Avian host defense cathelicidins: structure, expression, biological functions, and potential therapeutic applications

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ABSTRACT Host defense peptides (**HDP**) are multifunctional effectors of the innate immune system, which has antimicrobial and pleiotropic immunomodulatory functions. Although there is a very sophisticated superposition of adaptive immune systems in vertebrates, this system is still essential. As an important family of HDP, cathelicidins are also known for their broad-spectrum antibacterial activity against bacteria, fungi, and enveloped viruses. It has been found in humans and other species, including cattle, pigs, sheep, goats, chickens, rabbits, and some kind of fish. Among them, cathelicidins in birds were described for the first time in 2005. This review focuses on the structure, biological activities, expression, and regulation of avian cathelicidin, especially main effects of host defense cathelicidin on potential therapeutic applications. According to the results obtained both in vitro and in vivo, good perspectives have been opened for cathelicidin. Nevertheless, further studies are needed to better characterize the mechanisms of action underlying the beneficial effects of cathelicidin as novel therapeutic alternatives to antibiotics.

Key words: cathelicidin, biological functions, expression, regulation, avian

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INTRODUCTION

Traditionally, antibiotics are included in animal feed at subtherapeutic levels for promoting growth and preventing disease (Kelsy et al., 2018). However, in recent decades, the infection of multidrug-resistant bacteria caused by the abuse of antibiotics has become an urgent problem to be solved in contemporary clinical medicine (Van Boeckel et al., 2015; Wang et al., 2018). To this end, a ban of all antibiotics in livestock production by the European Union in 2006 and a removal of medically important antibiotics in animal feeds in the United States in January 2017 were put in force (Seal et al., 2013; Young-Speirs et al., 2018). According to the latest requirements of China's Ministry of Agriculture, pharmaceutical feed additives are also forbidden to be added in feed after 2020 and can also no longer be used in feed production. Therefore, there is an urgent need for antibiotic substitutes that can maintain animal health and productivity without triggering antimicrobial resistance (Young-Speirs et al., 2018).

The potential advantages of host defense peptides (**HDP**), also known as antimicrobial peptides (**AMP**), in the development of novel antimicrobials are beyond question (Hancock and Sahl, 2006). The HDP are a critical component of the animal innate immune system with direct antimicrobial and immunomodulatory activities (Kelsy et al., 2018; Chen et al., 2020). In addition, because the target of HDP are mainly located in the microbial cell membrane, it can induce microbial cell membrane depolarization, resulting in cell rupture and play a role which is not easy to cause microbial resistance (Moravej et al., 2018). Cathelicidin is a major family of HDP found in mammals, birds, reptiles, and fish (Van Dijk et al., 2005, 2011; Zanetti, 2005; Chang et al., 2006; Zhao et al., 2008; Moravej et al., 2018). It plays a critical role in animal innate immune system that can provide the first line of defense against a variety of microorganisms (Zanetti, 2005). Studies have found that that avian cathelicidins not only have a more broad spectrum and efficient antimicrobial activity but also have low hemolytic activity and cytotoxicity (Xiao et al., 2006; Wang et al., 2011; Zhang and Sunkara, 2014), which shows great potential in the field of new antimicrobial agents.

The aim of this article is to summarize the information about avian cathelicidins to provide an overview of the evolution, expression, regulation, biological activities, and potential application for human and veterinary medicine of these avian cathelicidin AMP.

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GENERAL ASPECTS OF AVIAN CATHELICIDINS

Cathelicidin is a short peptide of less than 40 amino acids, whose name is derived from the similarity of the cathelicidin large middle domain to cathelin, a cathepsin L inhibitor originally isolated from porcine leukocytes (Ritonja et al., 1989; Cheng et al., 2015). Cathelicidins have been found in vertebrates, such as mammals (Zanetti, 2005), reptiles (Zhao et al., 2008), fish (Uzzell et al., 2003; Chang et al., 2006), and birds (Lynn et al., 2004; Van Dijk et al., 2005, 2011; Xiao et al., 2006).

Structure and Classes

In chickens, the cathelicidin genes are densely clustered within the distance of 7.5 kb toward one end of chromosome 2 (Xiao et al., 2006; Kosciuczuk et al., 2012). Like the cathelicidin genes in mammal, all avian cathelicidin genes described so far consist of 4 exons and 3 introns (Figure 1A). The first exon encodes the part of the cathelin domain and signal peptide of 29–30 amino acid residues in size, whereas the second and third exon encode the major part of the cathelin domain of 99–114 amino acids. The fourth exon encodes the last few amino acid residues of the cathelin domain, and the mature peptide with the variable antimicrobial domain consisting of 12–100 amino acids (Zanetti et al., 1995; Zhang and Sunkara, 2014). Therefore, in brief, complete cathelicidins consist of inactive prepropeptides (include signal peptides and cathelin domain) and mature peptide. It has been reported that cathelicidin AMP are highly heterogeneous because their C-terminal mature peptides could be activated by proteolytic cleavage and exerts its antimicrobial and immunomodulatory activities after being released from the N-terminal cathelin portion of the holoprotein (Zanetti, 2004)

