Molecular Immunology 157 (2023) 53–69

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Evolutionary diversification of defensins and cathelicidins in birds and primates

Albert van Dijk [a,*], Rodrigo Guabiraba [b], Geoffrey Bailleul [b], Catherine Schouler [b], Henk P. Haagsman [a], Anne-Christine Lalmanach [b,*]

[a] Department Biomolecular Health Sciences, Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
[b] INRAE, Université de Tours, ISP, F-37380, Nouzilly, France

ARTICLE INFO

Keywords:
Avian
Primate
Defensins
Cathelicidins
Evolution

ABSTRACT

Divergent evolution for more than 310 million years has resulted in an avian immune system that is complex and more compact than that of primates, sharing much of its structure and functions. Not surprisingly, well conserved ancient host defense molecules, such as defensins and cathelicidins, have diversified over time. In this review, we describe how evolution influenced the host defense peptides repertoire, its distribution, and the relationship between structure and biological functions. Marked features of primate and avian HDPs are linked to species-specific characteristics, biological requirements, and environmental challenge.

1. Introduction

As a first line of defense, host defense peptides (HDPs) are intimately linked to maintenance of homeostasis. Yet, the number of functional defensins and cathelicidin genes vary considerably among the genomes of different animal species. Although this may be explained in part by differences in genome size, it also suggests that adaptation of organisms to different environments may have favored evolution of HDP members towards biological functions that help maintain this homeostasis. Here we compare the evolutionary distant clades of birds and primates. Birds possess a more condensed defensin repertoire then primates and despite of their smaller genome possess multiple members of the cathelicidin family in contrast to a single member in primates. These differences may contribute to host specificity of zoonotic bacteria such as *Salmonella* and *Campylobacter* spp. that are essentially intestinal commensals in birds but act as intestinal pathogens in primates.

The divergence between birds and mammals occurred at the Carboniferous period, around 310 million years ago (mya) (Hedges and Kumar, 2004). After the Cretaceous–Paleogene (K–Pg) mass extinction event approx. 66 mya, avian lineages evolved rapidly (Prum et al., 2015), while the evolution of primates occurred more gradually (Fig. 1). Despite their rapid evolutionary radiation, avian genomes are surprisingly well conserved when considering chromosomal organization, gene synteny and nucleotide sequences. Unique among vertebrates is the distinctly conserved large number of microchromosomes in the avian karyotype in addition to a haploid number of around 40 macrochromosomes (Griffin et al., 2007). In fact, about 7.5% of avian genomes are comprised of evolutionary constrained gene elements. Convergent evolution of protein-coding genes and their regulatory elements not only appears to have shaped similar behavioral and morphological traits in distantly related birds, but also the variation in specific gene families associated with avian traits and ecological adaptation (Zhang et al., 2014). The relatively higher recombination rate in birds has led to shorter introns and intergenic spaces and increased gene density (Nam and Ellegren, 2012). Due to genomic contraction, birds have the smallest genomes among amniotes, an adaptation shared with bats and probably associated with the rapid gene regulation necessary for flight (Zhang et al., 2014). Genome nuclear mass correlates with cell size in vertebrates, and the relatively small avian cells do have a larger surface to volume ratio which is advantageous for gas exchange at the high metabolic rate associated with flight (Hughes and Friedman, 2008). This raises questions about the consequences of this evolutionary path for the innate immune system of birds. Comparison of gene family sizes in chicken and five mammalian genomes revealed a massive loss of immune gene paralogs in the chicken genome, such as MHC and immunoglobulins, that had already begun in ancestral archosaurs (Hughes and Friedman, 2008). It stands to reason that during development into a more compact immune system over time a loss of immune-related genes must be compensated by diversification of the remaining immune genes and is driven by biological function. Here we examine how evolution

* Correspondence to: Department Biomolecular Health Sciences, Division Infectious Diseases and Immunology, the Netherlands.
E-mail addresses: a.vandijk1@uu.nl (A. van Dijk), anne-christine.lalmanach@inrae.fr (A.-C. Lalmanach).

https://doi.org/10.1016/j.molimm.2023.03.011
Received 26 October 2022; Received in revised form 9 March 2023; Accepted 14 March 2023
Available online 28 March 2023

impacted on the repertoire and biological functions of avian and primate host defense peptides.

As a first line of molecular defense against pathogens, the innate immune system encompasses a repertoire of peptides with antimicrobial and immunomodulatory activities including cathelicidins, histatins and defensins. Defensins and cathelicidins constitute the largest families of host defense peptides and are present at mucosal barriers between host and environment. The amphipathic and cationic properties of these 2–6 kDa peptides may confer broad-spectrum antimicrobial activity against Gram+ and Gram- bacteria, and against some fungi and enveloped viruses. The α-, β- and θ-defensin subfamilies are defined by the connectivity of the 3 intramolecular disulfide bridges (Ganz, 2003). Whereas β-defensins are ubiquitous in the animal kingdom, α- and θ-defensins are absent in birds and restricted, respectively, to mammals and primates. Characteristic for cathelicidins is the highly conserved propiece that contains a cathelin-like domain and is linked to a variable C-terminal bioactive peptide, usually less than 40 amino acid residues (Zanetti, 2005). Cathelicidins are present in mammals (Gudmundsson et al., 1996), reptiles (Wang et al., 2008), amphibians (Wei et al., 2013), birds (Lynn et al., 2004) and fish (Uzzell et al., 2003).

The aim of this review is thus to discuss the repertoire, tissue distribution, structures and biological functions of defensins and cathelicidins in these distantly related clades of birds and primates with an emphasis on common features and clade specificities.

2. Discovery of HDP genes and proteins

The repertoire of avian β-defensins (Hellgren and Ekblom, 2010; Ishige et al., 2016), including egg-specific defensin-like proteins (ovodefensins) (Zhang et al., 2019), comprises more than 30 members. Around 30 members of β-defensins have been identified in primates (Tu et al., 2015), with the first human β-defensin (HBD1) discovered in plasma more than twenty years ago (Bensch et al., 1995). Avian β-defensins (AvBDs) are composed of 36 to more than 70 amino acids, whereas primate β-defensins are composed of 35 to more than 100 amino acids both with the characteristic β-defensin cysteines connectivity (1–5, 2–4 and 3–6) providing stabilization of the peptide structure. Primate β-defensins such as human HBD1 to HBD4 were found to be present in many cell types including epithelial cells and leukocytes (Fang et al., 2003; Harder et al., 2004), with a marked conservation of sequence homology and chromosome localization in primates ranging from humans to New World Monkeys (Ventura et al., 2004). Avian defensins were first identified in heterophils, the equivalents of mammalian neutrophils in birds (Evans et al., 1994; Harwig et al., 1994), i.e. AvBD1 and AvBD2 in chicken (“gallinacins”) and Turkey Heterophil Peptides (THP) in turkey. Twelve additional avian defensins were discovered (Lynn et al., 2004; Xiao et al., 2004) upon the release of the chicken genome sequence in 2004 (Consortium, 2004) and resulted in an updated “avian β-defensin” (AvBD) based nomenclature that replaced the erroneous “gallinacin/GAL” system (Lynn et al., 2007). Defensin-like peptides identified in the hen egg white and not part of the defensin gene cluster were designated as ovodefensins (Gong et al., 2010).

Much more variable is the repertoire of cathelicidins (CATH) with 11 genes in pigs (Baumann et al., 2014) and a single gene in humans (Gudmundsson et al., 1996), non-human primates and rodents (Gallo et al., 1997; Nagaoka et al., 1997; Termen et al., 2003). Up to four different cathelicidin genes have been found in birds, with data-mining for cathelicidin-related structures in 55 avian genomes resulting in 52 identified cathelicidin genes divided over 21 species (Cheng et al., 2015; Yu et al., 2015). Mature avian cathelicidin peptides range from 23 to 42 amino acids. LL-37, the single human cathelicidin and its orthologs in non-human primates consist of 37 amino acid residues (Cheng et al., 2015; Gudmundsson et al., 1996).

3. Gene level

β-defensins are thought to originate at least 520 mya from an ancestral big defensin gene similar to the big defensin genes found in cephalochordates, molluscs and arthropods (Zhu and Gao, 2013). Fourteen avian β-defensin genes are located in a unique cluster on chicken chromosome 3 (Xiao et al., 2004). The genomic comparison of the β-defensin genes cluster of the chicken and of the zebra finch has shed light on gene duplication events that took place after the Galliformes-Passeriformes split 66 mya, increasing the repertoire from 14 in Galliformes to 22 in Passeriformes (Hellgren and Ekblom, 2010). Sixteen β-defensin genes were discovered in the duck genome (Huang et al., 2013). Nine avian β-defensin genes are well conserved, while others (AvBD1, AvBD3, AvBD7 and AvBD14) are probably the result of gene duplication or pseudogenisation events in specific avian lineages (Cheng et al., 2015). Genome analysis of the Japanese quail revealed that these birds lack the AvBD3 and − 7 loci, whereas the AvBD101α,

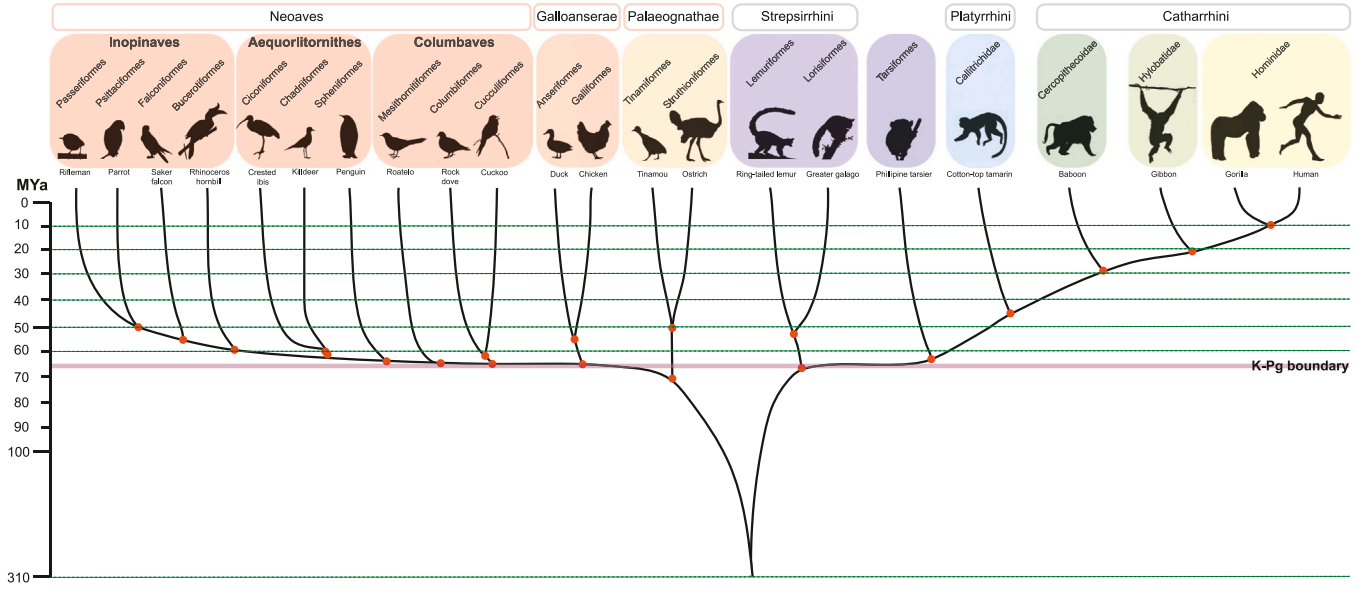

Fig. 1. Evolutionary tree of avian and primate evolution.
Adapted from Prum RO et al. (Prum et al., 2015) and Springer MS et al. (Springer et al., 2012).

– 101β, and – 101θ loci arose from gene duplication of the AvBD6 ortholog locus in the AvBD cluster after *Coturnix – Gallus* speciation (Ishige et al., 2016). A peculiar gene in birds is AvBD11, which consists of a long sequence encoding two successive β-defensin motifs (Herve--Grepinet et al., 2010). Recent genetic analysis of five defensin genes within waterfowl revealed a surprisingly low diversity at the individual-, population- and species-level with maintained alleles under purifying selection, which likely represents the optimal available evolutionary solution to maintain functional antimicrobial efficacy (Chapman et al., 2016). This study also revealed a balancing selection acting in AvBD3b, the most recently duplicated β-defensin gene, and shows critical amino acid changes that may enlarge the AvBD3b functional repertoire. This is in line with the functional allelic variation observed in the AvBD7 gene which occurs at high frequency in natural populations of the great tit (Passeriformes) (Hellgren et al., 2010).

Phylogenetic analysis of primate and avian β-defensin genes reveals a clear separation of primate and avian clades with clustering of orthologs within the primate and avian clades (Fig. 2). Despite the few number of species used in the analysis, avian defensins orthologs of AVBD1, AvBD2, AvBD3, AvBD4, AVBD5, AvBD6/7, AvBD8, AVBD9, AVBD10, AVBD11, AVBD13 in Galloansera and Neonaves were seen to cluster together, with exception of emperor penguin AVBD3. In addition, none of the (chicken, mallard and quail) AvBD14 orthologs clustered together, which is in accordance with AvBD3 and AVBD14 orthologs being less conserved in birds. As expected Galloansera ovodefensins clustered separately of other avian defensins.

Compared to β-defensin genes in primates and mammals in general that consist of two exons, avian β-defensin genes are composed of four exons and three short introns. Thus, in mammals, two exon fusions probably occurred. Human genome analysis has revealed the existence of four β-defensin clusters on three different chromosomes (6p21, 8p23, 20q11, 20p13) encoding more than 30 different β-defensins genes (Schutte et al., 2002). Homologous regions to the primate β-defensin genes on chromosome 8p23 were mapped in non-human primates (Ventura et al., 2004). Both primate and avian β-defensins are under the influence of positive selection. The human 8p22-p23 locus evolved

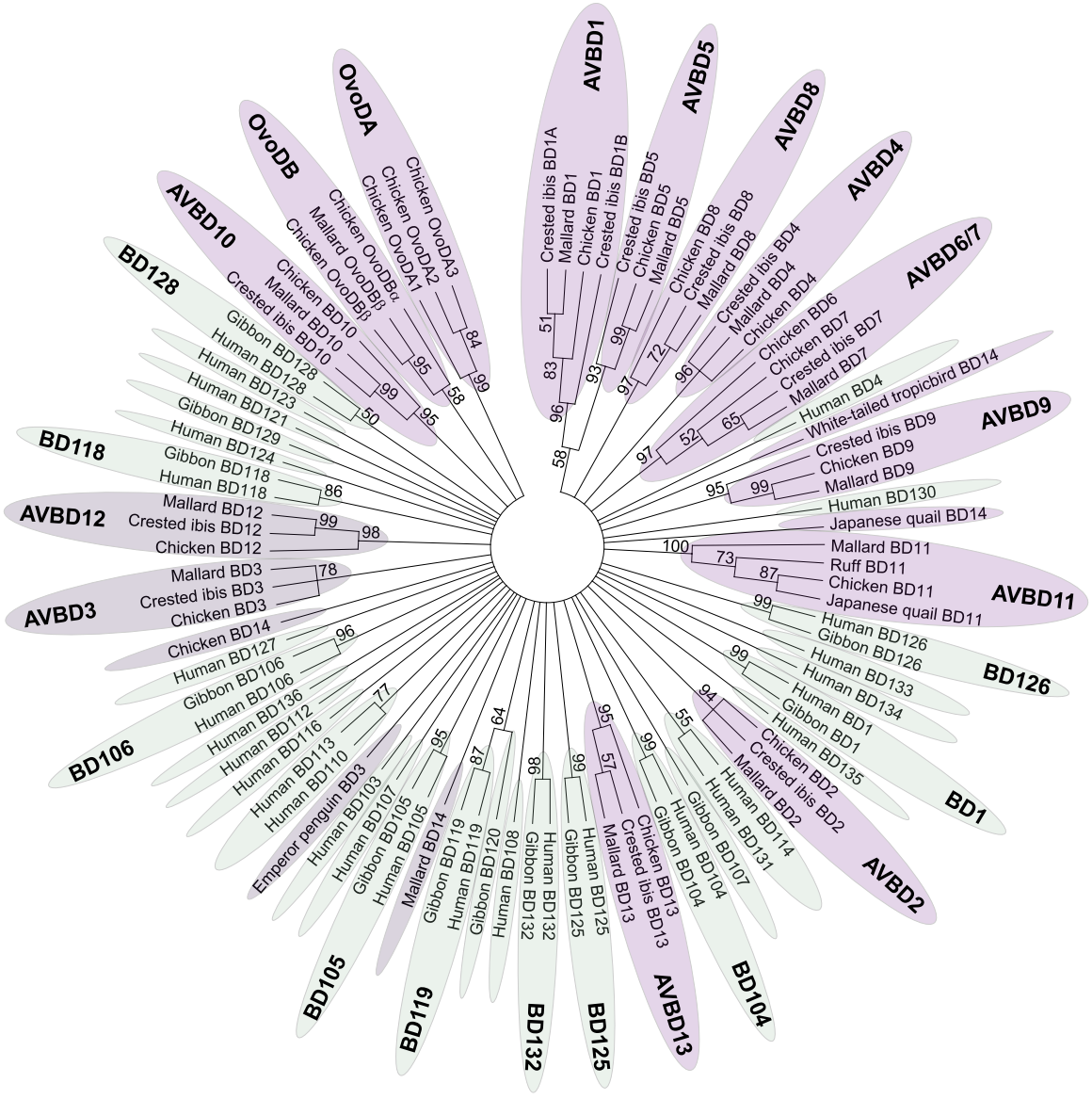

Fig. 2. Phylogenetic analyses of primate and avian β-defensin amino acid sequences using MEGA11 software version 11.03.13. Sequences were obtained from UNIPROT (https://www.uniprot.org/) and NCBI genome databases (https://www.ncbi.nlm.nih.gov/) and aligned using MUSCLE. Phylogenetic trees were constructed using Maximum Likelihood with the Jones-Taylor-Thornton (JTT) model of amino acid substitution (JTT + G + I). Nodal support of the unrooted trees was assessed by bootstrapping with 500 replicates. AVBD: avian β-defensin, OvoBD/OvoDA: ovodefensins, BD: primate β-defensin.

before the human-baboon divergence 23 mya, from a cluster of β-defensin paralogues by positive selection of the second exon, driving mature peptide charge alterations and divergence (Semple et al., 2003). Evidence suggests that after the human-macaque divergence 25mya, positive selection occurred in the macaque DEFB2L gene 3–9.5 mya and mostly in the exon encoding the mature peptide (Ottolini et al., 2014). In birds, weak positive selection sites were found in the mature peptide domains of AvBD9, AvBD11 and AvBD13. In contrast, for AvBD5, AvBD13 and AvBD14 more positive selection sites were found in the signal and propiece region. The apparent absence of strong positive selection among AvBDs suggests that positive selection probably occurred before the rapid radiation of the avian lineage (Cheng et al., 2015). It is probable that the environmental infectious pressure has contributed to the diversification of this family of genes in primates. If some coding sequences contain premature stop codons thus representing likely pseudogenes, the high frequency of gene duplication within the β-defensin gene cluster has however contributed to increase the repertoire of these antimicrobial molecules in Homo sapiens. It is noteworthy that primate genomes contain two loci on the same chromosome 20 encoding defensins related to the reproductive tract function (Radhakrishnan et al., 2005; Rodriguez-Jimenez et al., 2003). Similarly, chicken chromosome 3 contains, in addition to the locus of AvBD genes, another locus encoding ovodefensins (OvoDs) with OvoDA1, OvoDA2, OvoDA3, OvoDBα (Gong et al., 2010; Whenham et al., 2015) and an additional member recently identified named OvoDBβ (Yu et al., 2018). The repertoire of ovodefensin genes appears to vary between bird species (Zhang et al., 2019).

