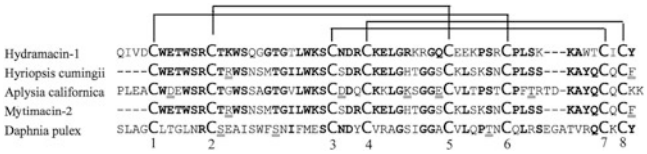

Fig. 9 How to recognize a macin. Hydramacin-1 is from the primitive coelenterate, *Hydra magnipapillata* (GI: 213424017). *Hyriopsis cunningii* is the triangle shell pearl mussel, and the peptide shown in this figure is called theromacin (AEC50045.1). *Aplysia californica* is also called a California sea hare, and the illustrated peptide is currently called "neuromacin-like" (NP_001 191629). Mytimacin-2 (CCC15016.1) comes from the Mediterranean mussel, *Mytilus galloprovincialis*. The final peptide is expressed by the tiny crustacean, *Daphnia pulex*, which is commonly called a "water flea" is. The cysteine connectivity was established in hydramacin-1

to hydramacin, an antimicrobial peptide expressed by the ancient cnidarian and basal metazoan, *Hydra magnipapillata* (Jung et al. 2009). Molluskan "macins" and hydramacins both likely possess a CSαβ-structure (Gerdol et al. 2011). The macin group is larger and more complex than the single *Mytilus* representative shown in Fig. 9. A peptide from *Daphnia pulex*, the "water flea," also appears in this figure. This microscopic crustacean played a crucial role in Eli Metchnikoff's discovery of phagocytosis. His experiments with Daphnia, which are described in his 1893 monograph "Lectures on the Comparative Pathology of Infection," remain worth reading.

6.4 Is eNAP-1 an AMP?

When equine neutrophils were examined two decades ago (Couto et al. 1992) instead of finding any defensins, two other cysteine-rich peptides with antimicrobial activity were discovered and named equine neutrophil antimicrobial peptides (eNAPs) 1 and 2. eNAP-2, which is described in Sect. 6.2, existed in relatively large amounts to allow it to function as an AMP. However, eNAP-1 was present in much smaller amounts, making it unlikely that its direct antimicrobial effects are functionally significant in vivo. The sequence of eNAP-1 and its homology to other members of the granulin family are shown in Fig. 10. The biological activities of granulins were recently reviewed (Bateman and Bennett 2009) and are distinct from direct antimicrobial activity. For these reasons, we would answer the rhetorical question introducing this paragraph with a simple "No."

6.5 Drosomycin

It is fitting to end this discussion with an AMP from *Drosophila*, a tiny fly whose contributions to genetics have been immense. Drosomycin, a 44-residue peptide, is produced after puncturing larval or adult *Drosophila* with a bacteria-soaked needle

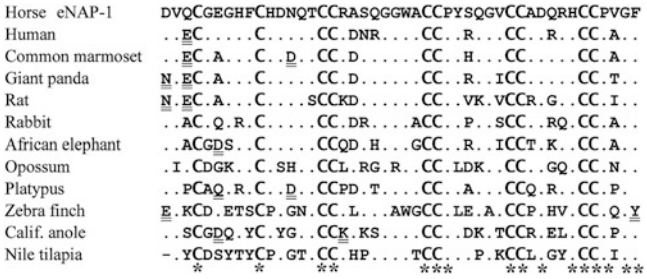

Fig. 10 eNAP-1 and granulins. Only the first 46 residues of eNAP-1 were sequenced. They are shown, along with the corresponding 46 residues of homologous peptides from other animals. Residues identical to those in eNAP1 are shown as *dots*, and conservative substitutions are *underlined*. The 16 *asterisks* beneath the sequences identify the most highly conserved residues. Full-length granulins have twelve cysteines and six disulfide bonds. Human granulin contains twelve additional C-terminal residues (including two cysteines that do not appear in the figure)