In addition, the mature peptides found after the protease cleaving steps are quite diverse. Based on amino acid sequences, mature cathelicidin peptides can be broadly categorized into peptides with α-helical, β-sheets, extended helical, and loop structure cathelicidins (Figure 1B): (1) α-helical cathelicidins—linear peptides with good amphiphilic properties, which usually have no intramolecular disulfide bridges; the N-terminal contains more hydrophilic amino acids, and the C-terminal

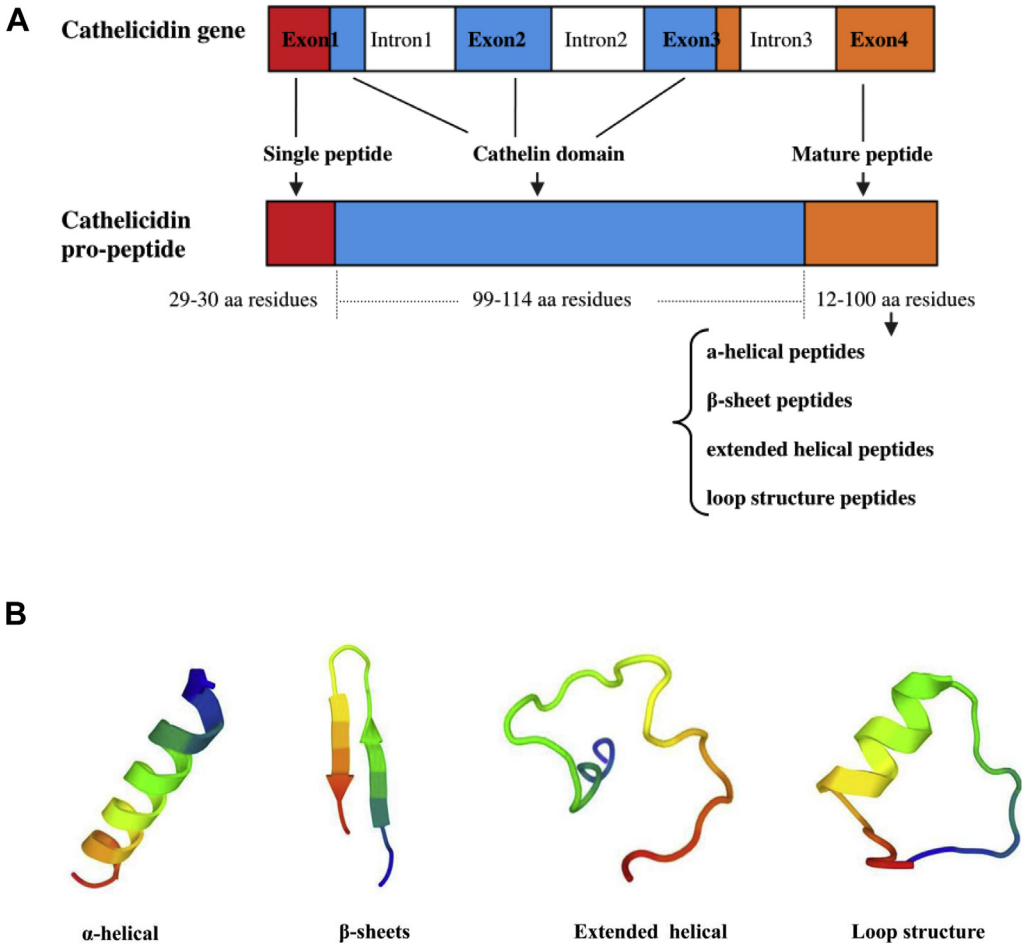

Figure 1. Gene and peptide structure of cathelicidins. (A) Schematic representation of gene and propeptide of the cathelicidin in vertebrate. (Adapted from related references (Zanetti et al., 1995; Zanetti, 2004; van Dijk et al., 2011; Cuperus et al., 2013; Zhang and Sunkara, 2014; Young-Speirs et al., 2018)). Corresponding colors of exons to propeptide regions indicate that exon codes for that specific region. (B) The schematic diagram of secondary structures of cathelicidin include α-helical, β-sheet, extended helical, and loop structure, which were predicted online by the Ressource Parisienne en BioInformatique Structurale web portal (Alexis et al., 2006).

Table 1. The gene name, amino acid sequences, and length of avian cathelicidins.