The polymorphism in human β-defensin genes has been related to the susceptibility to inflammatory and/or infectious diseases. This polymorphism can rely either on single nucleotide variation (SNPs), with emphasis in HBD1 gene (Jurevic et al., 2003; Kocsis et al., 2009; Tesse et al., 2008), or on gene copy number variation (CNV) (Hollox et al., 2008; Jones et al., 2014). Similarly, SNPs in chicken defensin genes have been associated to the susceptibility of birds to intestinal colonization by *Salmonella* Enteritidis (Hasenstein and Lamont, 2007), or to the host response to this bacteria (Hasenstein et al., 2006). CNV has also been recently identified for AvBD7 (Lee et al., 2016a), without effect on the gene transcription level in birds.

Cathelicidin-like genes have been found in bony fish and jawless fish suggesting that avian and primate cathelicidins may originate from a common ancestral gene existing before the split between cyclostomes and gnathostomes around 500 mya (Chang et al., 2006; Miyashita et al., 2019; Uzzell et al., 2003). The strong similarity of the cathelin-like domain to cystatins, a family of cysteine proteinases inhibitors, suggests a common ancestor for cathelicidins and cystatins (Zhu, 2008).

The evolutionary dynamics of avian cathelicidin genes have recently been reviewed by Cheng et al. (Cheng et al., 2015). A survey of 53 avian genomes revealed 44 cathelicidin genes in only 21 of the species. However, due to the low assembly quality of the avian genomes more cathelicidin genes may be expected. Initially, four cathelicidins were identified in chicken and designated CATH-1, − 2, − 3 and -B1 (Goitsuka et al., 2007; Lynn et al., 2004; van Dijk et al., 2005; Xiao et al., 2006a), which can be clustered in three clades: CATH-1/3, CATH-2 and CATH-B1 (Cheng et al., 2015). Homologues of the CATH-2 and CATH-B1 clades are most conserved across the examined avian orders. CATH-3 orthologs are present in fowl (Galliformes), gulls (Charadriformes), mesites (Mesitornithiformes), doves (Columbiformes), parrots (Psittaciformes), cuckoos (Cucculiformes), kingfishers (Coraciiformes) and as pseudogene in hawks (Falconiformes) orders, and due to CATH-3 gene duplication an additional CATH-1 gene is present in Galliformes (Cheng et al., 2015; Yu et al., 2015). CATH genes are ordered as CATH2, CATH3, CATH-B1 in most avian species and flanked by kelch-like family member 18 (KLHL18) and transforming growth β regulator 4 (TBRG4) (Cheng et al., 2015; Halper et al., 2004) and are mapped to chromosome 2p (Xiao et al., 2006a). The four avian cathelicidins share a similar genomic organization, consisting of four exons of which exons 1–3 encode the signal peptide and cathelin-like domain and exon 4 the mature peptide. Phylogenetic analysis of avian cathelicidin genes suggests that CATH-B1 predates CATH-2 and CATH-1/3 (Yu et al., 2015). Based on sequence similarity and close proximity to the KLHL18 gene, avian cathelicidins are most related to the mammalian neutrophilic granule proteins (NGPs) implying that 'classical' mammalian cathelicidins such as hCAP18/LL-37 came into existence after the split of mammalian and avian lineages (Xiao et al., 2006a).

By contrast, a single cathelicidin (CAMP) gene is present in all primates and encodes a remarkably conserved mature peptide (Zhu and Gao, 2017). Primate CAMP genes consist of four exons. Exons 1–3 encode the signal peptide and cathelin domain while exon 4 encodes the mature peptide and the locus has been mapped to human chromosome 3p21.3 (Gudmundsson et al., 1996, 1995) closely adjacent to KHLH18, as seen for avian cathelicidins (Xiao et al., 2006a). The human CAMP gene is present as a single copy per haploid genome and, in contrast to human β-defensin genes, does not exhibit copy number polymorphisms (Tecle et al., 2010). Single nucleotide polymorphisms (SNPs) in the CAMP gene have been reported, which was not associated with tuberculosis prevalence (Lopez Campos et al., 2014). Primate CAMP genes are subject to positive selection in the domains encoding the pro-piece and the mature peptide (Zelezetsky et al., 2006; Zhu and Gao, 2017). In contrast, avian cathelicidin genes have some positive selection sites in the signal peptide and cathelin domains, but completely lack these in the mature peptide domain (Cheng et al., 2015). It has been postulated that positive selection during evolution may also have served to maintain a balanced charge between the cationic mature peptide and anionic propiece (Cheng et al., 2015). For primate CAMP genes, positive selection seems not related to the microbicidal activities but associated with the modulatory functions towards immune cells, suggesting co-evolution of HDPs and endogenous receptors (Zhu and Gao, 2017). Phylogenetic analysis of primate and avian cathelicidin genes (Fig. 3) shows a separation between and within primate and avian clades that corresponds to their evolutionary diversification (Fig. 1). Within the rapidly diversified avian species, CATH-B1, CATH-1/3 and CATH-2 orthologs cluster for Galloansera separately from Neonaves. Primate CAP18 orthologs clustered in 6 distinct clades: Strepsirrhini (wet-nosed), Tarsiformes, Platyrhini (flat-nosed; New World monkeys) and Catharhini (down-nosed), which is subdivided in clades for Cercopithecidae (Old world monkeys), Hylobatidae (gibbons), and Hominidae (great apes).

4. Protein sequence level

Protein sequence alignment of representative primate and avian β-defensins (Supplementary Figure 1) reveals a high sequence variability in the propiece and mature peptide with few conserved residues (highlighted in blue) preceded by a highly conserved signal peptide (residues 5–24 in the consensus sequence). All mature avian and primate peptides share six strictly conserved cysteine residues (positions 58, 68, 74, 88, 95 and 96 of the consensus sequence), the hallmark of β-defensins, the pairing of which ensures compaction and structural stability (Fig. 2). A shared feature is the conserved glycine residue at position 66, in close proximity to the second cysteine residue that, due to its small side chain and minute steric hindrance, is involved in a β-bulge thought to be responsible for a twist in the β-sheet. This bulge may ensure proper folding as well as flexibility (Derache et al., 2012; Xie et al., 2005). The relatively well conserved glycine residue in proximity of the fourth cysteine residue may be involved in the appropriate folding of the peptide. A characteristic richness in cationic residues (mainly Lys and Arg), found in all β-defensins, is related to their antimicrobial activity by interacting with negatively charged surfaces in bacterial membranes. Some primate β-defensins have an extended C-terminal tail, which in the case of BD126 is highly glycosylated (Liu et al., 2013). Similarly, BD118 is equipped with an extended C-terminal tail containing multiple putative glycosylation sites and, like BD126, is epididymis-associated and involved in sperm motility (Tollner et al., 2011, 2008; Yenugu et al.,

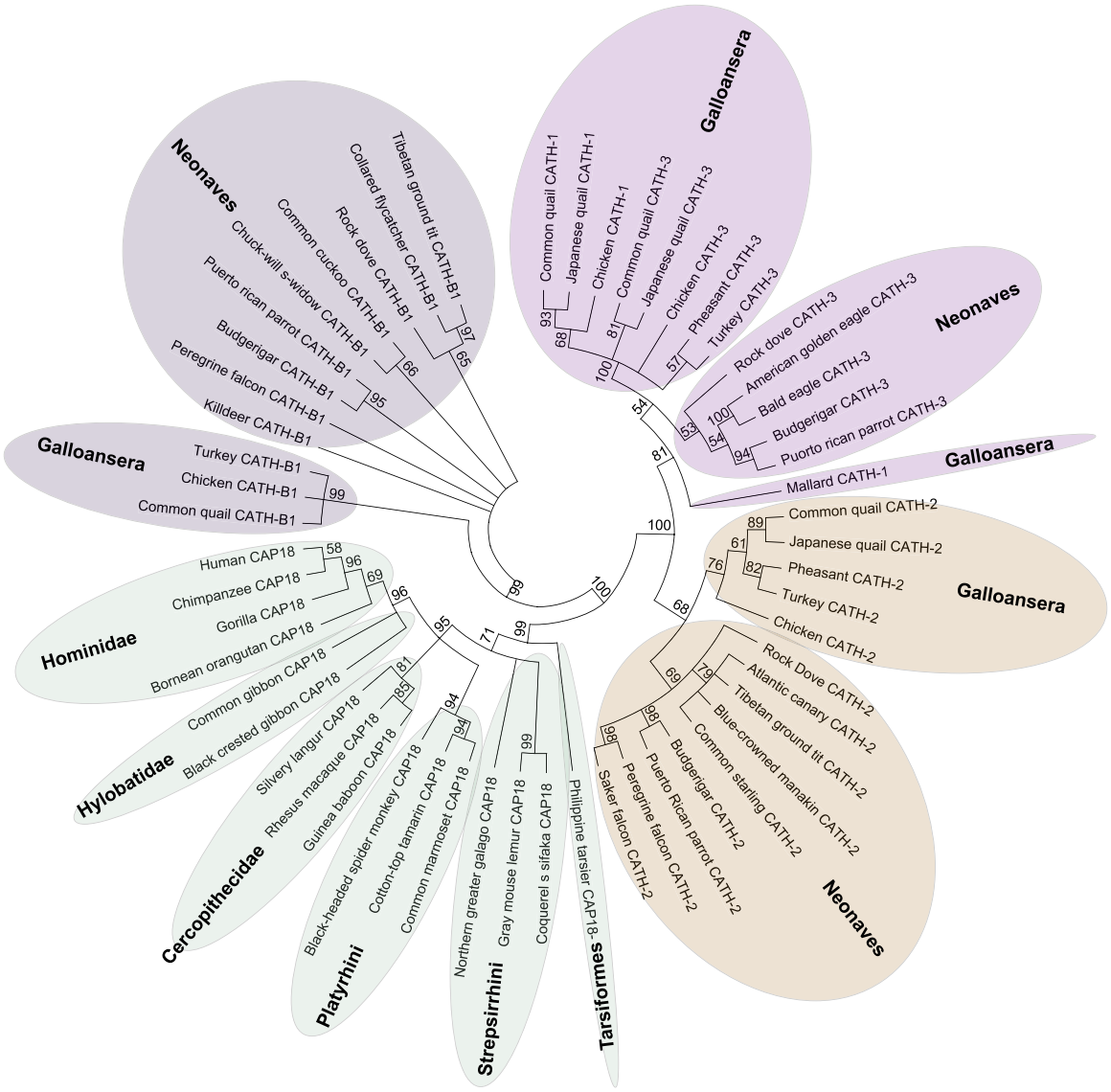

Fig. 3. Phylogenetic analyses of primate and avian cathelicidin amino acid sequences using MEGA11 software. version 11.03.13. Sequences were obtained from UNIPROT (https://www.uniprot.org/) and NCBI genome databases (https://www.ncbi.nlm.nih.gov/) and aligned using MUSCLE. Phylogenetic trees were constructed using Maximum Likelihood with the Jones-Taylor-Thornton (JTT) model of amino acid substitution (JTT + G). Nodal support of the unrooted trees was assessed by bootstrapping with 500 replicates.

2004). Chicken AvBD3, that contains an extended C-terminal tail, is also epididymis-associated (Das et al., 2011; Shimizu et al., 2008).

Alignment of primate CAP18 precursor sequences and avian cathelicidin precursor sequences (Supplementary Figure 2) reveals a high variability in all domains with the exception of four well-conserved cysteine residues in the cathelin-like domain (positions 215, 226, 237 and 254 of the consensus sequence). Signal peptide sequences are highly similar within the CATH-1/3 and − 2 clades but share little homology with those of the CATH-B1 clade. In Galliformes, CATH-B1 genes contain an additional region between the signal peptide and the cathelin-like domain consisting of six octamer repeats in chicken and turkey (ProGlyHAspGlySerXX; where 'H' corresponds to a hydrophobic residue), and twelve repeats in common quail, followed by three "ProXXXGlySerIleX" repeats in all three species. The first six common repeats are predicted to adopt β-turns (BetaTPred v3.0 software), which are known to be involved in protein folding. Within clades, there is a remarkable conservation of sequences of mature peptide segments. In the CATH-2 clade, the N-terminal part of the mature peptide including the proline residue is highly conserved and near identical for all 20 discovered CATH-2 analogues. Within the CATH-2 clade, C-terminal segments are also well conserved for Galliformes, whereas CATH-2 analogues in other birds are two residues longer and often contain an Asp/Asn residue at position 287. CATH-3 and CATH-1 orthologs are equally well conserved among Galliformes (93% and 96% identity, respectively).

A feature of mature CATH-2 orthologs is the presence of conserved aromatic residues (predominantly Phe residues at positions 270, 273, 280) encompassing cationic residues (Lys and Arg), and the central kink due to the presence of a single proline residue at position 282. By comparison, mature CATH-B1 orthologs in Galliformes contain multiple conserved tryptophan or tyrosine residues and charged residues. Overall, hydrophobic and polar residues, that enable the formation of a central amphipathic helix, are well conserved among primate LL-37 orthologs (Xhindoli et al., 2016; Zhu and Gao, 2017). Interestingly, the distribution of aromatic amino acids in mature primate LL-37 orthologs is evolutionary diversified; these orthologs consist mainly of Phe residues. A doublet of aromatic residues is exclusively present at the N-terminus of LL-37 orthologs in old world apes and monkeys at position

269 and 270, while the Phe residue at position 290 is found in almost all orthologs. Notably, most other LL-37 orthologs appear to compensate this with a C-terminal doublet of aromatic residues.

5. Protein folding level

Comparative analysis of human and avian β-defensin members based on 3D structures determined by NMR studies (Bailleul et al., 2016; Derache et al., 2012; Guyot et al., 2020; Herve et al., 2014; Hoover et al., 2001; Landon et al., 2004; Sawai et al., 2001; Schibli et al., 2002) show a common trefoil structure of β-sheets with three antiparallel strands that is remarkably conserved between these two evolutionary distant clades (Fig. 4). The typical cysteine pairing motif (Cys1-Cys5, Cys2-Cys4 and Cys3-Cys6) in structurally diverse β-defensin paralogues (Fig. 2) supports its role in structure stabilization. A notable feature in avian defensins is the absence of a small N-terminal alpha helix in all but one of the solved structures (Fig. 4). Hence, the cysteine stabilized αβ-motif, composed of an α-helix linked to an antiparallel β-sheet (Zhu and Gao, 2013), does not appear as well conserved in avian β-defensins as in their primate counterparts. This cannot be attributed to a shorter N-terminal end since the recently solved AvBD7 structure revealed an unusually long N-terminal extremity stabilized by a salt bridge, which does not adopt a helical conformation (Bailleul et al., 2016). The AvBD7 N-terminal strand appears to cover its C-terminal tail, which may explain its resistance to amino- or carboxy-peptidase activity (Bailleul et al., 2016). In contrast, HBD2 and HBD3 are rather susceptible to degradation by these proteases (Taggart et al., 2003). The trefoil β-sheet structure conservation in β-defensins in both avian and primate evolution connotes an importance in receptor interaction. The resemblance of the β-defensin trefoil structure to the antiparallel 3-stranded β-sheet of chemokine CCL20 and the fact that both can activate the CCR6 chemokine receptor (Yang et al., 1999) further supports a vital role of the trefoil structure in the interaction β-defensins with immune receptors.

The available 3D structures of avian and primate cathelicidins are depicted in Fig. 5. In aqueous environment chicken CATH-1, − 2 and − 3 lack a defined structure, while in a membrane-mimicking environment they adopt an α-helical structure consisting of two short helical segments separated by a flexible Gly-induced slightly kinked central hinge region (CATH-1, and −3) or by a Pro-induced extensively kinked central hinge region (CATH-2) (Bommineni et al., 2007; Xiao et al., 2006b, 2009). This is in accordance with human cathelicidin LL-37 in aqueous environment and low pH showing a random coiled state, while it adopts a helical formation at physiological pH and when interacting with membranes (Johansson et al., 1998). Predicted 3D structures of killdeer and chicken CATH-B1 suggest that the mature peptides adopt a conformation consisting of one large helical segment equipped with a coiled C-terminal tail whereas primate LL-37 orthologs adopt a strict helical configuration (Fig. 5).

In addition to basic residues, aromatic residues are commonly found in cathelicidins (Fig. 5) and other HDPs and play an important role in HDP-membrane interactions. Three-dimensional models of primate LL-37 analogs and avian cathelicidins all indicate an alignment of aromatic side chains in the same plane under conditions mimicking peptide interaction with membranes (Fig. 5). NMR studies with chicken CATH-1 analogs bound to lipopolysaccharides (LPS) show close proximity of Tyr and Trp residues with LPS (Bhunia et al., 2009). NMR studies of LL-37 bound to lipid micelles indicate direct interactions of all four Phe residues with D8PG micelles (Wang et al., 2008). In particular, Phe27 seems important for the membrane binding of LL-37 analogs, as it is highly conserved in primate LL-37 homologs. NMR studies of chicken CATH-1 analogs in complex with LPS also revealed close proximity of terminal basic residues to both glucosamine phosphate groups, which appear to form multiple hydrogen bond/salt bridge interactions (Bhunia et al., 2009). Similarly, profound interactions were observed for Arg residues in LL-37 with anionic phosphatidylglycerols (PGs) in bacterial membranes, with a prominent role for Arg23 positioned at the LL-37 hydrophobic/hydrophilic interface (Wang et al., 2014). This has been proposed to cause anionic lipid re-distribution in bacterial membranes

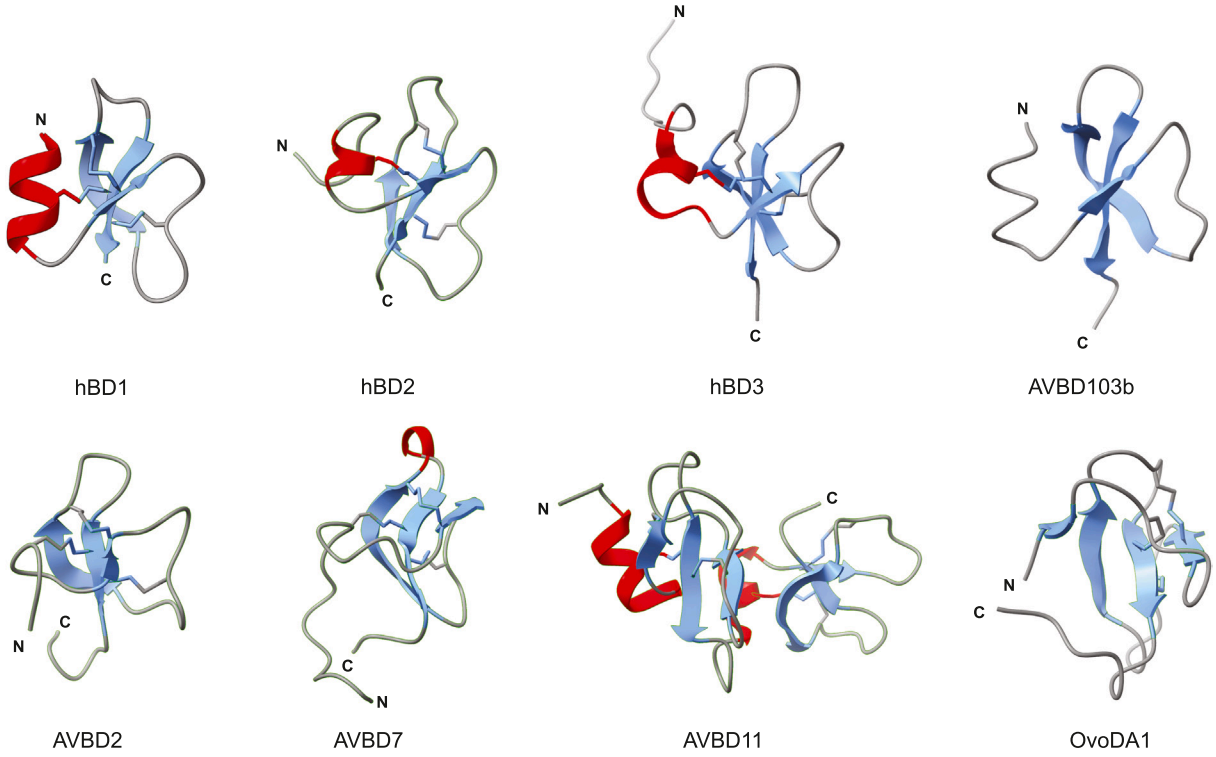

Fig. 4. Three-dimensional structure of human ß-defensins hBD1 (1IJU), hBD2 (1FQQ), hBD3 (1KJ6), emperor penguin AVBD103b (1UT3), chicken AVBD2 (2LG5), AVDB7 (5LCS), AVBD11 (6QEU) and chicken ovodefensin OvoDA1 (2MJK). Helical regions are indicated in red and strands in blue. Structures were obtained using the ChimeraX software.