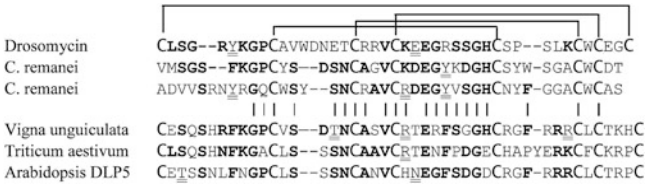

Fig. 11 Drosomycin. Drosomycin, from the fruit fly (*Drosophila melanogaster*), has homologs in the nematode, *Caenorhabditis remanei*, and it also has them in plants, including the cowpea (*Vina unguiculata*), wheat (*Triticum aestivum*), and mouse-ear cress (*Arabidopsis thaliana*). The disulfide bonds of drosomycin are indicated above its sequence. *Vertical lines* traversing the space between animal and plant sequences show conserved sites. Identical residues found on both sides of the central plant/animal divide are in *bold* font

(Fehlbaum et al. 1994). Synthesized in the fat body, it contains eight cysteines and four intramolecular disulfide bonds and is primarily antifungal. In their description, the authors recognized its structural resemblance and sequence homology to antifungal peptides produced by plants (Fig. 11) and wrote, "It is tempting to speculate that drosomycin and plant defensins have evolved from a common ancestor molecule that predated the separation of plants and animals." Additional pieces of the puzzle that have since been found, including the nematode (*Caenorhabditis remanei*) sequences in Fig. 10, leave little doubt that their initial speculation is correct.

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References

Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH (1995) FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. Proc Natl Acad Sci USA 92:195–199

Alibardi L, Celeghin A, Dalla VL (2012) Wounding in lizards results in the release of beta-defensins at the wound site and formation of an antimicrobial barrier. Dev Comp Immunol 36(3):557–565, PMID:22001772

Amiche M, Galanth C (2011) Dermaseptins as models for the elucidation of membrane-acting helical amphipathic antimicrobial peptides. Curr Pharm Biotechnol 12(1184):1193

Amid C, Rehaume LM, Brown KL, Gilbert JG, Dougan G, Hancock RE et al (2009) Manual annotation and analysis of the defensin gene cluster in the C57BL/6 J mouse reference genome. BMC Genomics 10:606. doi:10.1186/1471-2164-10-606

Andersson M, Gunne H, Agerberth B, Boman A, Bergman T, Sillard R et al (1995) NK-lysin, a novel effector peptide of cytotoxic T and NK cells. Structure and cDNA cloning of the porcine form, induction by interleukin 2, antibacterial and antitumour activity. EMBO J 14:1615–1625

Andersson M, Gunne H, Agerberth B, Boman A, Bergman T, Olsson B et al (1996) NK-lysin, structure and function of a novel effector molecule of porcine T and NK cells. Vet Immunol Immunopathol 54:123–126

Andra J, Hammer MU, Grotzinger J, Jakovkin I, Lindner B, Vollmer E et al (2009) Significance of the cyclic structure and of arginine residues for the antibacterial activity of arenicin-1 and its interaction with phospholipid and lipopolysaccharide model membranes. Biol Chem 390:337–349

Andreu D, Carreno C, Linde C, Boman HG, Andersson M (1999) Identification of an anti-mycobacterial domain in NK-lysin and granulysin. Biochem J 344(Pt 3):845–849

Arrese EL, Soulages JL (2010) Insect fat body: energy, metabolism, and regulation. Annu Rev Entomol 55:207–225

Aumelas A, Mangoni M, Roumestand C, Chiche L, Despaux E, Grassy G et al (1996) Synthesis and solution structure of the antimicrobial peptide protegrin-1. Eur J Biochem 237:575–583

Ayala FJ, Rzhetsky A, Ayala FJ (1998) Origin of the metazoan phyla: molecular clocks confirm paleontological estimates. Proc Natl Acad Sci USA 95:606–611

Bagella L, Scocchi M, Zanetti M (1995) cDNA sequences of three sheep myeloid cathelicidins. FEBS Lett 376(2):25–228

Bajoghli B, Guo P, Aghaallaei N, Hirano M, Strohmeier C, McCurley N et al (2011) A thymus candidate in lampreys. Nature 470:90–94

Baker EN, Baker HM, Kidd RD (2002) Lactoferrin and transferrin: functional variations on a common structural framework. Biochem Cell Biol 80:27–34

Basanez G, Shinnar AE, Zimmerberg J (2002) Interaction of hagfish cathelicidin antimicrobial peptides with model lipid membranes. FEBS Lett 532:115–120

Basir YJ, Conlon JM (2003) Peptidomic analysis of the skin secretions of the pickerel frog Rana palustris identifies six novel families of structurally-related peptides. Peptides 24:379–383