Source	Gene name	Peptide name	AA sequence	Mature peptide length	GenBank no.	Reference
Chicken (Gallus gallus)	CATH1	Cathelicidin1/fowlicidin-1	RVKRVWPLVIRTVIAGYNLYRAIKKK	26	HQ640431	Lynn et al., 2004; Xiao et al., 2006
	CMAP27/CATH2	Cathelicidin2/fowlicidin-2	LVQRGRFGRFLRKIRRFRPKVTITIQGSARFG	32	HQ640432	Van Dijk et al., 2005; Xiao et al., 2006
	CATH3	Cathelicidin3/fowlicidin-3	RVKRFWPLVPVAINTVAAGINLYKAIRRK	29	HQ640433	Bommineni et al., 2007; Veldhuizen et al., 2013
	CATHB1	Cathelicidin-B1	PIRNWWIRIWEWLNGIRKRVRQRSPFYVRGHLNVTSTPQP	40	AB915170	Goitsuka et al., 2007
Quail (*Coturnix coturnix*)	Cc-CATH1	Cathelicidin	RVKRVLPLVIRTVIAGYNLYRAIKRK	26	GU232858	Feng et al., 2011
	Cc-CATH2	Cathelicidin CATH2	LVQRGRFGRFLKKVRRFIPKVIIAAQIGSRFG	32	GU171373	
	Cc-CATH3	Cathelicidin CATH3	RVRRFWPLVPVAINTVAAGINLYKAIRRK	29	GU171374	
Pheasant (*Phasianus colchicus*)	Pc-CATH1	Cathelicidin CATH1	RIKRFWPLVPVAINTVAAGINLYKAIKRK	29	GU143407	Wang et al., 2011
	Pc-CATH2	cathelicidins	LVQRGRFGRFLSKIRRFRPKFTITIQGSGRFG	32	GU143408	
	Pc-CATH3	Cathelicidin CATH3	RIKRFWPVVIRTVVAGYNLYRAIKKK	26	GU171372	
Duck (*Anas platyrhynchos*)	dCATH	Cathelicidin	KRFWQLVPLAIKIYRAWKRR	20	KT230679	Gao et al., 2015
Turkey (*Meleagris gallopavo*)	CATH2	Cathelicidin-2	LVQRGRFGRFLSKFRRFRPRVTITIQGSARFG	32	XM003206909	Hamad et al., 2017; Ishige et al., 2017
	CATH3	Cathelicidin-3	RVKRFWPLVPVAINTVAAGINLYKAIKRK	29	XM010712309	
Rock pigeon (*Columba livia*)	Cl-CATH2	Cathelicidin 2	LIQRGRFGRFLGRIRRFRPRINFDIRARGSIRLG	34	KP645199	Yu et al., 2015
	Cl-CATH3	Cathelicidin 3	RVKRFWPLVPVAINTVAAGINLYKAIKRK	29	KP645200	
Japanese quail (*Coturnix japonica*)	Cj-CATH1	Cathelicidin	RVKRVLPLVIRTVIAGYNLYRAIKRK	26	LC136907	Ishige et al., 2017
	Cj-CATH2	Cathelicidin CATH2	LVQRGRFGRFLKKVRRFIPKVIIAAQIGSRFG	32	LC136907	
	Cj-CATH3	Cathelicidin CATH3	RVKRFWPLVPVAINTVAAGINLYKAIRRK	29	LC136907	
	Cj-CATB1	Cathelicidin-B1 precursor	PIRNWWTRIREWWDGIRRRLRQRSPFHVRGRLNISSTAQP	40	LC136907	

Abbreviation: AA, amino acid.

is rich in hydrophobic amino acids (Wang et al., 2017); (2) β-sheet cathelicidins—short peptides formed by 2 intramolecular disulfide bridges, which is developed by 4 conserved cysteines (Tossi et al., 1995; Wang et al., 2017); (3) extended helical cathelicidins—usually contain a high amount of specific amino acids, such as indocyanine rich in tryptophan, and PR-39 rich in proline/arginine (Ando et al., 2010; Kosciuczuk et al., 2012); (4) loop structure cathelicidins—contain a disulfide bond in its molecule and show a chain structure as a whole, which is the structure of most amphibian frog–derived cathelicidins and sheep and bovine cyclic dodecapeptide (Storici et al., 1992; Zanetti, 2005). Among these structural peptides, the α-helical cathelicidin peptides are the most widely spread and found in all investigated mammalian species (Van Dijk et al., 2011; Cuperus et al., 2013), also including all avian cathelicidins.