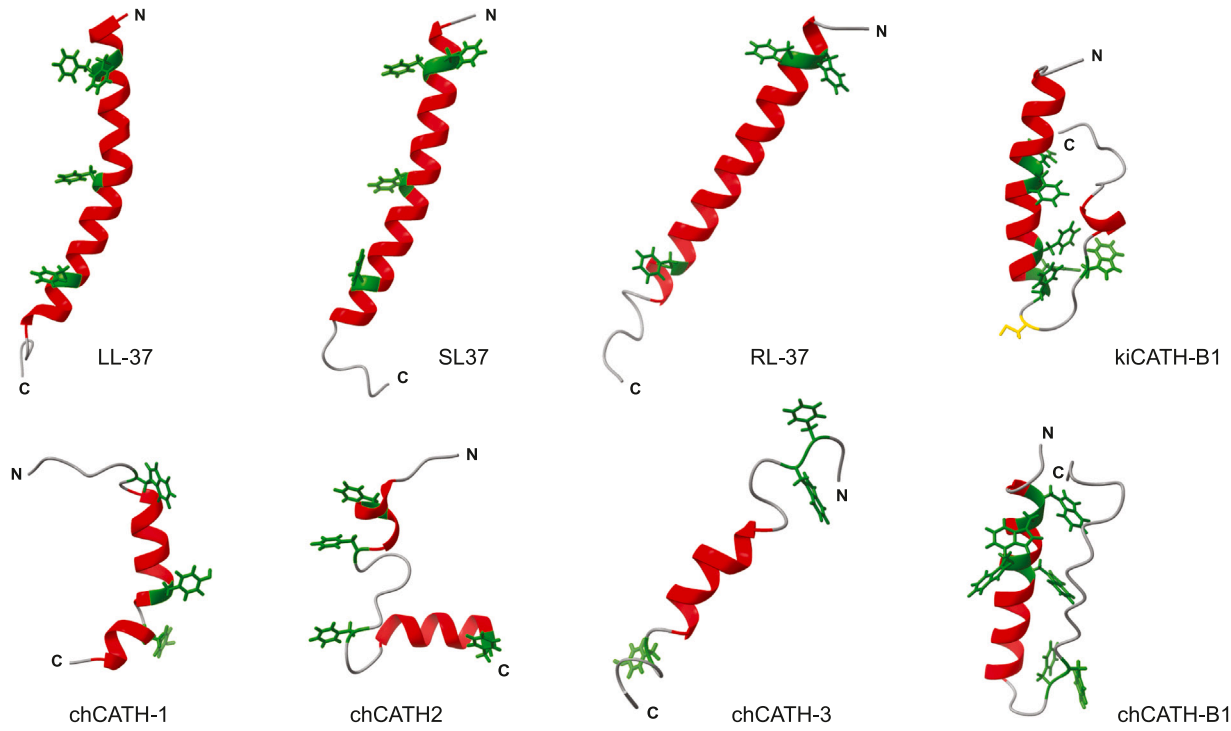

Fig. 5. Three-dimensional structure of primate and avian cathelicidins. Human LL-37 (2K6O), common gibbon SL-37 (iTasser), rhesus macaque RL-37 (iTasser), killdeer CATH-B1 (iTasser), chicken CATH-1 (2AMN), CATH-2 (2GDL), CATH-3 (2HFR) and CATH-B1 (iTasser). Helical regions are indicated in red and aromatic residues in green. Structures were obtained using the ChimeraX software.

and to impact on bacterial signal transduction (Wang et al., 2008).

6. Expression of HDPs in the organisms

6.1. Distribution of defensins in the host

Despite many similarities in expression (Cuperus et al., 2013; Pazgier et al., 2006), avian and primate defensins exhibit certain distinguishable features linked to physiological and anatomical peculiarities. For instance, primate α-defensins (Ganz et al., 1985; Selsted et al., 1985) are found in the granules of neutrophil granulocytes and intestinal crypt Paneth cells (Porter et al., 1997). In contrast, birds lack α-defensins and store β-defensins in heterophilic granulocytes (Evans et al., 1994; Harwig et al., 1994), whereas the existence of Paneth-like cells in chicken intestinal crypts is recently described (Wang et al., 2016; Yu et al., 2021). Heterophils are functional analogs of primate neutrophils, and have a very different granule composition, among others an absence of myeloperoxidase (Daimon and Caxton-Martins, 1977), and therefore lack an effective oxidative burst response. Peptide mapping of intact cells by mass spectrometry analysis revealed that heterophils contain at least three different defensins (Derache et al., 2009b) in addition to their cathelicidin content (van Dijk et al., 2009), which could compensate for the lack of myeloperoxidase in their antimicrobial activity.

Primate β-defensins are produced by epithelial cells lining mucosal surfaces and by skin cells. Particularly the human β-defensins HBD1-HBD4 have been extensively studied. HBD1 appears to be constitutively expressed (Zhao et al., 1996), while HBD2-HBD4 expression is induced by pathogen associated molecular patterns (PAMPs) and/or inflammatory cytokines (Garcia et al., 2001; Harder et al., 2001; Liu et al., 2002). Similarly, bird epithelial cells also express β-defensins (Derache et al., 2009a). Expression of AvBDs in the digestive tract of birds is constitutive but may also be regulated upon infection (Ramasamy et al., 2012; Saidi et al., 2021; Su et al., 2017). AvBD9 protein appears to be stored in the granules of enteroendocrine cells throughout the chicken intestine (Cuperus et al., 2016a).

A major discrepancy between primate and avian species relies on the relative abundance of defensins in the skin of primates (Schroder and Harder, 2006), while low β-defensin expression is detected in avian skin (Lynn et al., 2004; Xiao et al., 2004). In birds, the dense layers of feathers functions as a protective shield to protect a surprisingly thin skin. In addition, birds possess a single large sebaceous gland (uropygial gland of preen gland) at the base of their tail that secretes a waxy substance that is distributed onto skin and feathers at regular intervals during preening behavior and is rich in fatty acids and contains cathelicidins and bacteriocins (Ruiz-Rodriguez et al., 2013, 2012; van Dijk et al., 2005). Considering the presence of HBD1 and HBD2 in human sebaceous glands (Chronnell et al., 2001) it will be interesting to examine if preen glands produce defensins. Altered expression of human defensins (HBD2 and HBD3) is associated with dermatological pathologies such as atopic dermatitis and psoriasis (Marcinkiewicz and Majewski, 2016). Noteworthy is that these chronic inflammatory skin diseases have never been described in birds. In domestic birds, an often-described pathological skin inflammation is pododermatitis; its association with altered HDPs expression is not known.

β-defensins are abundantly expressed in the reproductive tract of birds and primates and with great repertoire diversity. In the hen oviduct, 11 different AvBDs are expressed in the region from infundibulum to cloaca, an area open to ascending bacterial pathogens (Mageed et al., 2008). The large variety of defensins and broad antimicrobial spectrum may be expected to contribute to the protection of gametes and embryos. For instance, during egg formation in the magnum, before deposition of egg white, the oocyte and yolk are encapsulated by a vitelline membrane that contains antimicrobial proteins including lysozyme and AvBD11 (Mann, 2008). Likewise, three major HBDs are expressed at high levels in the human vagina and uterus (King et al., 2007a; Valore et al., 1998; Zaga-Clavellina et al., 2012) and HBD1–3 are present in the placenta and in the feto-maternal interface composed of the placental and chorion trophoblast layers of fetal membranes (King et al., 2007b).

β-defensin genes are abundantly expressed in the male reproductive

tract of birds and primates, e.g. in chickens 9 genes are expressed in the testis (Anastasiadou et al., 2014; Watanabe et al., 2011) and 10 genes in the epididymis (Watanabe et al., 2011), whereas even more β-defensin genes have been found expressed in human testis and epididymis (Com et al., 2003; Patil et al., 2005; Rodriguez-Jimenez et al., 2003; Yamaguchi et al., 2002; Yenugu et al., 2004; Yudin et al., 2005). A glycocalyx protects mammalian and avian spermatocytes to maintain their viability and function during passage of the female reproductive tract. In primates, glycosylated β-defensin 126 covers the spermatocyte surface during its passage through the epididymal duct and strengthens the spermatocyte glycocalyx, necessary to prevent recognition by the female reproductive tract's immune system and safeguard fertility (125). The considerable contribution of the glycosylated C-terminal tail of BD126 to the spermatocyte glycocalyx is demonstrated by the impaired spermatocyte motility when the defensin is mutated there (Tollner et al., 2011). BD126 orthologs are found in the glycocalyx of primate (Tollner et al., 2011, 2008) and murine species (Jelinsky et al., 2007); therefore, one may speculate that avian orthologs may exist with similar functions. In chicken testis, AvBD11 peptide was found in Sertoli cells and AvBD11 and − 12 peptides were also found in efferent ducts of epididymis (Watanabe et al., 2011). Up to date, only AvBD3 peptide was detected on the mid-section and upperpart of the tail of chicken spermatocytes, but not on the head (Shimizu et al., 2008). It remains to be clarified which AvBDs are part of the avian spermatocyte glycocalyx and to what extent AvBD C-terminal tail glycosylation governs avian spermatocyte protection. Thus, β-defensins seem to play a dual role in the reproductive tracts of birds and primates, some having evolved to promote fertility while others are part of the protective barrier against pathogenic organisms.

6.2. *Distribution of cathelicidins in the host*

The tissue distribution of cathelicidins in birds is very different from that in primates. In humans, LL-37 is found produced by a variety of different cell types: widely expressed by epithelial cells, monocytes and neutrophils and to various extents by B and T cells, macrophages, dendritic cells, NK cells, and mast cells (Zanetti, 2004). In birds, the cathelicidin repertoire is more diverse and its distribution restricted. For instance, chicken cathelicidin-2 and -B1 are found in heterophils (van Dijk et al., 2009) and gut M cells (Goitsuka et al., 2007), respectively. Transcription profiles indicate that avian cathelicidins are widely dispersed throughout the gastrointestinal, respiratory and reproductive tracts (Achanta et al., 2012; Lynn et al., 2004; van Dijk et al., 2005; Yacoub et al., 2016) although in certain tissues this may be linked to residential or surveilling immune cells. For chicken CATH-1, − 2 and − 3 and pheasant CATH-1, mRNA levels are most abundant in bone marrow and bursa of Fabricius (Achanta et al., 2012; Lynn et al., 2004; van Dijk et al., 2005; Wang et al., 2011). Chicken CATH-B1 is primarily found in the bursa of Fabricius where B cells are produced (Goitsuka et al., 2007). This is not surprising, considering the importance of the bursa for B cell maturation and its proximity to the cloaca, a portal of entry for various pathogens. Immunohistochemical studies demonstrated that chicken CATH-2 protein synthesis is restricted to heterophils (Cuperus et al., 2016a; van Dijk et al., 2009), that are numerous in embryonic and neonatal bursal mesenchyme and remain there in small groups at the mesenchyme/follicle interphase of some follicles (Cuperus et al., 2016a). Chicken CATH-B1 protein is produced by bursal interfollicular secretory enterocytes and taken up by M cells via pinocytosis (Goitsuka et al., 2007).

Primate LL-37 is constitutively expressed in gastric (Hase et al., 2003) and colonic (Hase et al., 2002) epithelial cells and regulated by cyclic AMP signaling pathways (Chakraborty et al., 2009). LL-37 production by colonic epithelial cells is upregulated by short-chain fatty acids, such as butyrate, that are produced by gut microbiota (Hase et al., 2002). In monocytes and keratinocytes, TGF-β1 and TLR2 ligands induce LL-37 production through a vitamin D receptor dependent pathway (Gombart et al., 2005; Schauber et al., 2007). Independent of the vitamin D receptor, LL-37 expression may be upregulated via NF-κB-C/EBPα activation, as a result of ER stress in epithelial cells but not in myeloid cells (Park et al., 2011). LPS and lipoteichoic acid (LTA) stimulation of DT40 cells, a chicken bursal lymphoma cell line, induces CATH-B1 transcription (Takeda et al., 2014), and feed supplementation with vitamin D upregulates transcription of CATH-1 and -B1 in spleen (Rodriguez-Lecompte et al., 2016). The possibility that chCATH-1, − 2, − 3 and -B1 gene expression is also regulated via NF-κB-C/EBPα signaling (Lee et al., 2016b) still needs to be examined.

In adult human skin, LL-37 is expressed at low basal levels by keratinocytes and upregulated in case of injury and inflammatory skin diseases such as psoriasis and rosaceae (Gallo and Nizet, 2008). LL-37 is also produced by sweat and sebaceous glands and continuously secreted onto the skin surface where it is cleaved into active forms by kallikreins (Afshar and Gallo, 2013; Gallo and Nizet, 2008). Avian skin lacks sweat glands and contain a single sebaceous (uropygial) gland. Chicken CATH-2 was shown to be present in the epidermis localized to heterophils and abundantly present in uropygial gland tissue (van Dijk et al., 2005), the latter possibly to protect glandular epithelium.

Avian and human cathelicidins have in common their high level of expression in the male and female reproductive tracts and likely fulfill similar roles. All four chicken cathelicidins are expressed at moderate or high levels in testis (Achanta et al., 2012; van Dijk et al., 2005; Yacoub et al., 2016), while low transcript levels were found for pheasant CATH-1 (Wang et al., 2011). All chicken cathelicidins are expressed in ovary (Achanta et al., 2012) and CATH-1 and − 2 are highly expressed in oviduct (Yacoub et al., 2016). LL-37 precursor protein hCAP18 is expressed in epididymis epithelium and has been found in seminal plasma at high concentrations (9–32 μM) at a ratio of ~6.6 million hCAP-18 molecules per spermatocyte but proved to be absent in testis (Malm et al., 2000). Although seminal fluid could reduce the antimicrobial potency of HDPs, hCAP18 may aid to protect spermatocytes "en route" to the ovum during fertilization (Malm et al., 2000). hCAP18 is consistently expressed by squamous epithelia of cervix and vagina (Frohm Nilsson et al., 1999) and is found at low levels in vaginal fluid (0.4 – 220 nM) (Levinson et al., 2009; Valore et al., 2002).

7. Biological functions of HDPs

Many biological functions have been attributed to cathelicidins and β-defensins (Shelley et al., 2020; van Harten et al., 2018). Variation in length, composition and order of amino acids and (restricted) conformation of mature peptides have resulted in what appear tailor-made peptides. HDP interaction with bacterial and eukaryotic membranes is governed by charge, hydrophobicity, distribution thereof and flexibility (e.g. Gly, Pro residues, disulfide bonds). In the case of bacteria, membrane interaction is driven by peptide cationic charges binding to anionic outer membrane components e.g. LPS and LTA, which disrupts bacterial outer membrane organization, enabling binding to the bacterial inner membrane, membrane penetration and leakage and eventually cell death (Schmidt et al., 2011). The lower external electro-negativity and higher concentration of cholesterol in eukaryotic membranes, ensuring lipid leaflet rigidity, explain the weak lytic effect of defensins on host cells by comparison to bacterial cells (Matsuzaki, 1999). Antiviral activities of HDPs *in vitro* may include direct mechanisms such as inhibition of host cell infectivity by viral envelope destabilization or virion aggregation, inhibition of viral replication, prevention of viral capsid uncoating as well as host-mediated effects i.e. viral receptor blocking and modulation of immune cells (Mookherjee et al., 2020). The versatile immunomodulatory functions described for HDPs are likely explained by their differential intracellular uptake and complex interactions with epithelial, immune and other cells via various cell surface receptors.

7.1. Antimicrobial activity and cytotoxicity of defensins

The antibacterial activities of human and avian defensins have a similar range with minimum inhibitory concentration (MIC) values from 0.1 μM to ≈ 20 μM against both Gram-positive and Gram-negative bacteria (Ageitos et al., 2017), and are equally susceptible to inhibition by cations (Bals et al., 1998a; Goldman et al., 1997; Tomita et al., 2000; Yacoub et al., 2015; Yang et al., 2016). An exception to this rule is avian defensin AvBD103b that maintains its antimicrobial activity in the high salt environment of the penguin stomach, due to its highly cationic nature (Landon et al., 2004). Despite the unfavorable physiological conditions, this activity is made up by local release in large quantities of defensins and cathelicidins by neutrophils and heterophils after recruitment to the site of infection (Ganz, 1987; Ganz et al., 1985; Rice et al., 1987).

The N-terminal helix structure is not essential for the antibacterial activity of avian defensins. In human BD3 this region is associated with membrane selectivity (Dathe and Wieprecht, 1999), e.g. deletion of 3 N-terminal amino acids of HBD3 improved antimicrobial activity at high salt concentration (Li et al., 2015) and the helix forming propensity is correlated to host cell cytotoxicity (Zelezetsky and Tossi, 2006). The number of disulfide bonds and proper connectivity are not essential for antimicrobial activity of primate defensins as shown for HBD3 and HBD4 (Hoover et al., 2003; Kluver et al., 2005; Sharma and Nagaraj, 2015; Wu et al., 2003). Oxidized HBD1 displays only weak antimicrobial activity (Schroeder et al., 2011), but its naturally occurring five cysteine analog that contains only two intramolecular disulfide bonds has comparable antimicrobial activity (Circo et al., 2002). Similarly, blocking cysteine pairing in synthetic avian defensin AvBD2 by acetomethylation did not abolish antimicrobial activity against Gram-negative bacteria (Derache et al., 2012) and a loss of connectivity by reduction did not substantially reduce the antibacterial activity of AvBD6 or AvBD12 (Yang et al., 2016). Partial substitution of hydrophobic residues by cationic residues diminished antibacterial activity in duck AvBD2 (Soman et al., 2010), but increased activity in AvBD8 (Higgs et al., 2007). These findings suggest that, for primate and avian defensins, antimicrobial activity is more dependent on positive net charge and a higher overall hydrophobicity than on the disulfide bridges (Kluver et al., 2005; Yang et al., 2017). *In vivo* antimicrobial efficacy was demonstrated for human defensins: i. HBD2 gene therapy in a mouse model of infection by *Escherichia coli* (Huang et al., 2002), ii. HBD4 induction using a viral vector application in a burn wound mouse model and a secondary *Pseudomonas aeruginosa* infection (Park et al., 2014), and iii. intraperitoneally (*i.p.*) administered HBD1/HBD2 in a murine salmonellosis model (Maiti et al., 2014). Likewise, *i.p.* AvBD7 administration in a lethal systemic mouse salmonellosis model increased survival and reduced the liver bacterial load (Bailleul et al., 2019).

The antiviral activity of avian and primate defensins is well documented. Herpes simplex virus (HSV) is most susceptible to HBDs among the viruses tested (Daher et al., 1986). HDPs may block multiple steps of HSV infection as in the case of HBD3 with prevention of binding and entry (Hazrati et al., 2006). In chickens, infection with the emblematic herpes virus causing Marek's disease (MDV) increases expression levels of AvBD2, − 4 and − 7 (Niu et al., 2018). The antiviral effect of these avian defensins on MDV remains to be determined. HIV-1 infectivity is reduced via HBD-2 and − 3 binding to epithelial cell surface heparan sulfate proteoglycans (HSPGs) resulting in co-internalization with HSPG-bound HIV gp120 to endosomes and oligomer formation (Herrera et al., 2016). In peripheral blood mononuclear cells (PBMC) and T cells HBD2 and − 3 impair HIV infection by direct binding to the viral particle and through downregulation of the HIV-1 co-receptor CXCR4 (Quinones-Mateu et al., 2003) and HBD2 inhibits HIV replication in the intracellular environment (Sun et al., 2005). However, HIV-positive subjects also exhibit dramatically diminished levels of HBD2 expression in the oral mucosa (Sun et al., 2005).