Bateman A, Bennett HP (2009) The granulin gene family: from cancer to dementia. Bioessays 31:1245–1254

Batista CV, Scaloni A, Rigden DJ, Silva LR, Rodrigues RA, Dukor R et al (2001) A novel heterodimeric antimicrobial peptide from the tree-frog Phyllomedusa distincta. FEBS Lett 494:85–89

Bellamy W, Takase M, Wakabayashi H, Kawase K, Tomita M (1992) Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. J Appl Bacteriol 73:472–479

Bergquist DC, Williams FM, Fisher CR (2000) Longevity record for deep-sea invertebrate. Nature 403:499–500

Boman HG (1995) Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 13:61–92
Broekaert WF, Terras FR, Cammue BP, Osborn RW (1995) Plant defensins: novel antimicrobial peptides as components of the host defense system. Plant Physiol 108:1353–1358
Bruhn H (2005) A short guided tour through functional and structural features of saposin-like proteins. Biochem J 389:249–257
Bruhn H, Riekens B, Berninghausen O, Leippe M (2003) Amoebapores and NK-lysin, members of a class of structurally distinct antimicrobial and cytolytic peptides from protozoa and mammals: a comparative functional analysis. Biochem J 375:737–744
Bruhn O, Paul S, Tetens J, Thaller G (2009) The repertoire of equine intestinal alpha-defensins. BMC Genomics 10:631. doi:10.1186/1471-2164-10-631
Chakrabarti S, Sen PC, Sinha NK (1988) Purification and characterization of a low molecular weight basic protein from marine turtle egg white. Arch Biochem Biophys 262:286–292
Chattopadhyay S, Sinha NK, Banerjee S, Roy D, Chattopadhyay D, Roy S (2006) Small cationic protein from a marine turtle has beta-defensin-like fold and antibacterial and antiviral activity. Proteins 64:524–531
Chen T, Zhou M, Gagliardo R, Walker B, Shaw C (2006) Elements of the granular gland peptidome and transcriptome persist in air-dried skin of the South American orange-legged leaf frog, Phyllomedusa hypocondrialis. Peptides 27(21):29–2136
Clark DP, Durell S, Maloy WL, Zasloff M (1994) Ranalexin. A novel antimicrobial peptide from bullfrog (Rana catesbeiana) skin, structurally related to the bacterial antibiotic, polymyxin. J Biol Chem 269:10849–10855
Cole AM, Hong T, Boo LM, Nguyen T, Zhao C, Bristol G et al (2002) Retrocyclin: a primate peptide that protects cells from infection by T- and M-tropic strains of HIV-1. Proc Natl Acad Sci USA 99:1813–1818
Conlon JM (2011a) Structural diversity and species distribution of host-defense peptides in frog skin secretions. Cell Mol Life Sci 68:2303–2315
Conlon JM (2011b) The contribution of skin antimicrobial peptides to the system of innate immunity in anurans. Cell Tissue Res 343(201–21):2
Conlon JM, Kolodziejek J, Nowotny N (2004) Antimicrobial peptides from ranid frogs: taxonomic and phylogenetic markers and a potential source of new therapeutic agents. Biochim Biophys Acta 1696:1–14
Couto MA, Harwig SS, Cullor JS, Hughes JP, Lehrer RI (1992a) Identification of eNAP-1, an antimicrobial peptide from equine neutrophils. Infect Immun 60:3065–3071
Couto MA, Harwig SS, Cullor JS, Hughes JP, Lehrer RI (1992b) eNAP- 2, a novel cysteine-rich bactericidal peptide from equine leukocytes. Infect Immun 60(504):2–5047
Couto MA, Harwig SS, Lehrer RI (1993) Selective inhibition of microbial serine proteases by eNAP- 2, an antimicrobial peptide from equine neutrophils. Infect Immun 61:2991–2994
Cowland JB, Johnsen AH, Borregaard N (1995) hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. FEBS Lett 368:173–176
Dandekar T, Leippe M (1997) Molecular modeling of amoebapore and NK-lysin: a four-alpha-helix bundle motif of cytolytic peptides from distantly related organisms. Fold Des 2(47):52
Dassanayake RS, Silva Gunawardene YI, Tobe SS (2007) Evolutionary selective trends of insect/mosquito antimicrobial defensin peptides containing cysteine-stabilized alpha/beta motifs. Peptides 28:62–75
Dubin G (2002) Extracellular proteases of Staphylococcus spp. Biol Chem 383:1075–1086
Ebert TA (2008) Longevity and lack of senescence in the red sea urchin *Strongylocentrotus franciscanus*. Exp Gerontol 43:734–738
Ehret-Sabatier L, Loew D, Goyffon M, Fehlbaum P, Hoffmann JA, van Dorsselaer A et al (1996) Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. J Biol Chem 271:29537–29544
Fazio MA, Oliveira VX Jr, Bulet P, Miranda MT, Daffre S, Miranda A (2006) Structure-activity relationship studies of gomesin: importance of the disulfide bridges for conformation, bioactivities, and serum stability. Biopolymers 84:205–218