Discovery of Avian Cathelicidins (Evolution)

About a dozen of cathelicidin family members (Table 1) have been isolated in avian species. Four cathelicidins were identified in chickens (Gallus gallus cathelicidin CATH1, 2/CMAP27, 3, and CATH-B1) (Lynn et al., 2004; Van Dijk et al., 2005; Xiao et al., 2006), of which CATH1, 2, and 3 have also been independently reported as fowlicidin 1-3 (Xiao et al., 2006). Furthermore, peptides similar to cathelicidins were described in quail (*Coturnix coturnix* cathelicidin Cc-CATH1, 2, and 3) (Feng et al., 2011), pheasant (*Phasianus colchicus* cathelicidin Pc-CATH1, 2, and 3) (Wang et al., 2011), duck (*Anas platyrhynchos* cathelicidin dCATH) (Gao et al., 2015), turkey (*Meleagris gallopavo* cathelicidin CATH2 and 3) (Yacoub et al., 2016; Hamad et al., 2017; Ishige et al., 2017), rock pigeon (*Columba livia* cathelicidin Cl-CATH2 and 3) (Yu et al., 2015), and Japanese quail (*Coturnix japonica* cathelicidin Cj-CATH-1, -2, -3, and -B1) (Ishige et al., 2017). In addition to the completion of the cathelicidin amino acid sequencing of these avian species in Table 1, currently the cathelicidin sequences of some other avian species, such as budgerigar (*Melopsittacus undulatus*), peregrine falcon (*Falco peregrinus*), ground tit (*Pseudopodoces humilis*), and emperor penguin (*Aptenodytes forsteri)*, have been predicted and obtained in GenBank.

Phylogenetic analysis demonstrated that cathelicidins of mammals, avian species, and fish were classified into distinctly separated clusters (Goitsuka et al., 2007), and when comparing avian cathelicidins with mammalian proteins, the highest sequence similarity was found with neutrophilic granule proteins (**NGP**)-like cathelicidin, such as rabbit, mouse, and bovine NGP, suggesting that they may share a common ancestor. This hypothesis was confirmed in subsequent studies, that is, NGP and avian cathelicidin gene clusters were located in close proximity to the Kelch-like 18 gene

in both types of animals, with synteny (Xiao et al., 2006; Cuperus et al., 2013). Although they have evolved from a single, remotely related gene, the difference is that the C-terminal region of cathelicidins is highly variable across species and could cleave from the cathelicidin-like domain to become bioactive by proteolysis; the NGPs are conserved and functionally active in the whole sequence, and there is no proteolysis (Kosciuczuk et al., 2012; Zhang and Sunkara, 2014). In addition, it is worth noting that the chicken CATHB1 seems to be an outlier, located between the fish sequence and the group containing the other avian cathelicidins, not only a great distance from NGPs but also only 40% identity with CATH1, and sharing only 40% identity with CATH1 (Goitsuka et al., 2007; Zhang and Sunkara, 2014).

Currently, among the 4 identified chicken cathelicidins, CATH1 and CATH3 are most closely related with a >90% identity throughout the entire peptide sequence (Zhang and Sunkara, 2014). At the level of amino acid, the signal peptides of CATH1 and CATH2 show a high similarity of 94%, whereas the cathelin domains of the 2 genes share only 56% homology (Van Dijk et al., 2005; Xiao et al., 2006). Conversely, the mature peptides are highly differentiated, such as the CATH2 mature peptide have been found to have less than 10% homology with other chicken cathelicidins (Van Dijk et al., 2005). The orthologs of chicken CATH1-3 has also been reported in other avian species such as the *C. coturnix* and *P. colchicus* (Feng et al., 2011; Wang et al., 2011). The comparison of 3 Cc-CATHs from *C. coturnix* showed that Cc-CATH1 and Cc-CATH3 were more closely related, with 93% homology throughout the entire sequence, suggesting that the 2 genes were the result of gene duplication (Feng et al., 2011). Two cathelicidin genes, Cl-CATH-2 and -3, have been described in *C. livia*, and these 2 genes share a high degree of similarity with previously characterized CATH-2 and -3 from chicken (Yu et al., 2015). Furthermore, the identification and initial characterization of the *C. japonica* CATH genes (Cj-CATH-1, -2, -3, and -B1) were recently reported by Ishige et al. (2017). The percent identities between the coding sequences of Cj-CATHs and chicken cathelicidins is more than 85.3%, and the predicted amino acid sequences of Cj-CATHs exhibited >75.4% identity to chicken cathelicidin orthologs (Ishige et al., 2017).

BIOLOGICAL ACTIVITIES OF AVIAN CATHELICIDINS

Since the first identification of cathelicidin propeptides (Zanetti et al., 1993), many cathelicidins from different species have been studied. Avian cathelicidins not only have direct antibacterial activity but also can selectively enhance the host immune response by regulating the production of cytokines and the recruitment of immune cells (Cuperus et al., 2013; Zhang and Sunkara, 2014). Herein, we reviewed the biological activities and some internal mechanisms of avian cathelicidins.