HBDs have shown promising antiviral capacity against respiratory infection viruses such as influenza virus (IAV) and respiratory syncytial virus (RSV). The lectin-like properties of HBD3 enable it to block viral pore generation, by creating a protective barrier of immobilized surface glycoproteins (Leikina et al., 2005). A more direct interaction with virions was shown for HBD2, that was able to block RSV entry, possibly through the destabilization/disintegration of the viral envelope (Kota et al., 2008). In birds, β-defensin expression is upregulated upon avian influenza virus (AIV) vaccination (Kalenik et al., 2018) and AIV infection *in vitro* (Jang et al., 2015). AvBD11 was found to reduce AIV titer in infected chicken epithelial cells when pre-incubated with the virus (Guyot et al., 2020), while AvBD2, − 6 and − 12 peptides reduced the infectivity of infectious bronchitis virus in chicken embryos (Xu et al., 2015) and AvBD2 pre-incubation with Newcastle disease virus, another respiratory virus, greatly reduced infectivity in chicken embryo fibroblasts (Liu et al., 2018). Thus, the antiviral activities of primate and avian defensins appear to rely more on virus neutralizing effects than intracellular virus cycle interference.

7.2. Antimicrobial activity and cytotoxicity of cathelicidins

Primate and avian cathelicidins exhibit antibacterial activities at similar MIC (≈ 0.2–20 μM) (Ageitos et al., 2017). At sub MIC concentrations, chicken CATH-2 rapidly binds and permeabilizes *E. coli* membranes, localizes intracellularly, and induces DNA condensation, enhanced vesicle release and formation of membrane wrinkles (Schneider et al., 2016), while MIC concentrations of CATH-2 induce membrane rupture and cell lysis (Schneider et al., 2016). Moderate to potent antifungal activity against *Candida albicans* is described for chicken, quail and pigeon CATH-2 orthologs (Ordonez et al., 2014; van Dijk et al., 2009; Wang et al., 2011; Yu et al., 2015). LL-37 however only displays weak activity against this fungus (Ordonez et al., 2014). Both CATH-2 and LL-37 rapidly bind to the *C. albicans* cell membrane and start to internalize at sublethal concentrations, affecting the fungal membrane and nuclear envelope, while only CATH-2 caused cell shrinkage possibly through loss of transmembrane potential (Ordonez et al., 2014). At physiological salt concentrations, several species including methicillin-resistant *Staphylococcus aureus*, *Proteus mirabilis*, *E. coli* and *C. albicans* were shown to become more or completely resistant to LL-37 (Bals et al., 1998b; Travis et al., 2000; Turner et al., 1998). In contrast, the antibacterial activity of quail CATH-2 and − 3 (Feng et al., 2011), chicken CATH-1, and − 2 (Xiao et al., 2006a), pheasant CATH-1 (Wang et al., 2011) was not affected in the presence of 100 mM NaCl.

A single helical domain connected to the hinge region is mandatory for the antimicrobial action of avian cathelicidin peptides. Either helical segment of chicken CATH-2 including the central hinge region retains considerable antibacterial activity, whereas cytotoxicity is reduced (Xiao et al., 2009). The N-terminal helical segment plus hinge region is sufficient for the antibacterial activity of chicken CATH-3 (Qu et al., 2016), CATH-3 cytotoxicity is linked to the short hinge region. The short chicken CATH-1 C-terminal helical segment is critical for both antibacterial and cytotoxic activity (Xiao et al., 2006b). Chicken CATH-1 is much more cytotoxic than CATH-3, which may be due to participation of a 3 amino acid N-terminal stretch in cytotoxicity (Xiao et al., 2006b) and reduced hinge flexibility by steric hinderance of the central Gly residue by the flanking Tyr residue, which appears to be positively correlated with α-helical peptide toxicity in mammalian cells (Bommineni et al., 2007). Studies with truncated peptides (Molhoek et al., 2009; Nell et al., 2006; Wang et al., 2014) showed that the antibacterial activity, antiviral and antineoplastic activities of primate LL-37 are retained in a smaller segment, whereas the whole central helical section is required for immunomodulatory, LPS- and LTA-neutralizing activities. Additionally, it was shown that aromatic residues of importance for the microbicidal and immunomodulatory activities of avian and primate cathelicidins (Andrushchenko et al., 2008; Datta et al., 2016; Staubitz et al., 2001; van Dijk et al., 2016).

In vivo antimicrobial efficacy of primate cathelicidins was shown for the intratracheal therapeutic application of LL-37 (1 mg/kg) to mice infected with a multidrug-resistant strain of *Mycobacterium tuberculosis* that substantially reduced lung bacilli after 28–30 days of treatment (Rivas-Santiago et al., 2013). Using a similar route, LL-37 ameliorated methicillin-resistant *Staphylococcus aureus*-induced pneumonia in mice (Hou et al., 2013). Intravenous administration of LL-37 also improved the survival of septic mice possibly by inhibition of pyroptosis, modulation of inflammatory cytokine production and by reduction of bacterial burden (Hu et al., 2016). In birds, demonstration of activity of cathelicidins came from *in ovo* studies. Injection of embryonated chicken eggs with chicken CATH-2 analog DCATH-2 at day 18 (1 mg/kg bodyweight) followed by challenge with avian pathogenic *E. coli* at 7 days of age significantly reduced morbidity and respiratory bacterial load one week post infection (Cuperus et al., 2016b). Furthermore, in the absence of an infectious stimulus, *in ovo* administration of DCATH-2 altered the microbiota composition without affecting the chicks' immune system post-hatch (Cuperus et al., 2018).

LL-37 is the most potent antiviral peptide among cathelicidins and displays potent antiviral activity against influenza A virus (IAV) *in vitro* and *in vivo* (Barlow et al., 2011) and plays a role in the defense against a variety of other viral pathogens including RSV, Dengue virus, HIV, HSV, Vaccinia virus and Adenovirus (Bergman et al., 2007; Currie et al., 2013, 2016; Gordon et al., 2005; Howell et al., 2004; Yasin et al., 2000). The antiviral activity of chicken cathelicidins has been studied against avian influenza A virus, a major concern for poultry breeders. Compared to other cathelicidins (CATH-1, −2, −3, LL-37, PMAP-23, and K9CATH), chicken CATH-B1 has broad anti-IAV activity with inhibition of viral infection up to 80% against IAV strains of zoonotic interest (H1N1, H3N1, and H5N1) (Peng et al., 2020). Direct interaction of CATH-B1 with viral particles is required for anti-IAV activity and involves aggregation of viral particles and presumably hampers haemagglutinin binding to cell surface receptors. Primate cathelicidin LL-37 seems to bind AIV in a different manner, not inhibiting hemagglutinin (HA)-receptor binding and failing to inhibit the binding and cellular uptake of viral particles (Tripathi et al., 2013, 2015). In addition, antiviral activity of LL-37 is IAV strain dependent, being more efficient against H3N1 than against H1N1 and H5N1 strains (Peng et al., 2020), in contrast to avian CATH-B1 that exhibited similar antiviral activity against these IAVs subtypes. The antiviral activity against AIV, RSV and dengue viruses is sequence-specific as scrambled LL-37 peptide lacked antiviral activity (Alagarasu et al., 2017; Barlow et al., 2011; Currie et al., 2016), yet the exact molecular mechanisms involved need to be further elucidated.

7.2.1. Immune modulation by defensins

7.2.1.1. Immune cell recruitment. In the context of immune cell recruitment, HBD2 chemoattracts immature dendritic cells and memory T cells at low micromolar concentrations, thus linking innate to adaptive immunity (Yang et al., 1999). It can attract mast cells as well (Niyonsaba et al., 2002a). HBD3 may stimulate antigen-presenting cells through TLR1 and TLR2 to enhance expression of costimulatory molecules (Funderburg et al., 2007) that further link innate to adaptive immunity. Both HBD2 and HBD3 recruit monocytes, macrophages and neutrophils via chemokine receptor CCR2, which recognizes members of the MCP family of chemokines, e.g. CCL2/MCP-1 (Rohrl et al., 2010). Additionally, primate β-defensins can stimulate leukocytes to induce expression of cytokines and / or chemokines that in turn recruit various immune cells. HBD1–3 stimulate IL-8 and CCL2 production by PBMCs leading to recruitment of neutrophils and monocytes, while HBD2 more selectively induces IL-6 and IL-10 production (Boniotto et al., 2006). In birds, the organization of lymphoid tissue is somewhat different compared to that of primates. The absence of lymph nodes and presence of a more diffuse lymphoid tissue at mucosal sites positions avian lymphoid cells more closely to infectious foci thus less dependent on distant recruitment. However, recruitment of chicken B- and T-lymphocytes was shown for duck AvDB2 (Soman et al., 2009a, 2009b). Modest recruitment of chicken macrophages and potent recruitment of CCR2-positive CHO-K1 cells was observed for AvBD6 and AvBD12, whereas AvBD6 moderately recruited CCR6-positive CHO-K1 cells (Yang et al., 2016). At high concentrations AvBD12 attracted murine iDCs. Thus, avian orthologs of the mammalian CCR2 and CCR6 receptors appear involved in AvBD-mediated cell recruitment, implicating conserved co-evolution of ligands and CCR2/CCR6-like receptors in primates and birds. The capacity of defensins to recruit and activate phagocytes can be considered as a means for the host to amplify the local innate defense by a feedback loop system. Their intervention in many immune regulatory and physiological processes has been increasingly demonstrated (Hancock et al., 2016). The role of defensins in leucocyte recruitment and activation, as well as in angiogenesis and in wound repair are less known in birds, but suggest that these multifunctional effectors constitute key players in resistance to diseases.

7.2.1.2. LPS neutralization. The anti-inflammatory efficacy of primate β-defensins was demonstrated in vivo using lethal endotoxic shock murine models (Motzkus et al., 2006; Semple et al., 2010). Cationic properties of β-defensins enable high affinity binding to anionic LPS (Sawyer et al., 1988) and limit LPS access to its receptor TLR4 (Toll-like receptor 4) on the cell surface. HBD3, the most cationic human defensin, HBD123 and HBD126, but not HBD2, inhibited the LPS-induced production of inflammatory mediators such as TNFα and IL-6 by mammalian macrophages (Liu et al., 2013; Motzkus et al., 2006; Semple et al., 2010). The anti-inflammatory HBD3 activity was cAMP and IL-10 independent and maintained if peptide was added to macrophages 1 h after LPS stimulation and uptake (Semple et al., 2010), suggesting that β-defensins also inhibit LPS effects downstream of TLR4 activation. Avian β-defensin 6 and − 12 both neutralize LPS, the more cationic AvBD6 (+7) being more efficient (Yang et al., 2016); while reduced AvBD12 (+1), having the same net positive charge as its related unreduced peptide, is less able to neutralize LPS. Thus, in the interaction between AvBDs and LPS, electrostatic attraction is essential but not the only determining factor. Interestingly, the LPS-neutralizing capacity of AvBD6 and − 12 was fully functional at physiological salt conditions and not affected by increased NaCl concentrations. Thus, microbial clearance appears to be equally promoted by avian and primate β-defensins via direct and indirect recruitment of immune cells, while excess inflammation is dampened.

7.2.2. Immune modulation by cathelicidins

7.2.2.1. Immune cell recruitment. Local release of cathelicidins by neutrophils or other cells can directly and indirectly lead to the recruitment of immune cells to the site of infection. Chemotactic activity of human LL-37 has been demonstrated for peripheral blood monocytes, T cells (De et al., 2000), mast cells (Niyonsaba et al., 2002b), neutrophils and eosinophils (Tjabringa et al., 2006). *In vitro*, LL-37 chemoattracts human neutrophils, monocytes, T cells and eosinophils via the FPR2 receptor (De et al., 2000; Tjabringa et al., 2006) and mast cells via a high-affinity pertussis-sensitive receptor (Niyonsaba et al., 2002b), while mast cell degranulation by LL-37 is mediated via the GPCR MgrX2 (Yu et al., 2017). Indirect recruitment of immune cells by LL-37 can occur via activation of MAPK ERK1/2 and p38 signaling pathways in monocytes that initiate production of chemokines and cytokines such as IL-8, MCP-1 and − 3 (Bowdish et al., 2004) leading to recruitment of neutrophils and monocytes, and upregulation of chemokine receptor expression (CCR2, CXCR4) on macrophages (Scott et al., 2002). Interestingly, the threshold of LL-37-induced activation of monocytes is lowered by GM-CSF to 5–10 μg/ml, levels that are present at the onset of infection (Bowdish et al., 2004). GM-CSF is locally produced by various cells (Hamilton, 2002) and released in serum after LPS-activation

(Sheridan and Metcalf, 1972), suggesting that the immunomodulatory actions of LL-37 may be locally amplified by the presence of cytokines. LL-37-enhanced immune cell recruitment was also demonstrated in vivo. hLL-37 enhanced a protective pro-inflammatory response to acute *P. aeruginosa* lung infection in a murine model, effectively promoting bacterial clearance from the lung in the absence of direct microbicidal activity but through upregulation of the early neutrophil response (Beaumont et al., 2014). Notably, *Pseudomonas* clearance and early neutrophil response were impaired in cathelicidin-deficient mice. Direct recruitment of immune cells by avian cathelicidins was demonstrated for chicken CATH-B1 and CATH-1 analogs (Bommineni et al., 2014) and indirect immune cell recruitment may occur through activation of monocytes. Chicken CATH-2 induces transcription of MCP-3, CCLi4/RANTES and CXCLi2/IL-8 in the chicken macrophage-like HD11 cell line (van Dijk et al., 2016) and chicken CATH-1–3 induced MCP-1 production in murine RAW264.7 cells (Coorens et al., 2017b). Intraperitoneal administration of cathelicidin-derived peptides fowl-1(6−26) and fowl-1(8−26) in mice resulted in chemoattraction of neutrophils, but not of monocytes or lymphocytes, while chCATH-B1 induced mast cell migration (Takeda et al., 2014). These findings suggest that, like their mammalian counterparts, avian cathelicidins play an active role in the recruitment of leukocytes to inflammatory sites.

7.2.2.2. LPS neutralization. In particular highly cationic cathelicidins are potent inhibitors of endotoxin-induced activation of immune cells. LPS-induced TLR4 activation is strongly inhibited by chicken CATH-1, − 2, − 3 and –B1 (Coorens et al., 2017b; Takeda et al., 2014; van Dijk et al., 2016; Xiao et al., 2006a). LTA-induced TLR2 activation is inhibited by chicken CATH-2, -B1 and to a lesser extent CATH-1, but not by CATH-3 (Coorens et al., 2017b; Takeda et al., 2014). Correlation of isothermal titration calorimetry data with MIC values for chicken CATH-2 interaction with rough and smooth LPS type *E. coli* strains indicate that this peptide binds to the lipid A moiety as well as to the O-antigen (Schneider et al., 2016). Molecular docking of pigeon CATH-2 to the TLR4-MD-2 complex implicated that LPS-neutralization may be established by binding of cathelicidin peptides to the hydrophobic MD-2 pocket via ionic interactions (Yu et al., 2015). In LPS neutralization by primate cathelicidins, charge, (α-helical) conformation and mode of LPS interaction all contribute to the LPS neutralization capacity but appear to do this indirectly; most effective inhibition of LPS-induced NO production was found for leaf eater monkey RL-37 (+10) and to a lesser extent by human LL-37 (+6), whereas orang-utan LL-37 (+4) and rhesus macaque RL-37 (+8) neutralized poorly (Tomasinsig et al., 2009). However, LPS-neutralization by cathelicidins is more complex than just prevention of TLR4 activation by LPS blocking. LPS neutralization by LL-37 and chicken CATH-2 is in part independent of binding to LPS as both can partially or completely neutralize LPS in LPS-primed macrophages (van Dijk et al., 2016). In fact, alternative mechanisms have been unraveled for LPS neutralization by LL-37, including peptide binding to cell surface receptor CD14, inhibition of NF-kB p50 and P65 subunit translocation to the nucleus, inhibition of NF-kB activation via activation of ERK1/2-mediated CREB phosphorylation and differentially altered gene expression that excluded inhibition of negative regulators of NF-kB and certain chemokines (Mookherjee et al., 2006; Nagaoka et al., 2002; Wen et al., 2010).

7.2.2.3. Microbial clearance. Another mechanism by which cathelicidins can promote clearance of bacterial pathogens is by inducing apoptosis of infected host cells. LL-37 enhanced apoptosis of *Pseudomonas aeruginosa*-infected pulmonary epithelial cells via caspase- and Bax-dependent mitochondrial membrane depolarization only with bacterial invasion of the epithelial cells (Barlow et al., 2010). This has been recently characterized as a cell penetrating peptide mechanism in pulmonary epithelial cells infected by *P. aeruginosa*, with synergistic induction of caspase 1-dependent death of infected epithelial cells (McHugh et al., 2019). Intratracheal delivery of chicken CATH-2-killed *P. aeruginosa* in a murine lung infection model inhibited *P. aeruginosa*-induced neutrophil recruitment, cytokine and chemokine production and so dampened inflammation (Coorens et al., 2017a). Likewise, LL-37 can dampen inflammation by reduction of pro-inflammatory responses to stimuli (e.g. TNFα, NO), while maintaining the ability to phagocytose, to kill bacteria and to clear apoptotic cells (Brown et al., 2011). LL-37 has also shown to activate professional antigen-presenting cells as potent modifier of DC differentiation, increasing HLA-DR and co-stimulatory molecule expression (Bandholtz et al., 2006; Davidson et al., 2004). In a similar manner, chicken CATH-2 enhanced expression levels of mannose receptor MRC1, MHCII, CD40 and CD86 on mononuclear phagocytes (Kraaij et al., 2017).

8. Conclusions and perspectives

Rapid radiation of avian species after the C-Pg mass extinction event resulted in diversification of cathelicidin genes and fewer β-defensin genes due to genome size constraints. In primates the larger genome size limited the loss of β-defensin paralogs and the more gradual adaptive radiation resulted in a highly conserved, ubiquitously distributed and versatile single cathelicidin gene with multiple immunomodulatory functions. In birds, gene duplication generated multiple mature cathelicidin peptides that are equally well preserved among different clades. A plausible explanation for this gene expansion in birds could be an adaptation to fulfill niche functions such as the antiviral activity of CATH-B1 protein that is abundantly present near M cells, an immune surveillance portal for viral and bacterial microparticles. Another example of avian adaptation are heterophils, crucial for the innate immune response in birds to bacterial infections that lack an effective oxidative burst response but are loaded with cathelicidins and defensins that have demonstrated potent immunomodulatory and antimicrobial properties. Evidence of positive selection is found in both primate and avian peptide superfamilies and may be more correlated to co-evolution with endogenous receptors. The well-conserved β-sheet structure with three antiparallel strands in primate and avian β-defensins, reminiscent of chemokines, also supports convergent evolution towards receptor-mediated functions. Taken together, the dynamics in avian and primate cathelicidin and β-defensin evolution have led to HDP repertoires with the potential of broad range antimicrobial/antiviral activities as well as the ability to directly or indirectly recruit and activate immune cells, aid in dampening of inflammation, participate in antigen presentation and promote the clearance of pathogens (Table 1). In addition, simultaneously released endogenous defensins and cathelicidins may exhibit synergistic antibacterial, anti-inflammatory and immune modulatory effects that influence the outcome of infection. However, an expansion of avian-specific (antibody) tools is necessary to better understand the correlation between HDP distribution, regulation and gene-specific biological functions in birds. Considering that the multifunctional properties of defensins and cathelicidins are strongly influenced by their microenvironment it is fundamental to examine these in situ at physiological conditions. For this purpose, rapidly developing techniques such as single-cell spatial analysis may aid at identifying HDP production sites at cell level in tissues, to study co-expression patterns, inter- and intracellular interactions of HDPs with receptors and to perform spatial phenotyping.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability

Data will be made available on request.