Fehlbaum P, Bulet P, Michaut L, Lagueux M, Broekaert WF, Hetru C et al (1994) Insect immunity. Septic injury of Drosophila induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. J Biol Chem 269:33159–33163
Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L et al (1997) Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem 272:13088–13093
Ganz T, Nemeth E (2011) Hepcidin and disorders of iron metabolism. Annu Rev Med 2:347–360
Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF et al (1985) Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76(14):27–1435
Garcia AE, Osapay G, Tran PA, Yuan J, Selsted ME (2008) Isolation, synthesis, and antimicrobial activities of naturally occurring theta-defensin isoforms from baboon leukocytes. Infect Immun 76:5883–5891
Gennaro R, Dewald B, Horisberger U, Gubler HU, Baggiolini M (1983) A novel type of cytoplasmic granule in bovine neutrophils. J Cell Biol 96:1651–1661
Gerdol M, DeMoro G, Manfrin C, Venier P, Pallavicini A (2011) Big defensins and mytimacins, new AMP families of the Mediterranean mussel Mytilus galloprovincialis. Dev Comp Immunol 36(2):390–399, PMID:21871485
Gifford JL, Hunter HN, Vogel HJ (2005) Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. Cell Mol Life Sci 62:2588–2598
Gombart AF, Borregaard N, Koeffler HP (2005) Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1, 25-dihydroxyvitamin D3. FASEB J 19:1067–1077
Gombart AF, Saito T, Koeffler HP (2009) Exaptation of an ancient Alu short interspersed element provides a highly conserved vitamin D-mediated innate immune response in humans and primates. BMC Genomics 10:321
Gong D, Wilson PW, Bain MM, McDade K, Kalina J, Herve-Grepinet V et al (2010) Gallin; an antimicrobial peptide member of a new avian defensin family, the ovodefensins, has been subject to recent gene duplication. BMC Immunol 11:12. doi:10.1186/1471-2172-11-12
Hanzawa H, Shimada I, Kuzuhara T, Komano H, Kohda D, Inagaki F et al (1990) 1 H nuclear magnetic resonance study of the solution conformation of an antibacterial protein, sapecin. FEBS Lett 269:413–420
Harwig SS, Swiderek KM, Kokryakov VN, Tan L, Lee TD, Panyutich EA et al (1994) Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. FEBS Lett 342:281–285
Harwig SS, Waring A, Yang HJ, Cho Y, Tan L, Lehrer RI (1996) Intramolecular disulfide bonds enhance the antimicrobial and lytic activities of protegrins at physiological sodium chloride concentrations. Eur J Biochem 240:352–357
Henriques ST, Tan CC, Craik DJ, Clark RJ (2010) Structural and functional analysis of human liver-expressed antimicrobial peptide 2. Chembiochem 11:2148–2157
Hiemstra PS, Maassen RJ, Stolk J, Heinzel-Wieland R, Steffens GJ, Dijkman JH (1996) Antibacterial activity of antileukoprotease. Infect Immun 64:4520–4524
Hoek KS, Milne JM, Grieve PA, Dionysius DA, Smith R (1997) Antibacterial activity in bovine lactoferrin-derived peptides. Antimicrob Agents Chemother 41:54–59
Hou ZC, Romero R, Wildman DE (2009) Phylogeny of the Ferungulata (Mammalia: Laurasiatheria) as determined from phylogenomic data. Mol Phylogenet Evol 5(2):660–664
Hubert F, Noel T, Roch P (1996) A member of the arthropod defensin family from edible Mediterranean mussels (Mytilus galloprovincialis). Eur J Biochem 240(30):2–306
Huttner KM, Lambeth MR, Burkin HR, Burkin DJ, Broad TE (1998) Localization and genomic organization of sheep antimicrobial peptide genes. Gene 206:85–91
Iwanaga S, Kawabata S, Muta T (1998) New types of clotting factors and defense molecules found in horseshoe crab hemolymph: their structures and functions. J Biochem 1(23):1–15
Jang WS, Kim KN, Lee YS, Nam MH, Lee IH (2002) Halocidin: a new antimicrobial peptide from hemocytes of the solitary tunicate, *Halocynthia aurantium*. FEBS Lett 521:81–86