Antimicrobial Activities

Avian cathelicidins have been demonstrated to exhibit active antimicrobial activity toward both gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and so forth (Zhang and Sunkara, 2014; Ishige et al., 2017). The antibacterial spectrum and minimum inhibitory concentration (**MIC**) of some major avian cathelicidins AMP are summarized in Table 2. The MIC of mature chicken fowlicidins (CATH1-3) and CATH-B1 to most strains tested in a salt-independent manner are in the range of 0.4–2.5 μM (Xiao et al., 2006; Bommineni et al., 2007). Quail CATH-2 and -3, pheasant CATH-1, and duck CATH show MIC values in the range of 0.3–2.5, 0.1–2.95, and 2.0–4.0 μM for most gram-positive and gram-negative bacteria, respectively (Feng et al., 2011; Wang et al., 2011; Gao et al., 2015). Pigeon Cl-CATH2 exerted broad-spectrum but moderate antimicrobial abilities with most MIC ranging from 9.38 to 37.5 μg/mL, which is higher than CATH1, Cc-CATH3, and Pc-CATH1 (Yu et al., 2015). In addition, fungi such as *Candida albicans*, *Candida glabrata*, and slime mold are susceptible to avian cathelicidins, showing MIC values in the range of about 1–10 μM (Feng et al., 2011; Wang et al., 2011; Cuperus et al., 2013; Yu et al., 2015).

Avian cathelicidins are cationic polypeptides that play an important role in the initial response of invasive pathogens. The major bactericidal mechanism is killing microbes via disruption and lysis of their membrane integrity (Xiao et al., 2009; Derache et al., 2012). Currently, 3 main mechanisms for cationic peptides to penetrate microbial membranes have been proposed, that is, the barrel-stave, carpet, and toroidal pore model (Van Dijk et al., 2011), as shown in Figure 2. However, the above 3 mechanisms do not exist independently but are inter-related. It has been reported that the barrel-stave, carpet, and toroidal pore models are actually several continuous stages based on the effect of peptides on the cell membrane (Dathe and Wieprecht, 1999). In addition, the antibacterial activity of avian cathelicidins is closely related to its structural characteristics. The kink or hinge region of the peptide center and amphiphilic structure in the α-helical cathelicidins molecule plays a key role in antimicrobial activities (Oh et al., 2000; Tossi et al., 2000; Cuperus et al., 2013). The cationic side interacts with the cell membrane of bacteria and fungi with anions, the central hinge region (including proline or glycine, etc.) induces flexibility and inserts into the bacterial membrane resulting in pore formation, and the hydrophobic side also causes stomata in the cell membrane. Eventually, the phospholipid bilayer of the cell membrane was destroyed. A peptide can use different mechanisms depending on its concentration (Brogden, 2005; Nicolas, 2009). Generally, the mode of action of AMP in vitro depends on the high multiples of the MIC of peptides and (or) high peptide-to-lipid ratios. At low peptide-to-lipid ratios, peptides are bound parallel to a lipid bilayer. At a high

Table 2. Activity spectrum of avian cathelicidin peptides in a salt-independent manner described in literature.

	Gram positive[1]									
Gene name	*S. aureus*	*S. epidermidis*	*S. haemolyticus*	*N. asteroides*	*P. acnes*	*L. monocytogenes*	*E. faecium*	*B. cereus*	*B. subtilis*	Reference
CATH1	0.4-1.0	+	+			0.8-2.0	+			Bommineni et al., 2007; Xiao et al., 2006
CATH2	0.33-1.25	+	+			1.33	+			Veldhuizen et al., 2013; Xiao et al., 2006
CATH3	1.0-1.25	+	+			2.0	+			Bommineni et al., 2007; Veldhuizen et al., 2013
CATH-B1	1.25									Goitsuka et al., 2007
Cc-CATH2	0.3-1.3	2.5	+	1.3	1.3		+			Feng et al., 2011
Cc-CATH3	0.2-0.7	−	+	0.7	1.4		+			Feng et al., 2011
Pc-CATH1	0.18-0.74	2.95	+	0.74	0.74		+			Wang et al., 2011
dCATH	4.0	4.0					4.0		+	Gao et al., 2015
Cl-CATH2	2.27							+		Yu et al., 2015

	Gram negative[2]										
Gene name	*A. sobria*	*A. baumannii*	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*P. mirabilis*	*K. pneumoniae*	*K. oxytoca*	*S. typhimurium*	*S. enteritidis*	*S. maltophilia*	Reference
CATH1			0.8-2.0	3.18			0.4-1.0		0.4-2.0	2.0	+	Bommineni et al., 2007; Xiao et al., 2006
CATH2			0.66-2.66	5.32			0.66-1.25		0.66-1.33		+	Veldhuizen et al., 2013; Xiao et al., 2006
CATH3			2	1.25-2.5			0.6-1.0		2.0	2.0	+	Bommineni et al., 2007; Veldhuizen et al., 2013
CATH-B1			2.5	0.63								Goitsuka et al., 2007
Cc-CATH2	1.3	+	2.5-5.1	10.1	10.1	5.1	+	2.5	+		1.3	Feng et al., 2011
Cc-CATH3	1.4	+	−	5.6	> 29.6	1.4	−	22.2	−		1.4	Feng et al., 2011
Pc-CATH1	1.48	+	1.48-2.95	5.90	23.62	1.48	+	11.81	+		0.74	Wang et al., 2011
dCATH			2.0						4.0			Gao et al., 2015
Cl-CATH2			4.54	4.54								Yu et al., 2015

The minimum inhibitory concentrations (MIC) of peptides are indicated in μM. The presence of inhibition is denoted by "+". The lack of inhibition is denoted by "−".