Table 1
Features of bird and primate defensins and cathelicidins.

Features	Defensins	Cathelicidins
Repertoire diversity	• Absence of α-defensins in birds. • Functional θ-defensins only in non-human primates.	• Multiple members in birds. • Only one member in primates.
Molecular structures	• Beta-sheet stabilized by three disulfide bridges. • Conserved αβ-motif only in primates.	• Alpha-helical structure. adopted only in hydrophobic environment. • Conserved aromatic and cationic residues.
Biodistributions	• In granules of both bird heterophils and primate neutrophils. • Abundance in the skin of primates but not of birds. • Abundance in the uropygial gland of birds. • Abundance in the reproductive tract (male and female) of birds and primates.	• Restricted distribution (heterophils and epithelial cells) in birds. • Variety of producing cell types in primates. • Abundance in the reproductive tract (male and female) of both birds and primates.
Biological functions	• Similar MIC against bacteria. • Cysteine pairing not required for antimicrobial activity. • Activity dependent on cationicity. • Virus neutralizing effects. • Anti-inflammatory capacity through LPS neutralization. • CCR2- and CCR6-dependent recruitment of immune cells.	• Similar MIC against bacteria. • Salt-sensitivity only in primates. • More activity against fungi in birds. • Virus neutralizing effects with different mechanism against IAV between birds and primates. • Direct and indirect anti-LPS activity. • Direct and indirect (through chemokine induction) recruitment of leukocytes. • Activation of antigen-presenting cells. • Induction of apoptosis of infected host cells in humans.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.molimm.2023.03.011.

References

Achanta, M., Sunkara, L.T., Dai, G., Bommineni, Y.R., Jiang, W., Zhang, G., 2012. Tissue expression and developmental regulation of chicken cathelicidin antimicrobial peptides. J. Anim. Sci. Biotechnol. 3, 15. https://doi.org/10.1186/2049-1891-3-15.

Afshar, M., Gallo, R.L., 2013. Innate immune defense system of the skin. e38-39 Vet. Dermatol. 24, 32–38. https://doi.org/10.1111/j.1365-3164.2012.01082.x.

Ageitos, J.M., Sanchez-Perez, A., Calo-Mata, P., Villa, T.G., 2017. Antimicrobial peptides (AMPs): Ancient compounds that represent novel weapons in the fight against bacteria. Biochem. Pharmacol. 133, 117–138. https://doi.org/10.1016/j.bcp.2016.09.018.

Alagarasu, K., Patil, P.S., Shil, P., Seervi, M., Kakade, M.B., Tillu, H., Salunke, A., 2017. In-vitro effect of human cathelicidin antimicrobial peptide LL-37 on dengue virus type 2. Peptides 92, 23–30. https://doi.org/10.1016/j.peptides.2017.04.002.

Anastasiadou, M., Theodoridis, A., Michailidis, G., 2014. Effects of sexual maturation and Salmonella infection on the expression of avian beta-defensin genes in the chicken testis. Vet. Res. Commun. 38, 107–113. https://doi.org/10.1007/s11259-014-9591-4.

Andrushchenko, V.V., Aarabi, M.H., Nguyen, L.T., Prenner, E.J., Vogel, H.J., 2008. Thermodynamics of the interactions of tryptophan-rich cathelicidin antimicrobial peptides with model and natural membranes. Biochim. Biophys. Acta 1778, 1004–1014. https://doi.org/10.1016/j.bbamem.2007.12.022.

Bailleul, G., Kravtzoff, A., Joulin-Giet, A., Lecaille, F., Labas, V., Meudal, H., Loth, K., Teixeira-Gomes, A.P., Gilbert, F.B., Coquet, L., Jouenne, T., Bromme, D., Schouler, C., Landon, C., Lalmanach, G., Lalmanach, A.C., 2016. The unusual resistance of avian defensin AvBD7 to proteolytic enzymes preserves its antibacterial activity. PLoS One 11, e0161573. https://doi.org/10.1371/journal.pone.0161573.

Bailleul, G., Guabiraba, R., Virlogeux-Payant, I., Lantier, I., Trotereau, J., Gilbert, F.B., Wiedemann, A., Trotereau, A., Velge, P., Schouler, C., Lalmanach, A.C., 2019. Systemic administration of avian defensin 7: distribution, cellular target, and antibacterial potential in mice. Front. Microbiol. 10, 541. https://doi.org/10.3389/fmicb.2019.00541.

Bals, R., Wang, X., Wu, Z., Freeman, T., Bafna, V., Zasloff, M., Wilson, J.M., 1998a. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J. Clin. Invest 102, 874–880. https://doi.org/10.1172/JCI2410.

Bals, R., Wang, X., Zasloff, M., Wilson, J.M., 1998b. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc. Natl. Acad. Sci. U. S. A. 95, 9541–9546.

Bandholtz, L., Ekman, G.J., Vilhelmsson, M., Buentke, E., Agerberth, B., Scheynius, A., Gudmundsson, G.H., 2006. Antimicrobial peptide LL-37 internalized by immature human dendritic cells alters their phenotype. Scand. J. Immunol. 63, 410–419. https://doi.org/10.1111/j.1365-3083.2006.001752.x.

Barlow, P.G., Beaumont, P.E., Cosseau, C., Mackellar, A., Wilkinson, T.S., Hancock, R.E., Haslett, C., Govan, J.R., Simpson, A.J., Davidson, D.J., 2010. The human cathelicidin LL-37 preferentially promotes apoptosis of infected airway epithelium. Am. J. Respir. Cell Mol. Biol. 43, 692–702. https://doi.org/10.1165/rcmb.2009-0250OC.

Barlow, P.G., Svoboda, P., Mackellar, A., Nash, A.A., York, I.A., Pohl, J., Davidson, D.J., Donis, R.O., 2011. Antiviral activity and increased host defense against influenza infection elicited by the human cathelicidin LL-37. PLoS One 6, e25333. https://doi.org/10.1371/journal.pone.0025333.

Baumann, A., Demoulins, T., Python, S., Summerfield, A., 2014. Porcine cathelicidins efficiently complex and deliver nucleic acids to plasmacytoid dendritic cells and can thereby mediate bacteria-induced IFN-alpha responses. J. Immunol. 193, 364–371. https://doi.org/10.4049/jimmunol.1303219.

Beaumont, P.E., McHugh, B., Gwyer Findlay, E., Mackellar, A., Mackenzie, K.J., Gallo, R. L., Govan, J.R., Simpson, A.J., Davidson, D.J., 2014. Cathelicidin host defence peptide augments clearance of pulmonary Pseudomonas aeruginosa infection by its influence on neutrophil function in vivo. PLoS One 9, e99029. https://doi.org/10.1371/journal.pone.0099029.

Bensch, K.W., Raida, M., Magert, H.J., Schulz-Knappe, P., Forssmann, W.G., 1995. hBD-1: a novel beta-defensin from human plasma. FEBS Lett. 368, 331–335.

Bergman, P., Walter-Jallow, L., Broliden, K., Agerberth, B., Soderlund, J., 2007. The antimicrobial peptide LL-37 inhibits HIV-1 replication. Curr. HIV Res. 5, 410–415. https://doi.org/10.2174/157016207781023947.

Bhunia, A., Mohanram, H., Bhattacharjya, S., 2009. Lipopolysaccharide bound structures of the active fragments of fowlicidin-1, a cathelicidin family of antimicrobial and antiendotoxic peptide from chicken, determined by transferred nuclear Overhauser effect spectroscopy. Biopolymers 92, 9–22. https://doi.org/10.1002/bip.21104.

Bommineni, Y.R., Dai, H., Gong, Y.X., Soulages, J.L., Fernando, S.C., Desilva, U., Prakash, O., Zhang, G., 2007. Fowlicidin-3 is an alpha-helical cationic host defense peptide with potent antibacterial and lipopolysaccharide-neutralizing activities. FEBS J. 274, 418–428. https://doi.org/10.1111/j.1742-4658.2006.05589.x.

Bommineni, Y.R., Pham, G.H., Sunkara, L.T., Achanta, M., Zhang, G., 2014. Immune regulatory activities of fowlicidin-1, a cathelicidin host defense peptide. Mol. Immunol. 59, 55–63. https://doi.org/10.1016/j.molimm.2014.01.004.

Boniotto, M., Jordan, W.J., Eskdale, J., Tossi, A., Antcheva, N., Crovella, S., Connell, N. D., Gallagher, G., 2006. Human beta-defensin 2 induces a vigorous cytokine response in peripheral blood mononuclear cells. Antimicrob. Agents Chemother. 50, 1433–1441. https://doi.org/10.1128/AAC.50.4.1433-1441.2006.

Bowdish, D.M., Davidson, D.J., Speert, D.P., Hancock, R.E., 2004. The human cationic peptide LL-37 induces activation of the extracellular signal-regulated kinase and p38 kinase pathways in primary human monocytes. J. Immunol. 172, 3758–3765.

Brown, K.L., Poon, G.F., Birkenhead, D., Pena, O.M., Falsafi, R., Dahlgren, C., Karlsson, A., Bylund, J., Hancock, R.E., Johnson, P., 2011. Host defense peptide LL-37 selectively reduces proinflammatory macrophage responses. J. Immunol. 186, 5497–5505. https://doi.org/10.4049/jimmunol.1002508.

Chakraborty, K., Maity, P.C., Sil, A.K., Takeda, Y., Das, S., 2009. cAMP stringently regulates human cathelicidin antimicrobial peptide expression in the mucosal epithelial cells by activating cAMP-response element-binding protein, AP-1, and inducible cAMP early repressor. J. Biol. Chem. 284, 21810–21827. https://doi.org/10.1074/jbc.M109.001180.

Chang, C.I., Zhang, Y.A., Zou, J., Nie, P., Secombes, C.J., 2006. Two cathelicidin genes are present in both rainbow trout (Oncorhynchus mykiss) and atlantic salmon (Salmo salar). Antimicrob. Agents Chemother. 50, 185–195. https://doi.org/10.1128/AAC.50.1.185-195.2006.

Chapman, J.R., Hellgren, O., Helin, A.S., Kraus, R.H., Cromie, R.L., Waldenstrom, J., 2016. The evolution of innate immune genes: purifying and balancing selection on beta-defensins in waterfowl. Mol. Biol. Evol. 33, 3075–3087. https://doi.org/10.1093/molbev/msw167.

Cheng, Y., Prickett, M.D., Gutowska, W., Kuo, R., Belov, K., Burt, D.W., 2015. Evolution of the avian beta-defensin and cathelicidin genes. BMC Evol. Biol. 15, 188. https://doi.org/10.1186/s12862-015-0465-3.

Chronnell, C.M., Ghali, L.R., Ali, R.S., Quinn, A.G., Holland, D.B., Bull, J.J., Cunliffe, W. J., McKay, I.A., Philpott, M.P., Muller-Rover, S., 2001. Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions. J. Invest. Dermatol. 117, 1120–1125. https://doi.org/10.1046/j.0022-202x.2001.01569.x.

Circo, R., Skerlavaj, B., Gennaro, R., Amoroso, A., Zanetti, M., 2002. Structural and functional characterization of hBD-1(Ser35), a peptide deduced from a DEFB1 polymorphism. Biochem. Biophys. Res. Commun. 293, 586–592. https://doi.org/10.1016/S0006-291X(02)00267-X.

Com, E., Bourgeon, F., Evrard, B., Ganz, T., Colleu, D., Jegou, B., Pineau, C., 2003. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol. Reprod. 68, 95–104.
Consortium, I.C.G.S., 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432, 695–716. https://doi.org/10.1038/nature03154.
Coorens, M., Banaschewski, B.J.H., Baer, B.J., Yamashita, C., van Dijk, A., Haagsman, H. P., Veldhuizen, R.A.W., Veldhuizen, E.J.A., 2017a. Killing of P. aeruginosa by chicken cathelicidin-2 is immunogenically silent, preventing lung inflammation in vivo. Infect. Immun. https://doi.org/10.1128/IAI.00546-17.
Coorens, M., Scheenstra, M.R., Veldhuizen, E.J., Haagsman, H.P., 2017b. Interspecies cathelicidin comparison reveals divergence in antimicrobial activity, TLR modulation, chemokine induction and regulation of phagocytosis. Sci. Rep. 7, 40874. https://doi.org/10.1038/srep40874.
Cuperus, T., Coorens, M., van Dijk, A., Haagsman, H.P., 2013. Avian host defense peptides. Dev. Comp. Immunol. 41, 352–369. https://doi.org/10.1016/j.dci.2013.04.019.
Cuperus, T., van Dijk, A., Dwars, R.M., Haagsman, H.P., 2016a. Localization and developmental expression of two chicken host defense peptides: cathelicidin-2 and avian beta-defensin 9. Dev. Comp. Immunol. 61, 48–59. https://doi.org/10.1016/j.dci.2016.03.008.
Cuperus, T., van Dijk, A., Matthijs, M.G., Veldhuizen, E.J., Haagsman, H.P., 2016b. Protective effect of in ovo treatment with the chicken cathelicidin analog D-CATH-2 against avian pathogenic E. coli. Sci. Rep. 6, 26622. https://doi.org/10.1038/srep26622.
Cuperus, T., Kraaij, M.D., Zomer, A.L., van Dijk, A., Haagsman, H.P., 2018. Immunomodulation and effects on microbiota after in ovo administration of chicken cathelicidin-2. PLoS One 13, e0198188. https://doi.org/10.1371/journal.pone.0198188.
Currie, S.M., Findlay, E.G., McHugh, B.J., Mackellar, A., Man, T., Macmillan, D., Wang, H., Fitch, P.M., Schwarze, J., Davidson, D.J., 2013. The human cathelicidin LL-37 has antiviral activity against respiratory syncytial virus. PLoS One 8, e73659. https://doi.org/10.1371/journal.pone.0073659.
Currie, S.M., Gwyer Findlay, E., McFarlane, A.J., Fitch, P.M., Bottcher, B., Colegrave, N., Paras, A., Jozwik, A., Chiu, C., Schwarze, J., Davidson, D.J., 2016. Cathelicidins have direct antiviral activity against respiratory syncytial virus in vitro and protective function in vivo in mice and humans. J. Immunol. 196, 2699–2710. https://doi.org/10.4049/jimmunol.1502478.
Daher, K.A., Selsted, M.E., Lehrer, R.I., 1986. Direct inactivation of viruses by human granulocyte defensins. J. Virol. 60, 1068–1074. https://doi.org/10.1128/JVI.60.3.1068-1074.1986.
Daimon, T., Caxton-Martins, A., 1977. Electron microscopic and enzyme cytochemical studies on granules of mature chicken granular leucocytes. J. Anat. 123, 553–562.
Das, S.C., Isobe, N., Yoshimura, Y., 2011. Expression of Toll-like receptors and avian beta-defensins and their changes in response to bacterial components in chicken sperm. Poult. Sci. 90, 417–425. https://doi.org/10.3382/ps.2010-00850.
Dathe, M., Wieprecht, T., 1999. Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. Biochim. Biophys. Acta 1462, 71–87.
Datta, A., Bhattacharyya, D., Singh, S., Ghosh, A., Schmidtchen, A., Malmsten, M., Bhunia, A., 2016. Role of aromatic amino acids in lipopolysaccharide and membrane interactions of antimicrobial peptides for use in plant disease control. J. Biol. Chem. 291, 13301–13317. https://doi.org/10.1074/jbc.M116.719575.
Davidson, D.J., Currie, A.J., Reid, G.S., Bowdish, D.M., MacDonald, K.L., Ma, R.C., Hancock, R.E., Speert, D.P., 2004. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J. Immunol. 172, 1146–1156.
De, Y., Chen, Q., Schmidt, A.P., Anderson, G.M., Wang, J.M., Wooters, J., Oppenheim, J. J., Chertov, O., 2000. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J. Exp. Med 192, 1069–1074.
Derache, C., Esnault, E., Bonsergent, C., Le Vern, Y., Quere, P., Lalmanach, A.C., 2009a. Differential modulation of beta-defensin gene expression by Salmonella Enteritidis in intestinal epithelial cells from resistant and susceptible chicken inbred lines. Dev. Comp. Immunol. 33, 959–966. https://doi.org/10.1016/j.dci.2009.03.005.
Derache, C., Labas, V., Aucagne, V., Meudal, H., Landon, C., Delmas, A.F., Magallon, T., Lalmanach, A.C., 2009b. Primary structure and antibacterial activity of chicken bone marrow-derived beta-defensins. Antimicrob. Agents Chemother. 53, 4647–4655. https://doi.org/10.1128/AAC.00301-09.
Derache, C., Meudal, H., Aucagne, V., Mark, K.J., Cadene, M., Delmas, A.F., Lalmanach, A.C., Landon, C., 2012. Initial insights into structure-activity relationships of avian defensins. J. Biol. Chem. 287, 7746–7755. https://doi.org/10.1074/jbc.M111.312108.
Evans, E.W., Beach, G.G., Wunderlich, J., Harmon, B.G., 1994. Isolation of antimicrobial peptides from avian heterophils. J. Leukoc. Biol. 56, 661–665.
Fang, X.M., Shu, Q., Chen, Q.X., Book, M., Sahl, H.G., Hoeft, A., Stuber, F., 2003. Differential expression of alpha- and beta-defensins in human peripheral blood. Eur. J. Clin. Invest 33, 82–87.
Feng, F., Chen, C., Zhu, W., He, W., Guang, H., Li, Z., Wang, D., Liu, J., Chen, M., Wang, Y., Yu, H., 2011. Gene cloning, expression and characterization of avian cathelicidin orthologs, Cc-CATHs, from Coturnix coturnix. FEBS J. 278, 1573–1584. https://doi.org/10.1111/j.1742-4658.2011.08080.x.
Frohm Nilsson, M., Sandstedt, B., Sorensen, O., Weber, G., Borregaard, N., Stahle-Backdahl, M., 1999. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. Infect. Immun. 67, 2561–2566.
Funderburg, N., Lederman, M.M., Feng, Z., Drage, M.G., Jadlowsky, J., Harding, C.V., Weinberg, A., Sieg, S.F., 2007. Human -defensin-3 activates professional antigen-presenting cells via Toll-like receptors 1 and 2. Proc. Natl. Acad. Sci. U. S. A 104, 18631–18635. https://doi.org/10.1073/pnas.0702130104.
Gallo, R.L., Nizet, V., 2008. Innate barriers against infection and associated disorders. Drug Discov. Today Dis. Mech. 5, 145–152. https://doi.org/10.1016/j.ddmec.2008.04.009.
Gallo, R.L., Kim, K.J., Bernfield, M., Kozak, C.A., Zanetti, M., Merluzzi, L., Gennaro, R., 1997. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J. Biol. Chem. 272, 13088–13093.
Ganz, T., 1987. Extracellular release of antimicrobial defensins by human polymorphonuclear leukocytes. Infect. Immun. 55, 568–571.
Ganz, T., 2003. Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. 3, 710–720. https://doi.org/10.1038/nri1180.
Ganz, T., Selsted, M.E., Szklarek, D., Harwig, S.S., Daher, K., Bainton, D.F., Lehrer, R.I., 1985. Defensins. Natural peptide antibiotics of human neutrophils. J. Clin. Invest 76, 1427–1435. https://doi.org/10.1172/JCI112120.
Garcia, J.R., Krause, A., Schulz, S., Rodriguez-Jimenez, F.J., Kluver, E., Adermann, K., Forssmann, U., Frimpong-Boateng, A., Bals, R., Forssmann, W.G., 2001. Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. FASEB J. 15, 1819–1821.
Goitsuka, R., Chen, C.L., Benyon, L., Asano, Y., Kitamura, D., Cooper, M.D., 2007. Chicken cathelicidin-B1, an antimicrobial guardian at the mucosal M cell gateway. Proc. Natl. Acad. Sci. U. S. A 104, 15063–15068. https://doi.org/10.1073/pnas.0707037104.
Goldman, M.J., Anderson, G.M., Stolzenberg, E.D., Kari, U.P., Zasloff, M., Wilson, J.M., 1997. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 88, 553–560.
Gombart, A.F., Borregaard, N., Koeffler, H.P., 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. https://doi.org/10.1096/fj.04-3284com.
Gong, D., Wilson, P.W., Bain, M.M., McDade, K., Kalina, J., Herve-Grepinet, V., Nys, Y., Dunn, I.C., 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. https://doi.org/10.1186/1471-2172-11-12.
Gordon, Y.J., Huang, L.C., Romanowski, E.G., Yates, K.A., Proske, R.J., McDermott, A.M., 2005. Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. Curr. Eye Res. 30, 385–394. https://doi.org/10.1080/02713680590934111.
Griffin, D.K., Robertson, L.B., Tempest, H.G., Skinner, B.M., 2007. The evolution of the avian genome as revealed by comparative molecular cytogenetics. Cytogenet. Genome Res. 117, 64–77. https://doi.org/10.1159/000103166.
Gudmundsson, G.H., Magnusson, K.P., Chowdhary, B.P., Johansson, M., Andersson, L., Boman, H.G., 1995. Structure of the gene for porcine peptide antibiotic PR-39, a cathelin gene family member: comparative mapping of the locus for the human peptide antibiotic FALL-39. Proc. Natl. Acad. Sci. U. S. A. 92, 7085–7089.
Gudmundsson, G.H., Agerberth, B., Odeberg, J., Bergman, T., Olsson, B., Salcedo, R., 1996. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. Eur. J. Biochem. 238, 325–332.
Guyot, N., Meudal, H., Trapp, S., Iochmann, S., Silvestre, A., Jousset, G., Labas, V., Reverdiau, P., Loth, K., Herve, V., Aucagne, V., Delmas, A.F., Rehault-Godbert, S., Landon, C., 2020. Structure, function, and evolution of Gga-AvBD11, the archetype of the structural avian-double-beta-defensin family. Proc. Natl. Acad. Sci. U. S. A 117, 337–345. https://doi.org/10.1073/pnas.1912941117.
Halper, J., Burt, D.W., Romanov, M.N., 2004. On reassessment of the chicken TGFB4 gene as TGFB1. Growth Factors 22, 121–122.
Hamilton, J.A., 2002. GM-CSF in inflammation and autoimmunity. Trends Immunol. 23, 403–408.
Hancock, R.E., Haney, E.F., Gill, E.E., 2016. The immunology of host defence peptides: beyond antimicrobial activity. Nat. Rev. Immunol. 16, 321–334. https://doi.org/10.1038/nri.2016.29.
Harder, J., Bartels, J., Christophers, E., Schroder, J.M., 2001. Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J. Biol. Chem. 276, 5707–5713. https://doi.org/10.1074/jbc.M008557200.
Harder, J., Meyer-Hoffert, U., Wehkamp, K., Schwichtenberg, L., Schroder, J.M., 2004. Differential gene induction of human beta-defensins (hBD-1, -2, -3, and -4) in keratinocytes is inhibited by retinoic acid. J. Invest. Dermatol. 123, 522–529. https://doi.org/10.1111/j.0022-202X.2004.23234.x.
Harwig, S.S., Swiderek, K.M., Kokryakov, V.N., Tan, L., Lee, T.D., Panyutich, E.A., Aleshina, G.M., Shamova, O.V., Lehrer, R.I., 1994. Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. FEBS Lett. 342, 281–285.
Hase, K., Eckmann, L., Leopard, J.D., Varki, N., Kagnoff, M.F., 2002. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. Infect. Immun. 70, 953–963.
Hase, K., Murakami, M., Iimura, M., Cole, S.P., Horibe, Y., Ohtake, T., Obonyo, M., Gallo, R.L., Eckmann, L., Kagnoff, M.F., 2003. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against Helicobacter pylori. Gastroenterology 125, 1613–1625.
Hasenstein, J.R., Lamont, S.J., 2007. Chicken gallinacin gene cluster associated with Salmonella response in advanced intercross line. Avian Dis. 51, 561–567 doi: 10.1637/0005-2086(2007)51[561:CGGCAW]2.0.CO;2.