Jung S, Dingley AJ, Augustin R, Anton-Erxleben F, Stanisak M, Gelhaus C et al (2009) Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan Hydra. J Biol Chem 284:1896–1905

Koenig E, Bininda-Emonds OR (2011) Evidence for convergent evolution in the antimicrobial peptide system in anuran amphibians. Peptides 32:20–25

Kokryakov VN, Harwig SS, Panyutich EA, Shevchenko AA, Aleshina GM, Shamova OV et al (1993) Protegrins: leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins. FEBS Lett 327:231–236

Krause A, Sillard R, Kleemeier B, Kluver E, Maronde E, Conejo-Garcia JR et al (2003) Isolation and biochemical characterization of LEAP- 2, a novel blood peptide expressed in the liver. Protein Sci 12:143–152

Krensky AM (2000) Granulysin: a novel antimicrobial peptide of cytolytic T lymphocytes and natural killer cells. Biochem Pharmacol 59:317–320

Lambert J, Keppi E, Dimarcq JL, Wicker C, Reichhart JM, Dunbar B et al (1989) Insect immunity: isolation from immune blood of the dipteran Phormia terranovae of two insect antibacterial peptides with sequence homology to rabbit lung macrophage bactericidal peptides. Proc Natl Acad Sci USA 86:262–266

Lee IH, Lee YS, Kim CH, Kim CR, Hong T, Menzel L et al (2001) Dicynthaurin: an antimicrobial peptide from hemocytes of the solitary tunicate, *Halocynthia aurantium*. Biochim Biophys Acta 1527:141–148

Leippe M, Herbst R (2004) Ancient weapons for attack and defense: the pore-forming polypeptides of pathogenic enteric and free-living amoeboid protozoa. J Eukaryot Microbiol 51:516–521

Leonova L, Kokryakov VN, Aleshina G, Hong T, Nguyen T, Zhao C et al (2001) Circular minidefensins and posttranslational generation of molecular diversity. J Leukoc Biol 70:461–464

Li J, Zhang C, Xu X, Wang J, Yu H, Lai R et al (2007) Trypsin inhibitory loop is an excellent lead structure to design serine protease inhibitors and antimicrobial peptides. FASEB J 21:2466–2473

Linzmeier RM, Ganz T (2005) Human defensin gene copy number polymorphisms: comprehensive analysis of independent variation in alpha- and beta-defensin regions at 8p 2 2-p 23. Genomics 86:23–430

Linzmeier R, Ho CH, Hoang BV, Ganz T (1999) A 450-kb contig of defensin genes on human chromosome 8p 23. Gene 233:205–211

Lynn DJ, Bradley DG (2007) Discovery of alpha-defensins in basal mammals. Dev Comp Immunol 31:963–967

Matsuzaki K, Nakayama M, Fukui M, Otaka A, Funakoshi S, Fujii N et al (1993) Role of disulfide linkages in tachyplesin-lipid interactions. Biochemistry 32:11704–11710

Mitta G, Vandenbulcke F, Hubert F, Roch P (1999) Mussel defensins are synthesised and processed in granulocytes then released into the plasma after bacterial challenge. J Cell Sci 112:4233–4242

Miyakawa Y, Ratnakar P, Rao AG, Costello ML, Mathieu-Costello O, Lehrer RI et al (1996) In vitro activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against Mycobacterium tuberculosis. Infect Immun 64:926–932

Miyata T, Tokunaga F, Yoneya T, Yoshikawa K, Iwanaga S, Niwa M et al (1989) Antimicrobial peptides, isolated from horseshoe crab hemocytes, tachyplesin II, and polyphemusins I and II: chemical structures and biological activity. J Biochem 106:663–668

Nakamura T, Furunaka H, Miyata T, Tokunaga F, Muta T, Iwanaga S et al (1988) Tachyplesin, a class of antimicrobial peptide from the hemocytes of the horseshoe crab (*Tachypleus tridentatus*). Isolation and chemical structure. J Biol Chem 263:16709–16713