[1]*S. aureus, Staphylococcus aureus*; *S. epidermidis, Staphylococcus epidermidis*; *S. haemolyticus, Staphylococcus haemolyticus*; *N. asteroids, Nocardia asteroids*; *P. acnes, Propionibacterium acnes*; *L. monocytogenes, Listeria monocytogenes*; *E. faecium, Enterococcus faecium*; *B. cereus, Bacillus cereus*; *B. subtilis, Bacillus subtilis.*

[2]*A. sobria, Aeromonas sobria*; *A. baumannii, Acinetobacter baumannii*; *E. coli, Escherichia coli*; *P. aeruginosa, Pseudomonas aeruginosa*; *P. vulgaris, Proteus vulgaris*; *P. mirabilis, Proteus mirabilis*; *K. pneumoniae, Klebsiella pneumoniae*; *K. oxytoca, Klebsiella oxytoca*; *S. typhimurium, Salmonella typhimurium*; *S. enteritidis, Salmonella enteritidis*; *S. maltophilia, Stenotrophomonas maltophilia.*

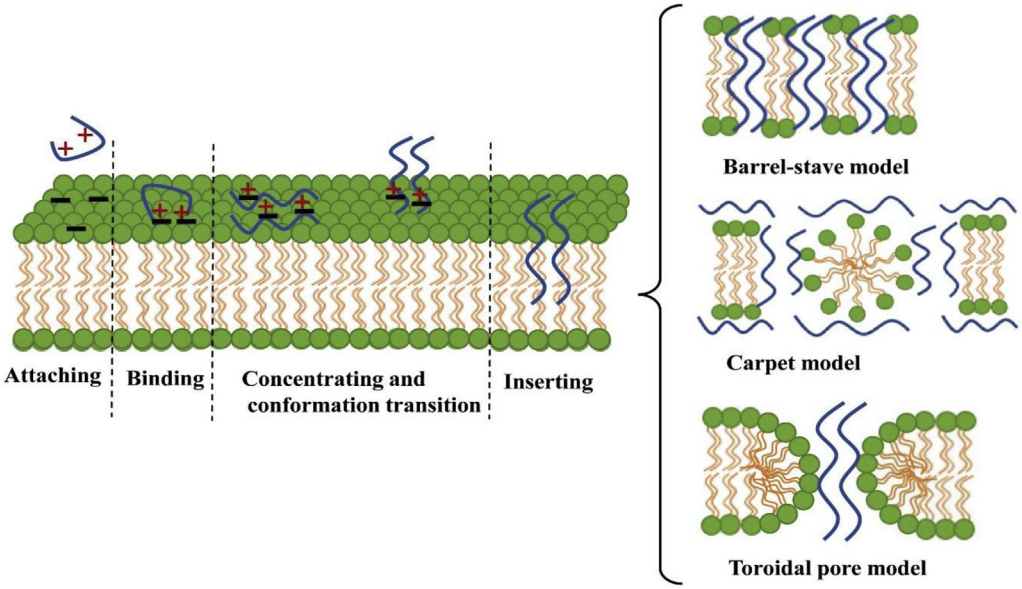

Figure 2. Three main mechanisms for cationic peptides to penetrate microbial membranes, including the barrel-stave model, carpet model, and toroidal pore model. Adapted with permission from Wang et al. (2018).

value of peptide-to-lipid ratios, peptide molecules are orientated perpendicular to the bilayer, forming transmembrane pores that are lethal to a cell (Huang, 2000; Yang et al., 2001; Brogden, 2005). This is consistent with the 2 general mechanisms of AMP entering microbial cells proposed by Nicolas (2009), which are spontaneous lipid-assisted translocation and stereospecific receptor–mediated membrane translocation, respectively. The results reported by Podda et al. (2006) suggest that proline-rich cathelicidin Bac7 may inactivate bacteria through 2 different modes of action, depending on its concentration: (1) a mechanism of stereospecific-dependent uptake and then binding to intracellular targets when close to MIC concentrations and (2) nonstereospecific membrane dissolution mechanism when concentrations are higher than the MIC value. Other microbial killing mechanisms have also been proposed, for example, some cathelicidins could bind to negatively charged biological macromolecules such as DNA, RNA, or protein, thus inhibiting DNA replication and impairing protein synthesis and function (Zasloff, 2002).