Hasenstein, J.R., Zhang, G., Lamont, S.J., 2006. Analyses of Five gallinacin genes and the Salmonella enterica serovar Enteritidis response in poultry. Infect. Immun. 74, 3375–3380. https://doi.org/10.1128/IAI.00027-06.

Hazrati, E., Galen, B., Lu, W., Wang, W., Ouyang, Y., Keller, M.J., Lehrer, R.I., Herold, B. C., 2006. Human alpha- and beta-defensins block multiple steps in herpes simplex virus infection. J. Immunol. 177, 8658–8666. https://doi.org/10.4049/jimmunol.177.12.8658.

Hedges, S.B., Kumar, S., 2004. Precision of molecular time estimates. Trends Genet 20, 242–247. https://doi.org/10.1016/j.tig.2004.03.004.

Hellgren, O., Ekblom, R., 2010. Evolution of a cluster of innate immune genes (beta-defensins) along the ancestral lines of chicken and zebra finch. Immunome Res. 6, 3. https://doi.org/10.1186/1745-7580-6-3.

Hellgren, O., Sheldon, B.C., Buckling, A., 2010. In vitro tests of natural allelic variation of innate immune genes (avian beta-defensins) reveal functional differences in microbial inhibition. J. Evol. Biol. 23, 2726–2730.

Herrera, R., Morris, M., Rosbe, K., Feng, Z., Weinberg, A., Tugizov, S., 2016. Human beta-defensins 2 and -3 cointernalize with human immunodeficiency virus via heparan sulfate proteoglycans and reduce infectivity of intracellular virions in tonsil epithelial cells. Virology 487, 172–187. https://doi.org/10.1016/j.virol.2015.09.025.

Herve, V., Meudal, H., Labas, V., Rehault-Godbert, S., Gautron, J., Berges, M., Guyot, N., Delmas, A.F., Nys, Y., Landon, C., 2014. Three-dimensional NMR structure of Hen Egg Gallin (Chicken Ovodefensin) reveals a new variation of the beta-defensin fold. J. Biol. Chem. 289, 7211–7220. https://doi.org/10.1074/jbc.M113.507046.

Herve-Grepinet, V., Rehault-Godbert, S., Labas, V., Magallon, T., Derache, C., Lavergne, M., Gautron, J., Lalmanach, A.C., Nys, Y., 2010. Purification and characterization of avian beta-defensin 11, an antimicrobial peptide of the hen egg. Antimicrob. Agents Chemother. 54, 4401–4409. https://doi.org/10.1128/AAC.00204-10.

Higgs, R., Lynn, D.J., Cahalane, S., Alana, I., Hewage, C.M., James, T., Lloyd, A.T., O'Farrelly, C., 2007. Modification of chicken avian beta-defensin-8 at positively selected amino acid sites enhances specific antimicrobial activity. Immunogenetics 59, 573–580. https://doi.org/10.1007/s00251-007-0219-5.

Hollox, E.J., Huffmeier, U., Zeeuwen, P.L., Palla, R., Lascorz, J., Rodijk-Olthuis, D., van de Kerkhof, P.C., Traupe, H., de Jongh, G., den Heijer, M., Reis, A., Armour, J.A., Schalkwijk, J., 2008. Psoriasis is associated with increased beta-defensin genomic copy number. Nat. Genet. 40, 23–25. https://doi.org/10.1038/ng.2007.48.

Hoover, D.M., Chertov, O., Lubkowski, J., 2001. The structure of human beta-defensin-1: new insights into structural properties of beta-defensins. J. Biol. Chem. 276, 39021–39026. https://doi.org/10.1074/jbc.M103830200.

Hoover, D.M., Wu, Z., Tucker, K., Lu, W., Lubkowski, J., 2003. Antimicrobial characterization of human beta-defensin 3 derivatives. Antimicrob. Agents Chemother. 47, 2804–2809.

Hou, M., Zhang, N., Yang, J., Meng, X., Yang, R., Li, J., Sun, T., 2013. Antimicrobial peptide LL-37 and IDR-1 ameliorate MRSA pneumonia in vivo. Cell Physiol. Biochem. 32, 614–623. https://doi.org/10.1159/000354465.

Howell, M.D., Jones, J.F., Kisich, K.O., Streib, J.E., Gallo, R.L., Leung, D.Y., 2004. Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum. J. Immunol. 172, 1763–1767. https://doi.org/10.4049/jimmunol.172.3.1763.

Hu, Z., Murakami, T., Suzuki, K., Tamura, H., Reich, J., Kuwahara-Arai, K., Iba, T., Nagaoka, I., 2016. Antimicrobial cathelicidin peptide LL-37 inhibits the pyroptosis of macrophages and improves the survival of polybacterial septic mice. Int. Immunol. 28, 245–253. https://doi.org/10.1093/intimm/dxv113.

Huang, G.T., Zhang, H.B., Kim, D., Liu, L., Ganz, T., 2002. A model for antimicrobial gene therapy: demonstration of human beta-defensin 2 antimicrobial activities in vivo. Hum. Gene Ther. 13, 2017–2025. https://doi.org/10.1089/10430340260395875.

Huang, Y., Li, Y., Burt, D.W., Chen, H., Zhang, Y., 2013. The duck genome and transcriptome provide insight into an avian influenza virus reservoir species. Nat. Gen. 45, 776–784. https://doi.org/10.1038/ng.2657.

Hughes, A.L., Friedman, R., 2008. Genome size reduction in the chicken has involved massive loss of ancestral protein-coding genes. Mol. Biol. Evol. 25, 2681–2688. https://doi.org/10.1093/molbev/msn207.

Ishige, T., Hara, H., Hirano, T., Mannen, H., Kono, T., Hanzawa, K., 2016. Basic characterization of avian beta-defensin genes in the Japanese quail, Coturnix japonica. Anim. Sci. J. 87, 311–320. https://doi.org/10.1111/asj.12436.

Jang, H.J., Lee, H.J., Kang, K.S., Song, K.D., Kim, T.H., Song, C.S., Park, M.N., 2015. Molecular responses to the influenza A virus in chicken trachea-derived cells. Poult. Sci. 94, 1190–1201. https://doi.org/10.3382/ps/pev033.

Jelinsky, S.A., Turner, T.T., Bang, H.J., Finger, J.N., Solarz, M.K., Wilson, E., Brown, E.L., Kopf, G.S., Johnston, D.S., 2007. The rat epididymal transcriptome: comparison of segmental gene expression in the rat and mouse epididymides. Biol. Reprod. 76, 561–570. https://doi.org/10.1095/biolreprod.106.057323.

Johansson, J., Gudmundsson, G.H., Rottenberg, M.E., Berndt, K.D., Agerberth, B., 1998. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. J. Biol. Chem. 273, 3718–3724.

Jones, E.A., Kananurak, A., Bevins, C.L., Hollox, E.J., Bakaletz, L.O., 2014. Copy number variation of the beta defensin gene cluster on chromosome 8p influences the bacterial microbiota within the nasopharynx of otitis-prone children. PLoS One 9, e98269. https://doi.org/10.1371/journal.pone.0098269.

Jurevic, R.J., Bai, M., Chadwick, R.B., White, T.C., Dale, B.A., 2003. Single-nucleotide polymorphisms (SNPs) in human beta-defensin 1: high-throughput SNP assays and association with Candida carriage in type I diabetics and nondiabetic controls. J. Clin. Microbiol 41, 90–96.

Kalenik, B.M., Gora-Sochacka, A., Stachyra, A., Pietrzak, M., Kopera, E., Fogtman, A., Sirko, A., 2018. Transcriptional response to a prime/boost vaccination of chickens with three vaccine variants based on HA DNA and Pichia-produced HA protein. Dev. Comp. Immunol. 88, 8–18. https://doi.org/10.1016/j.dci.2018.07.001.

King, A.E., Kelly, R.W., Sallenave, J.M., Bocking, A.D., Challis, J.R., 2007a. Innate immune defences in the human uterus during pregnancy. Placenta 28, 1099–1106. https://doi.org/10.1016/j.placenta.2007.06.002.

King, A.E., Paltoo, A., Kelly, R.W., Sallenave, J.M., Bocking, A.D., Challis, J.R., 2007b. Expression of natural antimicrobials by human placenta and fetal membranes. Placenta 28, 161–169. https://doi.org/10.1016/j.placenta.2006.01.006.

Kluver, E., Schulz-Maronde, S., Scheid, S., Meyer, B., Forssmann, W.G., Adermann, K., 2005. Structure-activity relation of human beta-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity. Biochemistry 44, 9804–9816. https://doi.org/10.1021/bi050272k.

Kocsis, A.K., Kiss, Z.F., Tiszlavicz, L., Tiszlavicz, Z., Mandi, Y., 2009. Potential role of human beta-defensin 1 in Helicobacter pylori-induced gastritis. Scand. J. Gastroenterol. 44, 289–295. https://doi.org/10.1080/00365520802530879.

Kota, S., Sabbah, A., Chang, T.H., Harnack, R., Xiang, Y., Meng, X., Bose, S., 2008. Role of human beta-defensin-2 during tumor necrosis factor-alpha/NF-kappaB-mediated innate antiviral response against human respiratory syncytial virus. J. Biol. Chem. 283, 22417–22429. https://doi.org/10.1074/jbc.M710415200.

Kraaij, M.D., van Dijk, A., Haagsman, H.P., 2017. CATH-2 and LL-37 increase mannose receptor expression, antigen presentation and the endocytic capacity of chicken mononuclear phagocytes. Mol. Immunol. 90, 118–125. https://doi.org/10.1016/j.molimm.2017.07.005.

Landon, C., Thouzeau, C., Labbe, H., Bulet, P., Vovelle, F., 2004. Solution structure of spheniscin, a beta-defensin from the penguin stomach. J. Biol. Chem. 279, 30433–30439. https://doi.org/10.1074/jbc.M401338200.

Lee, M.O., Bornelov, S., Andersson, L., Lamont, S.J., Chen, J., Womack, J.E., 2016a. Duplication of chicken defensin7 gene generated by gene conversion and homologous recombination. Proc. Natl. Acad. Sci. U. S. A 113, 13815–13820. https://doi.org/10.1073/pnas.1616948113.

Lee, S.I., Jang, H.J., Jeon, M.H., Lee, M.O., Kim, J.S., Jeon, I.S., Byun, S.J., 2016b. Transcriptional regulation of cathelicidin genes in chicken bone marrow cells. Poult. Sci. 95, 912–919. https://doi.org/10.3382/ps/pev361.

Leikina, E., Delanoe-Ayari, H., Melikov, K., Cho, M.S., Chen, A., Waring, A.J., Wang, W., Xie, Y., Loo, J.A., Lehrer, R.I., Chernomordik, L.V., 2005. Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoproteins. Nat. Immunol. 6, 995–1001. https://doi.org/10.1038/ni1248.

Levinson, P., Kaul, R., Kimani, J., Ngugi, E., Moses, S., MacDonald, K.S., Broliden, K., Hirbod, T., 2009. Levels of innate immune factors in genital fluids: association of alpha defensins and LL-37 with genital infections and increased HIV acquisition. AIDS 23, 309–317. https://doi.org/10.1097/QAD.0b013e328321809c.

Li, T., Guo, F., Wang, Q., Fang, H., Li, Z., Wang, D., Wang, H., 2015. N-terminus three residues deletion mutant of human beta-defensin 3 with remarkably enhanced salt-resistance. PLoS One 10, e0117913. https://doi.org/10.1371/journal.pone.0117913.

Liu, A.Y., Destoumieux, D., Wong, A.V., Park, C.H., Valore, E.V., Liu, L., Ganz, T., 2002. Human beta-defensin-2 production in keratinocytes is regulated by interleukin-1, bacteria, and the state of differentiation. J. Invest. Dermatol. 118, 275–281. https://doi.org/10.1046/j.0022-202x.2001.01651.x.

Liu, C., Jiang, L., Liu, L., Sun, L., Zhao, W., Chen, Y., Qi, T., Han, Z., Shao, Y., Liu, S., Ma, D., 2018. Induction of avian beta-defensin 2 is possibly mediated by the p38 MAPK signal pathway in chicken embryo fibroblasts after newcastle disease virus infection. Front. Microbiol. 9, 751. https://doi.org/10.3389/fmicb.2018.00751.

Liu, H., Yu, H., Gu, Y., Xin, A., Zhang, Y., Diao, H., Lin, D., 2013. Human beta-defensin DEFB126 is capable of inhibiting LPS-mediated inflammation. Appl. Microbiol. Biotechnol. 97, 3395–3408. https://doi.org/10.1007/s00253-012-4588-9.

Lopez Campos, G.N., Velarde Felix, J.S., Sandoval Ramirez, L., Cazares Salazar, S., Corona Nakamura, A.L., Amaya Tapia, G., Prado Montes de Oca, E., 2014. Polymorphism in cathelicidin gene (CAMP) that alters Hypoxia-inducible factor (HIF-1alpha::ARNT) binding is not associated with tuberculosis. Int. J. Immunogenet. 41, 54–62. https://doi.org/10.1111/iji.12080.

Lynn, D.J., Higgs, R., Gaines, S., Tierney, J., James, T., Lloyd, A.T., Fares, M.A., Mulcahy, G., O'Farrelly, C., 2004. Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 56, 170–177. https://doi.org/10.1007/s00251-004-0675-0.

Lynn, D.J., Higgs, R., Lloyd, A.T., O'Farrelly, C., Herve-Grepinet, V., Nys, Y., Brinkman, F.S., Yu, P.L., Soulier, A., Kaiser, P., Zhang, G., Lehrer, R.I., 2007. Avian beta-defensin nomenclature: a community proposed update. Immunol. Lett. 110, 86–89. https://doi.org/10.1016/j.imlet.2007.03.007.

Mageed, A.M., Isobe, N., Yoshimura, Y., 2008. Expression of avian beta-defensins in the oviduct and effects of lipopolysaccharide on their expression in the vagina of hens. Poult. Sci. 87, 979–984. https://doi.org/10.3382/ps.2007-00283.

Maiti, S., Patro, S., Purohit, S., Jain, S., Senapati, S., Dey, N., 2014. Effective control of Salmonella infections by employing combinations of recombinant antimicrobial human beta-defensins hBD-1 and hBD-2. Antimicrob. Agents Chemother. 58, 6896–6903. https://doi.org/10.1128/AAC.03628-14.

Malm, J., Sorensen, O., Persson, T., Frohm-Nilsson, M., Johansson, B., Bjartell, A., Lilja, H., Stahle-Backdahl, M., Borregaard, N., Egesten, A., 2000. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. Infect. Immun. 68, 4297–4302.

Mann, K., 2008. Proteomic analysis of the chicken egg vitelline membrane. Proteomics 8, 2322–2332. https://doi.org/10.1002/pmic.200800032.

Marcinkiewicz, M., Majewski, S., 2016. The role of antimicrobial peptides in chronic inflammatory skin diseases. Post. Dermatol. Alergol. 33, 6–12. https://doi.org/10.5114/pdia.2015.48066.

Matsuzaki, K., 1999. Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim. Biophys. Acta 1462, 1–10.

McHugh, B.J., Wang, R., Li, H.N., Beaumont, P.E., Kells, R., Stevens, H., Young, L., Rossi, A.G., Gray, R.D., Dorin, J.R., Gwyer Findlay, E.L., Brough, D., Davidson, D.J., 2019. Cathelicidin is a "fire alarm", generating protective NLRP3-dependent airway epithelial cell inflammatory responses during infection with Pseudomonas aeruginosa. PLoS Pathog. 15, e1007694 https://doi.org/10.1371/journal.ppat.1007694.