Naknukool S, Hayakawa S, Sun Y, Ogawa M (2008) Structural and physicochemical characteristics of novel basic proteins isolated from duck egg white. Biosci Biotechnol Biochem 72:2082–2091

Nguyen TX, Cole AM, Lehrer RI (2003) Evolution of primate theta-defensins: a serpentine path to a sweet tooth. Peptides 24:1647–1654

Odani S, Koide T, Ono T, Takahashi Y, Suzuki J (1989) Covalent structure of a low-molecular-mass protein, meleagrin, present in a turkey (*Meleagris gallopavo*) ovomucoid preparation. J Biochem 105:660–663

Ogata K, Linzer BA, Zuberi RI, Ganz T, Lehrer RI, Catanzaro A (1992) Activity of defensins from human neutrophilic granulocytes against *Mycobacterium avium-Mycobacterium intracellulare*. Infect Immun 60:4720–4725

Ohta M, Ito H, Masuda K, Tanaka S, Arakawa Y, Wacharotayankun R et al (1992) Mechanisms of antibacterial action of tachyplesins and polyphemusins, a group of antimicrobial peptides isolated from horseshoe crab hemocytes. Antimicrob Agents Chemother 36:1460–1465

Orth D, Ehrlenbach S, Brockmeyer J, Khan AB, Huber G, Karch H et al (2010) EspP, a serine protease of enterohemorrhagic Escherichia coli, impairs complement activation by cleaving complement factors C3/C3b and C5. Infect Immun 78(4):294–4301

Ovchinnikova TV, Aleshina GM, Balandin SV, Krasnosdembskaya AD, Markelov ML, Frolova EI et al (2004) Purification and primary structure of two isoforms of arenicin, a novel antimicrobial peptide from marine polychaeta Arenicola marina. FEBS Lett 577:209–214

Ovchinnikova TV, Shenkarev ZO, Nadezhdin KD, Balandin SV, Zhmak MN, Kudelina IA et al (2007) Recombinant expression, synthesis, purification, and solution structure of arenicin. Biochem Biophys Res Commun 360:156–162

Pellegrini A, Hülsmeier AJ, Hunziker P, Thomas U (2004) Proteolytic fragments of ovalbumin display antimicrobial activity. Biochim Biophys Acta 1672:76–85

Raimondo D, Andreotti G, Saint N, Amodeo P, Renzone G, Sanseverino M et al (2005) A folding-dependent mechanism of antimicrobial peptide resistance to degradation unveiled by solution structure of distinctin. Proc Natl Acad Sci USA 102:6309–6314

Rinaldi AC (2002) Antimicrobial peptides from amphibian skin: an expanding scenario. Curr Opin Chem Biol 6:799–804

Ritonja A, Kopitar M, Jerala R, Turk V (1989) Primary structure of a new cysteine proteinase inhibitor from pig leucocytes. FEBS Lett 255:211–214

Romeo D, Skerlavaj B, Bolognesi M, Gennaro R (1988) Structure and bactericidal activity of an antibiotic dodecapeptide purified from bovine neutrophils. J Biol Chem 263:9573–9575

Rorman EG, Scheinker V, Grabowski GA (1992) Structure and evolution of the human prosaposin chromosomal gene. Genomics 13:312–318

Rosa RD, Santini A, Fievet J, Bulet P, Destoumieux-Garzon D, Bachere E (2011) Big defensins, a diverse family of antimicrobial peptides that follows different patterns of expression in hemocytes of the oyster *Crassostrea gigas*. PLoS One 6:e25594

Roskill MW (1989) The attribution of paintings: some case histories. In: What is art history?, 2nd edn. University of Massachusetts Press, p 19

Saito T, Kawabata S, Shigenaga T, Takayenoki Y, Cho J, Nakajima H et al (1995) A novel big defensin identified in horseshoe crab hemocytes: isolation, amino acid sequence, and antibacterial activity. J Biochem 117:1131–1137

Sang Y, Blecha F (2009) Porcine host defense peptides: expanding repertoire and functions. Dev Comp Immunol 33:334–343

Schade AL, Caroline L (1946) An iron-binding component in human blood plasma. Science 104:340–341