Immunomodulatory Activities

In addition to directly killing bacteria, the immunomodulatory activity is the main biological function of HDP, which has been proven by more studies (Afacan et al., 2012; Wang, 2014). More recently, it has become evident that avian cathelicidins have a diverse range of functions in modulating immunity. The immunomodulatory function of avian cathelicidins mainly includes the following 2 aspects: (1) enhance anti-inflammatory immune modulation. It has been reported that chicken CATH2 and CATH3 can prevent toll-like receptor signaling by directly blinding to endotoxins lipopolysaccharide (**LPS**), which can in turn inhibit LPS-induced tumor necrosis factor-α production in macrophages, monocytes, or dendritic cells (Van Dijk et al., 2009a; Coorens et al., 2017a). Chicken CATH1-3 can inhibit the expression of cytokines IL-1β, IL-6, and nitric oxide production induced by LPS in both chicken peripheral blood mononuclear cells and mouse RAW264.7 cells (Coorens et al., 2017b; Van Harten et al., 2018). Besides inhibiting the production of proinflammatory cytokine-mediated by endotoxin, avian cathelicidins could also stimulate the production of anti-inflammatory cytokines (Van Harten et al., 2018). For example, Kraaij et al. reported that chicken CATH2 upregulates IL-10 mRNA levels in chicken peripheral blood mononuclear cells (Kraaij et al., 2017). (2) Modulation of the expression of chemokines—on the one hand, cathelicidins could directly bind to chemokine receptors on immune cells and induce migration. Chicken CATH1 was found to have specific chemotaxis to neutrophils after injection into the mouse peritoneum (Bommineni et al., 2014). Cathelicidins, released from infected sites where neutrophils gather mainly, will attract other immune cells. These immune cells may then also secrete cathelicidins, which can lead to a local high concentration of cathelicidin peptides. It was reported that this cumulative local concentration, even in the presence of salt, might be high enough to make cathelicidins bactericidal (Van Harten et al., 2018). On the other hand, in addition to direct chemotactic function, cathelicidins can also indirectly induce influx of a variety of innate and adaptive immune cells to gather to inflammatory sites by stimulating the expression of chemokines and chemokine receptors. For example, chicken CATH1 can activate the ability of RAW264.7 mouse macrophages by inducing the expression of inflammatory mediators including IL-1β, CCL2, and CCL3 (Bommineni et al., 2014).

EXPRESSION AND REGULATION OF AVIAN CATHELICIDINS

Tissue Expression Pattern

Most of the avian cathelicidins are derived from the various epithelial cells or bone marrow, with expression in most tissues except for the breast muscle (Selsted and Ouellette, 2005; Achanta et al., 2012). In chickens, CATH-1, -2, and -3 mRNAs are primarily of the myeloid origin and are expressed in a wide range of tissues, including the respiratory tract, gastrointestinal tract, and multiple lymphoid organs (Van Dijk et al., 2005; Lynn et al., 2007; Achanta et al., 2012). Van Dijk et al. (2005) and Achanta et al. (2012) have found that the high expression of CATH1-3 in the bone marrow, bursa of Fabricius, and cecal tonsils. Interestingly, although no CATH-1 has not been detected in skin tissue, there is a certain amount of CATH-2 expression in normal intact skin, which can assist in wound repair when the injury is induced (Dorschner et al., 2001; Heilborn et al., 2003; Lynn et al., 2004; Van Dijk et al., 2005). Immunohistochemical staining showed CATH-2 protein in heterophilic granulocytes, whereas not in other blood cells, such as monocytes, thrombocytes, or lymphocytes (Van Dijk et al., 2009a,b). Van et al. (Van Dijk et al., 2009b) observed the sections of gastrointestinal tissues and found that there was no expression of CATH-2 protein either in the control group or in the infected intestinal tissues, and the expression was equally restricted to heterotrophic cells.

In contrast, CATH-B1, as a distant member of avian cathelicidins, is derived from epithelial cells. It was reported that CATH-B1 mRNA displays a more restricted expression pattern, preferentially expressed in the secretory epithelial cells of the bursa of Fabricius (Goitsuka et al., 2007). Mature CATH-B1 peptide in the chicken bursa is secreted from the epithelial cells that are in close to proximity of M cells (Goitsuka et al., 2007; Achanta et al., 2012). In addition to selective expression in the bursa of Fabricius, studies have also shown that there are certain transcriptional levels of CATH-B1 in other organs, including the thymus, jejunum, colon, and peripheral blood leukocyte (Meade et al., 2009a; Van Dijk et al., 2011; Cuperus et al., 2013).