Miyashita, T., Coates, M.I., Farrar, R., Larson, P., Manning, P.L., Wogelius, R.A., Edwards, N.P., Anne, J., Bergmann, U., Palmer, A.R., Currie, P.J., 2019. Hagfish from the Cretaceous Tethys Sea and a reconciliation of the morphological-molecular conflict in early vertebrate phylogeny. Proc. Natl. Acad. Sci. U. S. A. 116, 2146–2151. https://doi.org/10.1073/pnas.1814794116.

Molhoek, E.M., den Hertog, A.L., de Vries, A.M., Nazmi, K., Veerman, E.C., Hartgers, F. C., Yazdanbakhsh, M., Bikker, F.J., van der Kleij, D., 2009. Structure-function relationship of the human antimicrobial peptide LL-37 and LL-37 fragments in the modulation of TLR responses. Biol. Chem. 390, 295–303. https://doi.org/10.1515/BC.2009.037.

Mookherjee, N., Wilson, H.L., Doria, S., Popowych, Y., Falsafi, R., Yu, J.J., Li, Y., Veatch, S., Roche, F.M., Brown, K.L., Brinkman, F.S., Hokamp, K., Potter, A., Babiuk, L.A., Griebel, P.J., Hancock, R.E., 2006. Bovine and human cathelicidin cationic host defense peptides similarly suppress transcriptional responses to bacterial lipopolysaccharide. J. Leukoc. Biol. 80, 1563–1574. https://doi.org/10.1189/jlb.0106048.

Mookherjee, N., Anderson, M.A., Haagsman, H.P., Davidson, D.J., 2020. Antimicrobial host defence peptides: functions and clinical potential. Nat. Rev. Drug Discov. 19, 311–332. https://doi.org/10.1038/s41573-019-0058-8.

Motzkus, D., Schulz-Maronde, S., Heitland, A., Schulz, A., Forssmann, W.G., Jubner, M., Maronde, E., 2006. The novel beta-defensin DEFB123 prevents lipopolysaccharide-mediated effects in vitro and in vivo. FASEB J. 20, 1701–1702. https://doi.org/10.1096/fj.05-4970fje.

Nagaoka, I., Tsutsumi-Ishii, Y., Yomogida, S., Yamashita, T., 1997. Isolation of cDNA encoding guinea pig neutrophil cationic antibacterial polypeptide of 11 kDa (CAP11) and evaluation of CAP11 mRNA expression during neutrophil maturation. J. Biol. Chem. 272, 22742–22750.

Nagaoka, I., Hirota, S., Niyonsaba, F., Hirata, M., Adachi, Y., Tamura, H., Tanaka, S., Heumann, D., 2002. Augmentation of the lipopolysaccharide-neutralizing activities of human cathelicidin CAP18/LL-37-derived antimicrobial peptides by replacement with hydrophobic and cationic amino acid residues. Clin. Diagn. Lab. Immunol. 9, 972–982.

Nam, K., Ellegren, H., 2012. Recombination drives vertebrate genome contraction. PLoS Genet 8, e1002680. https://doi.org/10.1371/journal.pgen.1002680.

Nell, M.J., Tjabringa, G.S., Wafelman, A.R., Verrijk, R., Hiemstra, P.S., Drijfhout, J.W., Grote, J.J., 2006. Development of novel LL-37 derived antimicrobial peptides with LPS and LTA neutralizing and antimicrobial activities for therapeutic application. Peptides 27, 649–660. https://doi.org/10.1016/j.peptides.2005.09.016.

Niu, S., Jahejo, A.R., Jia, F.J., Li, X., Ning, G.B., Zhang, D., Ma, H.L., Hao, W.F., Gao, W. W., Zhao, Y.J., Gao, S.M., Li, G.L., Li, J.H., Yan, F., Gao, R.K., Bi, Y.H., Han, L.X., Gao, G.F., Tian, W.X., 2018. Transcripts of antibacterial peptides in chicken erythrocytes infected with Marek's disease virus. BMC Vet. Res. 14, 363. https://doi.org/10.1186/s12917-018-1678-7.

Niyonsaba, F., Iwabuchi, K., Matsuda, H., Ogawa, H., Nagaoka, I., 2002a. Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. Int. Immunol. 14, 421–426.

Niyonsaba, F., Iwabuchi, K., Someya, A., Hirata, M., Matsuda, H., Ogawa, H., Nagaoka, I., 2002b. A cathelicidin family of human antibacterial peptide LL-37 induces mast cell chemotaxis. Immunology 106, 20–26.

Ordonez, S.R., Amarullah, I.H., Wubbolts, R.W., Veldhuizen, E.J., Haagsman, H.P., 2014. Fungicidal mechanisms of cathelicidins LL-37 and CATH-2 revealed by live-cell imaging. Antimicrob. Agents Chemother. 58, 2240–2248. https://doi.org/10.1128/AAC.01670-13.

Ottolini, B., Hornsby, M.J., Abujaber, R., MacArthur, J.A., Badge, R.M., Schwarzacher, T., Albertson, D.G., Bevins, C.L., Solnick, J.V., Hollox, E.J., 2014. Evidence of convergent evolution in humans and macaques supports an adaptive role for copy number variation of the beta-defensin-2 gene. Genome Biol. Evol. 6, 3025–3038. https://doi.org/10.1093/gbe/evu236.

Park, K., Elias, P.M., Oda, Y., Mackenzie, D., Mauro, T., Holleran, W.M., Uchida, Y., 2011. Regulation of cathelicidin antimicrobial peptide expression by an endoplasmic reticulum (ER) stress signaling, vitamin D receptor-independent pathway. J. Biol. Chem. 286, 34121–34130. https://doi.org/10.1074/jbc.M111.250431.

Park, S., Kim, J.I., Lee, I., Bae, J.Y., Hwang, M.W., Kim, D., Jang, S.I., Kim, H., Park, M.S., Kwon, H.J., Song, J.W., Cho, Y.S., Chun, W., Park, M.S., 2014. Inhibition of Pseudomonas aeruginosa with a recombinant RNA-based viral vector expressing human beta-defensin 4. BMC Microbiol 14, 237. https://doi.org/10.1186/s12866-014-0237-z.

Patil, A.A., Cai, Y., Sang, Y., Blecha, F., Zhang, G., 2005. Cross-species analysis of the mammalian beta-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. Physiol. Genom. 23, 5–17. https://doi.org/10.1152/physiolgenomics.00104.2005.

Pazgier, M., Hoover, D.M., Yang, D., Lu, W., Lubkowski, J., 2006. Human beta-defensins. Cell Mol. Life Sci. 63, 1294–1313. https://doi.org/10.1007/s00018-005-5540-2.

Peng, L., Du, W., Balhuizen, M.D., Haagsman, H.P., de Haan, C.A.M., Veldhuizen, E.J.A., 2020. Antiviral activity of chicken cathelicidin B1 against influenza A virus. Front. Microbiol. 11, 426. https://doi.org/10.3389/fmicb.2020.00426.

Porter, E.M., Liu, L., Oren, A., Anton, P.A., Ganz, T., 1997. Localization of human intestinal defensin 5 in Paneth cell granules. Infect. Immun. 65, 2389–2395.

Prum, R.O., Berv, J.S., Dornburg, A., Field, D.J., Townsend, J.P., Lemmon, E.M., Lemmon, A.R., 2015. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. Nature 526, 569–573. https://doi.org/10.1038/nature15697.

Qu, P., Gao, W., Chen, H., Li, D., Yang, N., Zhu, J., Feng, X., Liu, C., Li, Z., 2016. The central hinge link truncation of the antimicrobial peptide fowlicidin-3 enhances its cell selectivity without antibacterial activity loss. Antimicrob. Agents Chemother. 60, 2798–2806. https://doi.org/10.1128/AAC.02351-15.

Quinones-Mateu, M.E., Lederman, M.M., Feng, Z., Chakraborty, B., Weber, J., Rangel, H. R., Marotta, M.L., Mirza, M., Jiang, B., Kiser, P., Medvik, K., Sieg, S.F., Weinberg, A., 2003. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. AIDS 17, F39–F48. https://doi.org/10.1097/00002030-200311070-00001.

Radhakrishnan, Y., Hamil, K.G., Yenugu, S., Young, S.L., French, F.S., Hall, S.H., 2005. Identification, characterization, and evolution of a primate beta-defensin gene cluster. Genes Immun. 6, 203–210. https://doi.org/10.1038/sj.gene.6364184.

Ramasamy, K.T., Verma, P., Reddy, M.R., 2012. Differential gene expression of antimicrobial peptides beta defensins in the gastrointestinal tract of Salmonella serovar Pullorum infected broiler chickens. Vet. Res. Commun. 36, 57–62. https://doi.org/10.1007/s11259-011-9512-8.

Rice, W.G., Ganz, T., Kinkade Jr., J.M., Selsted, M.E., Lehrer, R.I., Parmley, R.T., 1987. Defensin-rich dense granules of human neutrophils. Blood 70, 757–765.

Rivas-Santiago, B., Rivas Santiago, C.E., Castaneda-Delgado, J.E., Leon-Contreras, J.C., Hancock, R.E., Hernandez-Pando, R., 2013. Activity of LL-37, CRAMP and antimicrobial peptide-derived compounds E2, E6 and CP26 against Mycobacterium tuberculosis. Int. J. Antimicrob. Agents 41, 143–148. https://doi.org/10.1016/j.ijantimicag.2012.09.015.

Rodriguez-Jimenez, F.J., Krause, A., Schulz, S., Forssmann, W.G., Conejo-Garcia, J.R., Schreeb, R., Motzkus, D., 2003. Distribution of new human beta-defensin genes clustered on chromosome 20 in functionally different segments of epididymis. Genomics 81, 175–183.

Rodriguez-Lecompte, J.C., Yitbarek, A., Cuperus, T., Echeverry, H., van Dijk, A., 2016. The immunomodulatory effect of vitamin D in chickens is dose-dependent and influenced by calcium and phosphorus levels. Poult. Sci. 95, 2547–2556. https://doi.org/10.3382/ps/pew186.

Rohrl, J., Yang, D., Oppenheim, J.J., Hehlgans, T., 2010. Human beta-defensin 2 and 3 and their mouse orthologs induce chemotaxis through interaction with CCR2. J. Immunol. 184, 6688–6694. https://doi.org/10.4049/jimmunol.0903984.

Ruiz-Rodriguez, M., Valdivia, E., Martin-Vivaldi, M., Martin-Platero, A.M., Martinez-Bueno, M., Mendez, M., Peralta-Sanchez, J.M., Soler, J.J., 2012. Antimicrobial activity and genetic profile of Enteroccoci isolated from hoopoes uropygial gland. PLoS One 7, e41843. https://doi.org/10.1371/journal.pone.0041843.

Ruiz-Rodriguez, M., Martinez-Bueno, M., Martin-Vivaldi, M., Valdivia, E., Soler, J.J., 2013. Bacteriocins with a broader antimicrobial spectrum prevail in enterococcal symbionts isolated from the hoopoe's uropygial gland. FEMS Microbiol. Ecol. 85, 495–502. https://doi.org/10.1111/1574-6941.12138.

Saidi, A., Gaboriaud, P., Lalmanach, A.C., Vanderlynden, L., Fessard, A., Vettori, P., Fort, G., Guabiraba, R., Schouler, C., Laurent, F., Guitton, E., Lecaille, F., Bussiere, F. I., Lalmanach, G., 2021. Upregulation of gut cathepsin L during Eimeria tenella infection. Res. Vet. Sci. 140, 109–116. https://doi.org/10.1016/j.rvsc.2021.08.013.

Sawai, M.V., Jia, H.P., Liu, L., Aseyev, V., Wiencek, J.M., McCray Jr., P.B., Ganz, T., Kearney, W.R., Tack, B.F., 2001. The NMR structure of human beta-defensin-2 reveals a novel alpha-helical segment. Biochemistry 40, 3810–3816.

Sawyer, J.G., Martin, N.L., Hancock, R.E., 1988. Interaction of macrophage cationic proteins with the outer membrane of Pseudomonas aeruginosa. Infect. Immun. 56, 693–698.

Schauber, J., Dorschner, R.A., Coda, A.B., Buchau, A.S., Liu, P.T., Kiken, D., Helfrich, Y. R., Kang, S., Elalieh, H.Z., Steinmeyer, A., Zugel, U., Bikle, D.D., Modlin, R.L., Gallo, R.L., 2007. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. J. Clin. Invest. 117, 803–811. https://doi.org/10.1172/JCI30142.

Schibli, D.J., Hunter, H.N., Aseyev, V., Starner, T.D., Wiencek, J.M., McCray Jr., P.B., Tack, B.F., Vogel, H.J., 2002. The solution structures of the human beta-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against Staphylococcus. aureus. J. Biol. Chem. 277, 8279–8289. https://doi.org/10.1074/jbc.M108830200.

Schmidt, N.W., Mishra, A., Lai, G.H., Davis, M., Sanders, L.K., Tran, D., Garcia, A., Tai, K. P., McCray, P.B., Ouellette, A.J., Selsted, M.E., Wong, G.C., 2011. Criterion for amino acid composition of defensins and antimicrobial peptides based on geometry of membrane destabilization. J. Am. Chem. Soc. 133, 6720–6727. https://doi.org/10.1021/ja200079a.

Schneider, V.A., Coorens, M., Ordonez, S.R., Tjeerdsma-van Bokhoven, J.L., Posthuma, G., van Dijk, A., Haagsman, H.P., Veldhuizen, E.J., 2016. Imaging the antimicrobial mechanism(s) of cathelicidin-2. Sci. Rep. 6, 32948. https://doi.org/10.1038/srep32948.

Schroder, J.M., Harder, J., 2006. Antimicrobial skin peptides and proteins. Cell Mol. Life Sci. 63, 469–486. https://doi.org/10.1007/s00018-005-5364-0.

Schroeder, B.O., Wu, Z., Nuding, S., Groscurth, S., Marcinowski, M., Beisner, J., Buchner, J., Schaller, M., Stange, E.F., Wehkamp, J., 2011. Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1. Nature 469, 419–423. https://doi.org/10.1038/nature09674.

Schutte, B.C., Mitros, J.P., Bartlett, J.A., Walters, J.D., Jia, H.P., Welsh, M.J., Casavant, T. L., McCray Jr., P.B., 2002. Discovery of five conserved beta -defensin gene clusters using a computational search strategy. Proc. Natl. Acad. Sci. U. S. A 99, 2129–2133. https://doi.org/10.1073/pnas.042692699.

Scott, M.G., Davidson, D.J., Gold, M.R., Bowdish, D., Hancock, R.E., 2002. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. J. Immunol. 169, 3883–3891. https://doi.org/10.4049/jimmunol.169.7.3883.

Selsted, M.E., Harwig, S.S., Ganz, T., Schilling, J.W., Lehrer, R.I., 1985. Primary structures of three human neutrophil defensins. J. Clin. Invest 76, 1436–1439. https://doi.org/10.1172/JCI112121.

Semple, C.A., Rolfe, M., Dorin, J.R., 2003. Duplication and selection in the evolution of primate beta-defensin genes. Genome Biol. 4, R31. https://doi.org/10.1186/gb-2003-4-5-r31.

Semple, F., Webb, S., Li, H.N., Patel, H.B., Perretti, M., Jackson, I.J., Gray, M., Davidson, D.J., Dorin, J.R., 2010. Human beta-defensin 3 has immunosuppressive activity in vitro and in vivo. Eur. J. Immunol. 40, 1073–1078. https://doi.org/10.1002/eji.200940041.

Sharma, H., Nagaraj, R., 2015. Human beta-defensin 4 with non-native disulfide bridges exhibit antimicrobial activity. PLoS One 10, e0119525. https://doi.org/10.1371/journal.pone.0119525.

Shelley, J.R., Davidson, D.J., Dorin, J.R., 2020. The dichotomous responses driven by beta-defensins. Front. Immunol. 11, 1176. https://doi.org/10.3389/fimmu.2020.01176.

Sheridan, J.W., Metcalf, D., 1972. Studies on the bone marrow colony stimulating factor (CSF): relation of tissue CSF to serum CSF. J. Cell Physiol. 80, 129–140. https://doi.org/10.1002/jcp.1040800114.

Shimizu, M., Watanabe, Y., Isobe, N., Yoshimura, Y., 2008. Expression of avian beta-defensin 3, an antimicrobial peptide, by sperm in the male reproductive organs and oviduct in chickens: an immunohistochemical study. Poult. Sci. 87, 2653–2659. https://doi.org/10.3382/ps.2008-00210.

Soman, S.S., Arathy, D.S., Sreekumar, E., 2009a. Discovery of Anas platyrhynchos avian beta-defensin 2 (Apl_AvBD2) with antibacterial and chemotactic functions. Mol. Immunol. 46, 2029–2038. https://doi.org/10.1016/j.molimm.2009.03.003.

Soman, S.S., Nair, S., Issac, A., Arathy, D.S., Niyas, K.P., Anoop, M., Sreekumar, E., 2009b. Immunomodulation by duck defensin, Apl_AvBD2: in vitro dendritic cell immunoreceptor (DCIR) mRNA suppression, and B- and T-lymphocyte chemotaxis. Mol. Immunol. 46, 3070–3075. https://doi.org/10.1016/j.molimm.2009.06.003.

Soman, S.S., Sivakumar, K.C., Sreekumar, E., 2010. Molecular dynamics simulation studies and in vitro site directed mutagenesis of avian beta-defensin Apl_AvBD2. BMC Bioinforma. 11 (Suppl 1), S7. https://doi.org/10.1186/1471-2105-11-S1-S7.

Springer, M.S., Meredith, R.W., Gatesy, J., Emerling, C.A., Park, J., Rabosky, D.L., Stadler, T., Steiner, C., Ryder, O.A., Janecka, J.E., Fisher, C.A., Murphy, W.J., 2012. Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS One 7, e49521. https://doi.org/10.1371/journal.pone.0049521.

Staubitz, P., Peschel, A., Nieuwenhuizen, W.F., Otto, M., Gotz, F., Jung, G., Jack, R.W., 2001. Structure-function relationships in the tryptophan-rich, antimicrobial peptide indolicidin. J. Pept. Sci. 7, 552–564. https://doi.org/10.1002/psc.351.

Su, S., Dwyer, D.M., Miska, K.B., Fetterer, R.H., Jenkins, M.C., Wong, E.A., 2017. Expression of host defense peptides in the intestine of Eimeria-challenged chickens. Poult. Sci. 96, 2421–2427. https://doi.org/10.3382/ps/pew468.

Sun, L., Finnegan, C.M., Kish-Catalone, T., Blumenthal, R., Garzino-Demo, P., La Terra Maggiore, G.M., Berrone, S., Kleinman, C., Wu, Z., Abdelwahab, S., Lu, W., Garzino-Demo, A., 2005. Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. J. Virol. 79, 14318–14329. https://doi.org/10.1128/JVI.79.22.14318-14329.2005.

Taggart, C.C., Greene, C.M., Smith, S.G., Levine, R.L., McCray Jr., P.B., O'Neill, S., McElvaney, N.G., 2003. Inactivation of human beta-defensins 2 and 3 by elastolytic cathepsins. J. Immunol. 171, 931–937.

Takeda, A., Tsubaki, T., Sagae, N., Onda, Y., Inada, Y., Mochizuki, T., Okumura, K., Kikuyama, S., Kobayashi, T., Iwamuro, S., 2014. Bacterial toxin-inducible gene expression of cathelicidin-B1 in the chicken bursal lymphoma-derived cell line DT40: functional characterization of cathelicidin-B1. Peptides 59, 94–102. https://doi.org/10.1016/j.peptides.2014.06.012.

Tecle, T., Tripathi, S., Hartshorn, K.L., 2010. Review: defensins and cathelicidins in lung immunity. Innate Immun. 16, 151–159. https://doi.org/10.1177/1753425910365734.