Scheetz T, Bartlett JA, Walters JD, Schutte BC, Casavant TL, McCray PB Jr (2002) Genomics-based approaches to gene discovery in innate immunity. Immunol Rev 190:137–145

Scocchi M, Wang S, Zanetti M (1997) Structural organization of the bovine cathelicidin gene family and identification of a novel member. FEBS Lett 417:311–315

Scott A, Weldon S, Taggart CC (2011) SLPI and elafin: multifunctional antiproteases of the WFDC family. Biochem Soc Trans 39:1437–1440

Selsted ME, Harwig SS, Ganz T, Schilling JW, Lehrer RI (1985) Primary structures of three human neutrophil defensins. J Clin Invest 76:1436–1439

Selsted ME, Tang YQ, Morris WL, McGuire PA, Novotny MJ, Smith W et al (1993) Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J Biol Chem 268:6641–6648

Selvaraj P (2011) Vitamin D, vitamin D receptor, and cathelicidin in the treatment of tuberculosis. Vitam Horm 86:307–325

Silva PI Jr, Daffre S, Bulet P (2000) Isolation and characterization of gomesin, an 18-residue cysteine-rich defense peptide from the spider Acanthoscurria gomesiana hemocytes with sequence similarities to horseshoe crab antimicrobial peptides of the tachyplesin family. J Biol Chem 275:33464–33470

Simpson RJ, Morgan FJ (1983) Isolation and complete amino acid sequence of a basic low molecular weight protein from black swan egg white. Int J Pept Protein Res 22:476–481

Solis-Cohen S (1901) The true role of drugs in the management of consumptives, 36 edn, pp 482–486

Sonawane A, Santos JC, Mishra BB, Jena P, Progida C, Sorensen OE et al (2011) Cathelicidin is involved in the intracellular killing of mycobacteria in macrophages. Cell Microbiol 13:1601–1617

Stegemann C, Tsvetkova EV, Aleshina GM, Lehrer RI, Kokryakov VN, Hoffmann R (2010) De novo sequencing of two new cyclic theta-defensins from baboon (*Papio hamadryas*) leukocytes by matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 24:599–604

Steinberg DA, Hurst MA, Fujii CA, Kung AH, Ho JF, Cheng FC et al (1997) Protegrin-1: a broad-spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob Agents Chemother 41:1738–1742

Stensvag K, Haug T, Sperstad SV, Rekdal O, Indrevoll B, Styrvold OB (2008) Arasin 1, a proline-arginine-rich antimicrobial peptide isolated from the spider crab, Hyas araneus. Dev Comp Immunol 32:275–285

Storici P, Del SG, Schneider C, Zanetti M (1992) cDNA sequence analysis of an antibiotic dodecapeptide from neutrophils. FEBS Lett 314:187–190

Tamamura H, Ikoma R, Niwa M, Funakoshi S, Murakami T, Fujii N (1993) Antimicrobial activity and conformation of tachyplesin I and its analogs. Chem Pharm Bull (Tokyo) 41:978–980

Tang YQ, Yuan J, Osapay G, Osapay K, Tran D, Miller CJ et al (1999) A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science 286(498):502

Teng L, Gao B, Zhang S (2012) The first chordate big defensin: Identification, expression and bioactivity. Fish Shellfish Immunol 32:572–577

Thomma BP, Cammue BP, Thevissen K (2002) Plant defensins. Planta 216:193–202

Tomasinsig L, Zanetti M (2005) The cathelicidins–structure, function and evolution. Curr Protein Pept Sci 6:23–34

Tomee JF, Koeter GH, Hiemstra PS, Kauffman HF (1998) Secretory leukoprotease inhibitor: a native antimicrobial protein presenting a new therapeutic option? Thorax 53:114–116

Tomita M, Takase M, Bellamy W, Shimamura S (1994) A review: the active peptide of lactoferrin. Acta Paediatr Jpn 36:585–591

Tongaonkar P, Tran P, Roberts K, Schaal J, Osapay G, Tran D et al (2011) Rhesus macaque theta-defensin isoforms: expression, antimicrobial activities, and demonstration of a prominent role in neutrophil granule microbicidal activities. J Leukoc Biol 89:283–290

Tran D, Tran PA, Tang YQ, Yuan J, Cole T, Selsted ME (2002) Homodimeric theta-defensins from rhesus macaque leukocytes: isolation, synthesis, antimicrobial activities, and bacterial binding properties of the cyclic peptides. J Biol Chem 277:3079–3084

van Dijk A, Veldhuizen EJ, Haagsman HP (2008) Avian defensins. Vet Immunol Immunopathol 124:1–18

Venkataraman N, Cole AL, Ruchala P, Waring AJ, Lehrer RI, Stuchlik O et al (2009) Reawakening retrocyclins: ancestral human defensins active against HIV-1. PLoS Biol 7:e95

Vouille V, Amiche M, Nicolas P (1997) Structure of genes for dermaseptins B, antimicrobial peptides from frog skin Exon 1-encoded prepropeptide is conserved in genes for peptides of highly different structures and activities. FEBS Lett 414:27–32

Wang W, Cole AM, Hong T, Waring AJ, Lehrer RI (2003) Retrocyclin, an antiretroviral theta-defensin, is a lectin. J Immunol 170:4708–4716

Welkos S, Cote CK, Hahn U, Shastak O, Jedermann J, Bozue J et al (2011) Humanized theta-defensins (retrocyclins) enhance macrophage performance and protect mice from experimental anthrax infections. Antimicrob Agents Chemother 55:4238–4250

Williams CJB (1849) On the use and administration of cod-liver oil in pulmonary consumption. Lond J Med 1–18

Williams SE, Brown TI, Roghanian A, Sallenave JM (2006) SLPI and elafin: one glove, many fingers. Clin Sci (Lond) 110:21–35

Wong JH, Xia L, Ng TB (2007) A review of defensins of diverse origins. Curr Protein Pept Sci 8:446–459

Xiao Y, Hughes AL, Ando J, Matsuda Y, Cheng JF, Skinner-Noble D et al (2004) A genome-wide screen identifies a single beta-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. BMC Genomics 5:56

Yan X, Liu H, Yang X, Che Q, Liu R, Yang H et al (2011) Bi-functional peptides with both trypsin-inhibitory and antimicrobial activities are frequent defensive molecules in Ranidae amphibian skins. Amino Acids 43(1):309–16, PMID:21927839

Yang YS, Mitta G, Chavanieu A, Calas B, Sanchez JF, Roch P et al (2000) Solution structure and activity of the synthetic four-disulfide bond Mediterranean mussel defensin (MGD-1). Biochemistry 39:14436–14447

Zanetti M (2004) Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol 75:39–48

Zanetti M (2005) The role of cathelicidins in the innate host defenses of mammals. Curr Issues Mol Biol 7:179–196

Zanetti M, Litteri L, Gennaro R, Horstmann H, Romeo D (1990) Bactenecins, defense polypeptides of bovine neutrophils, are generated from precursor molecules stored in the large granules. J Cell Biol 111:1363–1371

Zanetti M, Gennaro R, Romeo D (1995) Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett 374:1–5

Zhai Y, Saier MH Jr (2000) The amoebapore superfamily. Biochim Biophys Acta 1469:87–99

Zhang H, Kato Y (2003) Common structural properties specifically found in the CSalphabeta-type antimicrobial peptides in nematodes and molluscs: evidence for the same evolutionary origin? Dev Comp Immunol 27:499–503

Zhao C, Ganz T, Lehrer RI (1995) The structure of porcine protegrin genes. FEBS Lett 368:197–202

Zhu S (2007) Evidence for myxobacterial origin of eukaryotic defensins. Immunogenetics 59:949–954

Zhu S (2008a) Did cathelicidins, a family of multifunctional host-defense peptides, arise from a cysteine protease inhibitor? Trends Microbiol 16:353–360

Zhu S (2008b) Discovery of six families of fungal defensin-like peptides provides insights into origin and evolution of the CSalphabeta defensins. Mol Immunol 45:828–838

Zhu S (2008c) Positive selection targeting the cathelin-like domain of the antimicrobial cathelicidin family. Cell Mol Life Sci 65:1285–1294

Zhu S, Gao B (2009) A fossil antibacterial peptide gives clues to structural diversity of cathelicidin-derived host defense peptides. FASEB J 23:13–20

Zhu S, Wei L, Yamasaki K, Gallo RL (2008) Activation of cathepsin L by the cathelin-like domain of protegrin-3. Mol Immunol 45:2531–2536

Zou J, Mercier C, Koussounadis A, Secombes C (2007) Discovery of multiple beta-defensin like homologues in teleost fish. Mol Immunol 44:638–647