Termen, S., Tollin, M., Olsson, B., Svenberg, T., Agerberth, B., Gudmundsson, G.H., 2003. Phylogeny, processing and expression of the rat cathelicidin rCRAMP: a model for innate antimicrobial peptides. Cell Mol. Life Sci. 60, 536–549.

Tesse, R., Cardinale, F., Santostasi, T., Polizzi, A., Manca, A., Mappa, L., Iacoviello, G., De Robertis, F., Logrillo, V.P., Armenio, L., 2008. Association of beta-defensin-1 gene polymorphisms with Pseudomonas aeruginosa airway colonization in cystic fibrosis. Genes Immun. 9, 57–60. https://doi.org/10.1038/sj.gene.6364440.

Tjabringa, G.S., Ninaber, D.K., Drijfhout, J.W., Rabe, K.F., Hiemstra, P.S., 2006. Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. Int. Arch. Allergy Immunol. 140, 103–112. https://doi.org/10.1159/000092305.

Tollner, T.L., Yudin, A.I., Tarantal, A.F., Treece, C.A., Overstreet, J.W., Cherr, G.N., 2008. Beta-defensin 126 on the surface of macaque sperm mediates attachment of sperm to oviductal epithelia. Biol. Reprod. 78, 400–412. https://doi.org/10.1095/biolreprod.107.064071.

Tollner, T.L., Venners, S.A., Hollox, E.J., Yudin, A.I., Liu, X., Tang, G., Xing, H., Kays, R. J., Lau, T., Overstreet, J.W., Xu, X., Bevins, C.L., Cherr, G.N., 2011. A common mutation in the defensin DEFB126 causes impaired sperm function and subfertility. Sci. Transl. Med. 3, 92ra65. https://doi.org/10.1126/scitranslmed.3002289.

Tomasinsig, L., Morgera, F., Antcheva, N., Pacor, S., Skerlavaj, B., Zanetti, M., Tossi, A., 2009. Structure dependence of biological activities for primate cathelicidins. J. Pept. Sci. 15, 576–582. https://doi.org/10.1002/psc.1143.

Tomita, T., Hitomi, S., Nagase, T., Matsui, H., Matsuse, T., Kimura, S., Ouchi, Y., 2000. Effect of ions on antibacterial activity of human beta defensin 2. Microbiol. Immunol. 44, 749–754.

Travis, S.M., Anderson, N.N., Forsyth, W.R., Espiritu, C., Conway, B.D., Greenberg, E.P., McCray Jr., P.B., Lehrer, R.I., Welsh, M.J., Tack, B.F., 2000. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect. Immun. 68, 2748–2755.

Tripathi, S., Tecle, T., Verma, A., Crouch, E., White, M., Hartshorn, K.L., 2013. The human cathelicidin LL-37 inhibits influenza A viruses through a mechanism distinct from that of surfactant protein D or defensins. J. Gen. Virol. 94, 40–49. https://doi.org/10.1099/vir.0.045013-0.

Tripathi, S., Wang, G., White, M., Qi, L., Taubenberger, J., Hartshorn, K.L., 2015. Antiviral activity of the human cathelicidin, LL-37, and derived peptides on seasonal and pandemic Influenza A viruses. PLoS One 10, e0124706. https://doi.org/10.1371/journal.pone.0124706.

Tu, J., Li, D., Li, Q., Zhang, L., Zhu, Q., Gaur, U., Fan, X., Xu, H., Yao, Y., Zhao, X., Yang, M., 2015. Molecular evolutionary analysis of beta-defensin peptides in vertebrates. Evol. Bioinform. Online 11, 105–114. https://doi.org/10.4137/EBO.S25580.

Turner, J., Cho, Y., Dinh, N.N., Waring, A.J., Lehrer, R.I., 1998. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. Antimicrob. Agents Chemother. 42, 2206–2214.

Uzzell, T., Stolzenberg, E.D., Shinnar, A.E., Zasloff, M., 2003. Hagfish intestinal antimicrobial peptides are ancient cathelicidins. Peptides 24, 1655–1667. https://doi.org/10.1016/j.peptides.2003.08.024.

Valore, E.V., Park, C.H., Quayle, A.J., Wiles, K.R., McCray Jr., P.B., Ganz, T., 1998. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J. Clin. Invest 101, 1633–1642. https://doi.org/10.1172/JCI1861.

Valore, E.V., Park, C.H., Igreti, S.L., Ganz, T., 2002. Antimicrobial components of vaginal fluid. Am. J. Obstet. Gynecol. 187, 561–568.

van Dijk, A., Veldhuizen, E.J., van Asten, A.J., Haagsman, H.P., 2005. CMAP27, a novel chicken cathelicidin-like antimicrobial protein. Vet. Immunol. Immunopathol. 106, 321–327. https://doi.org/10.1016/j.vetimm.2005.03.003.

van Dijk, A., Tersteeg-Zijderveld, M.H., Tjeerdsma-van Bokhoven, J.L., Jansman, A.J., Veldhuizen, E.J., Haagsman, H.P., 2009. Chicken heterophils are recruited to the site of Salmonella infection and release antibacterial mature Cathelicidin-2 upon stimulation with LPS. Mol. Immunol. 46, 1517–1526. https://doi.org/10.1016/j.molimm.2008.12.015.

van Dijk, A., van Eldik, M., Veldhuizen, E.J., Tjeerdsma-van Bokhoven, H.L., de Zoete, M. R., Bikker, F.J., Haagsman, H.P., 2016. Immunomodulatory and anti-inflammatory activities of chicken cathelicidin-2 derived peptides. PLoS One 11, e0147919. https://doi.org/10.1371/journal.pone.0147919.

van Harten, R.M., van Woudenbergh, E., van Dijk, A., Haagsman, H.P., 2018. Cathelicidins: Immunomodulatory Antimicrobials. Vaccines (Basel) 6 doi: 10.3390/vaccines6030063.

Ventura, M., Boniotto, M., Pazienza, M., Palumbo, V., Cardone, M.F., Rocchi, M., Tossi, A., Amoroso, A., Crovella, S., 2004. Localization of beta-defensin genes in non human primates. Eur. J. Histochem. 48, 185–190.

Wang, G., Mishra, B., Epand, R.F., Epand, R.M., 2014. High-quality 3D structures shine light on antibacterial, anti-biofilm and antiviral activities of human cathelicidin LL-37 and its fragments. Biochim. Biophys. Acta 1838, 2160–2172. https://doi.org/10.1016/j.bbamem.2014.01.016.

Wang, L., Li, J., Li Jr., J., Li, R.X., Lv, C.F., Li, S., Mi, Y.L., Zhang, C.Q., 2016. Identification of the Paneth cells in chicken small intestine. Poult. Sci. 95, 1631–1635. https://doi.org/10.3382/ps/pew079.

Wang, Y., Hong, J., Liu, X., Yang, H., Liu, R., Wu, J., Wang, A., Lin, D., Lai, R., 2008. Snake cathelicidin from Bungarus fasciatus is a potent peptide antibiotics. PLoS One 3, e3217. https://doi.org/10.1371/journal.pone.0003217.

Wang, Y., Lu, Z., Feng, F., Zhu, W., Guang, H., Liu, J., He, W., Chi, L., Li, Z., Yu, H., 2011. Molecular cloning and characterization of novel cathelicidin-derived myeloid antimicrobial peptide from Phasianus colchicus. Dev. Comp. Immunol. 35, 314–322. https://doi.org/10.1016/j.dci.2010.10.004.

Watanabe, Y., Isobe, N., Yoshimura, Y., 2011. Detection of avian beta-defensins mRNA and proteins in male reproductive organs in chicken. J. Poult. Sci. 48, 275–280. https://doi.org/10.2141/jpsa.011042.

Wei, L., Yang, J., He, X., Mo, G., Hong, J., Yan, X., Lin, D., Lai, R., 2013. Structure and function of a potent lipopolysaccharide-binding antimicrobial and anti-inflammatory peptide. J. Med. Chem. 56, 3546–3556. https://doi.org/10.1021/jm4004158.

Wen, A.Y., Sakamoto, K.M., Miller, L.S., 2010. The role of the transcription factor CREB in immune function. J. Immunol. 185, 6413–6419. https://doi.org/10.4049/jimmunol.1001829.

Whenham, N., Lu, T.C., Maidin, M.B., Wilson, P.W., Bain, M.M., Stevenson, M.L., Stevens, M.P., Bedford, M.R., Dunn, I.C., 2015. Ovodefensins, an oviduct-specific antimicrobial gene family, have evolved in birds and reptiles to protect the egg by both sequence and intra-six-cysteine sequence motif spacing. Biol. Reprod. 92, 154. https://doi.org/10.1095/biolreprod.114.126839.

Wu, Z., Hoover, D.M., Yang, D., Boulegue, C., Santamaria, F., Oppenheim, J.J., Lubkowski, J., Lu, W., 2003. Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human beta-defensin 3. Proc. Natl. Acad. Sci. U. S. A 100, 8880–8885. https://doi.org/10.1073/pnas.1533186100.

Xhindoli, D., Pacor, S., Benincasa, M., Scocchi, M., Gennaro, R., Tossi, A., 2016. The human cathelicidin LL-37–A pore-forming antibacterial peptide and host-cell modulator. Biochim. Biophys. Acta 1858, 546–566. https://doi.org/10.1016/j.bbamem.2015.11.003.

Xiao, Y., Hughes, A.L., Ando, J., Matsuda, Y., Cheng, J.F., Skinner-Noble, D., Zhang, G., 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 Genom. 5, 56. https://doi.org/10.1186/1471-2164-5-56.
Xiao, Y., Cai, Y., Bommineni, Y.R., Fernando, S.C., Prakash, O., Gilliland, S.E., Zhang, G., 2006a. Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. J. Biol. Chem. 281, 2858–2867. https://doi.org/10.1074/jbc.M507180200.
Xiao, Y., Dai, H., Bommineni, Y.R., Soulages, J.L., Gong, Y.X., Prakash, O., Zhang, G., 2006b. Structure-activity relationships of fowlicidin-1, a cathelicidin antimicrobial peptide in chicken. FEBS J. 273, 2581–2593. https://doi.org/10.1111/j.1742-4658.2006.05261.x.
Xiao, Y., Herrera, A.I., Bommineni, Y.R., Soulages, J.L., Prakash, O., Zhang, G., 2009. The central kink region of fowlicidin-2, an alpha-helical host defense peptide, is critically involved in bacterial killing and endotoxin neutralization. J. Innate Immun. 1, 268–280. https://doi.org/10.1159/000174822.
Xie, C., Prahl, A., Ericksen, B., Wu, Z., Zeng, P., Li, X., Lu, W.Y., Lubkowski, J., Lu, W., 2005. Reconstruction of the conserved beta-bulge in mammalian defensins using D-amino. Acids J. Biol. Chem. 280, 32921–32929. https://doi.org/10.1074/jbc.M503084200.
Xu, Y., Zhang, T., Xu, Q., Han, Z., Liang, S., Shao, Y., Ma, D., Liu, S., 2015. Differential modulation of avian beta-defensin and Toll-like receptor expression in chickens infected with infectious bronchitis virus. Appl. Microbiol. Biotechnol. 99, 9011–9024. https://doi.org/10.1007/s00253-015-6786-8.
Yacoub, H.A., Elazzazy, A.M., Abuzinadah, O.A., Al-Hejin, A.M., Mahmoud, M.M., Harakeh, S.M., 2015. Antimicrobial activities of chicken beta-defensin (4 and 10) peptides against pathogenic bacteria and fungi. Front. Cell Infect. Microbiol. 5, 36. https://doi.org/10.3389/fcimb.2015.00036.
Yacoub, H.A., Elazzazy, A.M., Mahmoud, M.M., Baeshen, M.N., Al-Maghrabi, O.A., Alkarim, S., Ahmed, E.S., Almehdar, H.A., Uversky, V.N., 2016. Chicken cathelicidins as potent intrinsically disordered biocides with antimicrobial activity against infectious pathogens. Dev. Comp. Immunol. 65, 8–24. https://doi.org/10.1016/j.dci.2016.06.012.
Yamaguchi, Y., Nagase, T., Makita, R., Fukuhara, S., Tomita, T., Tominaga, T., Kurihara, H., Ouchi, Y., 2002. Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice. J. Immunol. 169, 2516–2523.
Yang, D., Chertov, O., Bykovskaia, S.N., Chen, Q., Buffo, M.J., Shogan, J., Anderson, M., Schroder, J.M., Wang, J.M., Howard, O.M., Oppenheim, J.J., 1999. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286, 525–528.
Yang, M., Zhang, C., Zhang, X., Zhang, M.Z., Rottinghaus, G.E., Zhang, S., 2016. Structure-function analysis of Avian beta-defensin-6 and beta-defensin-12: role of charge and disulfide bridges. BMC Microbiol 16, 210. https://doi.org/10.1186/s12866-016-0828-y.
Yang, M., Zhang, C., Zhang, M.Z., Zhang, S., 2017. Novel synthetic analogues of avian beta-defensin-12: the role of charge, hydrophobicity, and disulfide bridges in biological functions. BMC Microbiol 17, 43. https://doi.org/10.1186/s12866-017-0959-9.
Yasin, B., Pang, M., Turner, J.S., Cho, Y., Dinh, N.N., Waring, A.J., Lehrer, R.I., Wagar, E. A., 2000. Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides. Eur. J. Clin. Microbiol. Infect. Dis. 19, 187–194. https://doi.org/10.1007/s100960050457.
Yenugu, S., Hamil, K.G., Radhakrishnan, Y., French, F.S., Hall, S.H., 2004. The androgen-regulated epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is an antimicrobial beta-defensin. Endocrinology 145, 3165–3173. https://doi.org/10.1210/en.2003-1698.
Yu, H., Lu, Y., Qiao, X., Wei, L., Fu, T., Cai, S., Wang, C., Liu, X., Zhong, S., Wang, Y., 2015. Novel cathelicidins from pigeon highlights evolutionary convergence in avain cathelicidins and functions in modulation of innate immunity. Sci. Rep. 5, 11082. https://doi.org/10.1038/srep11082.
Yu, L., Xie, X., Jiang, K., Hong, Y., Zhou, Z., Mi, Y., Zhang, C., Li, J., 2021. Paneth cells mediated the response of intestinal stem cells at the early stage of intestinal inflammation in the chicken. Poult. Sci. 100, 615–622. https://doi.org/10.1016/j.psj.2020.11.055.
Yu, L.T., Xiao, Y.P., Li, J.J., Ran, J.S., Yin, L.Q., Liu, Y.P., Zhang, L., 2018. Molecular characterization of a novel ovodefensin gene in chickens. Gene 678, 233–240. https://doi.org/10.1016/j.gene.2018.08.029.
Yu, Y., Zhang, Y., Zhang, Y., Lai, Y., Chen, W., Xiao, Z., Zhang, W., Jin, M., Yu, B., 2017. LL-37-induced human mast cell activation through G protein-coupled receptor MrgX2. Int. Immunopharmacol. 49, 6–12. https://doi.org/10.1016/j.intimp.2017.05.016.
Yudin, A.I., Generao, S.E., Tollner, T.L., Treece, C.A., Overstreet, J.W., Cherr, G.N., 2005. Beta-defensin 126 on the cell surface protects sperm from immunorecognition and binding of anti-sperm antibodies. Biol. Reprod. 73, 1243–1252. https://doi.org/10.1095/biolreprod.105.042432.
Zaga-Clavellina, V., Ruiz, M., Flores-Espinosa, P., Vega-Sanchez, R., Flores-Pliego, A., Estrada-Gutierrez, G., Sosa-Gonzalez, I., Morales-Mendez, I., Osorio-Caballero, M., 2012. Tissue-specific human beta-defensins (HBD)-1, HBD-2 and HBD-3 secretion profile from human amniochorionic membranes stimulated with Candida albicans in a two-compartment tissue culture system. Reprod. Biol. Endocrinol. 10, 70. https://doi.org/10.1186/1477-7827-10-70.
Zanetti, M., 2004. Cathelicidins, multifunctional peptides of the innate immunity. J. Leukoc. Biol. 75, 39–48. https://doi.org/10.1189/jlb.0403147.
Zanetti, M., 2005. The role of cathelicidins in the innate host defenses of mammals. Curr. Issues Mol. Biol. 7, 179–196.
Zelezetsky, I., Tossi, A., 2006. Alpha-helical antimicrobial peptides–using a sequence template to guide structure-activity relationship studies. Biochim. Biophys. Acta 1758, 1436–1449. https://doi.org/10.1016/j.bbamem.2006.03.021.
Zelezetsky, I., Pontillo, A., Puzzi, L., Antcheva, N., Segat, L., Pacor, S., Crovella, S., Tossi, A., 2006. Evolution of the primate cathelicidin. Correlation between structural variations and antimicrobial activity. J. Biol. Chem. 281, 19861–19871. https://doi.org/10.1074/jbc.M511108200.
Zhang, G., Li, C., Li, Q., Li, B., Larkin, D.M., Lee, C., Storz, J.F., Antunes, A., Greenwold, M.J., Meredith, R.W., Odeen, A., Cui, J., Zhou, Q., Xu, L., Pan, H., Wang, Z., Jin, L., Zhang, P., Hu, H., Yang, W., Hu, J., Xiao, J., Yang, Z., Liu, Y., Xie, Q., Yu, H., Lian, J., Wen, P., Zhang, F., Li, H., Zeng, Y., Xiong, Z., Liu, S., Zhou, L., Huang, Z., An, N., Wang, J., Zheng, Q., Xiong, Y., Wang, G., Wang, B., Wang, J., Fan, Y., da Fonseca, R.R., Alfaro-Nunez, A., Schubert, M., Orlando, L., Mourier, T., Howard, J.T., Ganapathy, G., Pfenning, A., Whitney, O., Rivas, M.V., Hara, E., Smith, J., Farre, M., Narayan, J., Slavov, G., Romanov, M.N., Borges, R., Machado, J.P., Khan, I., Springer, M.S., Gatesy, J., Hoffmann, F.G., Opazo, J.C., Hastad, O., Sawyer, R.H., Kim, H., Kim, K.W., Kim, H.J., Cho, S., Li, N., Huang, Y., Bruford, M.W., Zhan, X., Dixon, A., Bertelsen, M.F., Derryberry, E., Warren, W., Wilson, R.K., Li, S., Ray, D.A., Green, R.E., O'Brien, S.J., Griffin, D., Johnson, W.E., Haussler, D., Ryder, O.A., Willerslev, E., Graves, G.R., Alstrom, P., Fjeldsa, J., Mindell, D.P., Edwards, S.V., Braun, E.L., Rahbek, C., Burt, D.W., Houde, P., Zhang, Y., Yang, H., Wang, J., Avian Genome, C., Jarvis, E.D., Gilbert, M.T., Wang, J., 2014. Comparative genomics reveals insights into avian genome evolution and adaptation. Science 346, 1311–1320. https://doi.org/10.1126/science.1251385.
Zhang, L., Chen, D., Yu, L., Wei, Y., Li, J., Zhou, C., 2019. Genome-wide analysis of the ovodefensin gene family: Monophyletic origin, independent gene duplication and presence of different selection patterns. Infect. Genet. Evol. 68, 265–272. https://doi.org/10.1016/j.meegid.2019.01.001.
Zhao, C., Wang, I., Lehrer, R.I., 1996. Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett. 396, 319–322.
Zhu, S., 2008. Did cathelicidins, a family of multifunctional host-defense peptides, arise from a cysteine protease inhibitor. Trends Microbiol 16, 353–360. https://doi.org/10.1016/j.tim.2008.05.007.
Zhu, S., Gao, B., 2013. Evolutionary origin of beta-defensins. Dev. Comp. Immunol. 39, 79–84. https://doi.org/10.1016/j.dci.2012.02.011.
Zhu, S., Gao, B., 2017. Positive selection in cathelicidin host defense peptides: adaptation to exogenous pathogens or endogenous receptors. Heredity 118, 453–465. https://doi.org/10.1038/hdy.2016.117.