For pheasant, the expression level of Pc-CATH-1 mRNA is the highest in the bone marrow and bursa of Fabricius, moderate to high in the brain, heart, lung, spleen, and testis, and lower in the liver and thymus (Wang et al., 2011). In addition, the Pc-CATH-2 (-3), Cc-CATH, and d-CATH genes were cloned from pheasant and *Coturnix* spleen and duck bone marrow cDNA library, respectively (Feng et al., 2011; Gao et al., 2015; Wang et al., 2018). However, their expression levels in the spleen, bone marrow, and other tissues have not been widely investigated. Our preliminary study found that the expression of duck HDPs d-CATH was higher in the bone marrow, liver, and brain but lower in the bursa of Fabricius (unpublished).

Developmental Regulation

It was reported that the mRNA expression levels of chicken cathelicidin were closely related to their phylogenetic relationship during the embryonic development (Meade et al., 2009b). Through the whole embryo gene expression profile analysis of 4 known avian cathelicidin genes (including CATH1-3 and CATH-B1) across the development process, it was found that most cathelicidin mRNAs were expressed at day 3 of embryonic stage, expect for CATH-B1, which was not expressed until day 9, and increased at day 12 (Meade et al., 2009b; Zhang and Sunkara, 2014). In contrast to 3 d after laying, expression of CATH-1, CATH-2, and CATH-3 was increased by between 6- and 9-folds at day 6, reduced at day 9, and was also subsequently increased again at day 12 (Meade et al., 2009b). All 4 chicken cathelicidins mRNA expression was generally increased as embryos developed (Meade et al., 2009b). There was no significant evidence of preferential expression in either the head or the abdomen of the embryo. *CATH1-3* and *CATH-B1* gene expression in the head of the embryo increased by 12-, 6-, 6-, and 3-fold, respectively, and the expression of these genes in the abdomen increased by 21-, 8-, 5-, and 3-fold, respectively (Meade et al., 2009b; Van Dijk et al., 2011).

Chicken cathelicidins are also developmentally regulated in both gene and tissue-specific patterns after hatching. During the first 28 d, CATH1-3 in the cecal tonsil and lung increased in an age-dependent manner, whereas all 4 cathelicidins peaked in the bursa on day 4 after hatching and gradually declined on the 28th d (Achanta et al., 2012). Moreover, the peak expression of CATH1-3 appeared in the cecum on day 28, whereas CATH-B1 showed the highest expression in the lung and cecum tonsil on day 14 (Achanta et al., 2012). In summary, although the 4 cathelicidins are widely expressed in a variety of tissues in chicken, the bursa and bone marrow are the primary sites for synthesis of CATH-B1 and CATH1-3, respectively, suggesting their important innate defense role.

Nutritional Manipulation

The expression of cathelicidins is inducible and regulated by microbial infection, inflammatory stimulation, and nutritive active substances. Studies have confirmed that there are multiple regulatory elements in the 5′ upstream regions of the cathelicidins gene, such as nuclear factor-κB, NF-IL-6, bacterial LPS binding site, IL-6, and γ-interferon response factor (Wang et al., 2004a). Exogenous trace components could act on these sites directly or indirectly to regulate the expression of cathelicidins. It has been found that exogenous pathogenic factors can affect the expression level of cathelicidins. The expression levels of CATH1 in chick cecal tonsils were significantly increased in response to *Salmonella typhimurium* infection on day 3 and 5 (Akbari et al., 2008). However, parasitic poultry pathogen *Eimeria praecox* downregulated CATH3 expression in the jejunum of

infected chickens on day 3 after infection (Sumners et al., 2011). Avian cathelicidin levels are also affected by *Campylobacter jejuni*. Chicken CATH2 and CATH3 gene expression at 6 h post-infection in peripheral blood leukocytes significantly reduced in response to *C. jejuni* infection with oral challenge (Meade et al., 2009a). A decrease in CATH2 mRNA expression levels was observed in the small intestine of *C. jejuni*–challenged broiler chicks at 48 h p.i (Van Dijk et al., 2012). Based on the different results observed in these experiments, it could be inferred that the expression of cathelicidins is related to the infection load and the inhibition of the cathelicidin level may be part of the immune escape strategy of pathogenic microorganisms.

It is an effective method to regulate the expression of endogenous HDP by nutritional means. The expression of avian cathelicidins is regulated by butyrate, vitamin D_3, and other substances. Butyrate, a major type of short-chain fatty acids produced by bacterial fermentation of undigested dietary fiber, not only plays a positive role in energy supply, inflammation, barrier integrity, and gut health but also regulates intestinal immunity and enhances disease resistance by inducing endogenous HDP in chickens (Sunkara et al., 2011; Chen et al., 2020). Sunkara et al. (2011) found that butyrate could significantly increase the expression of CATH-B1 in chicken immune cells (HD11 macrophage cells and primary monocytes) and the small intestine (the jejunum and ceca), whereas CATH1-3 were essentially not modulated by butyrate in either cell type. This indicates that butyrate has interspecific differences in regulating the expression and mechanism of cathelicidins, and it is also related to the types of AMP. Notably, although butyrate at the concentration used had no direct antibacterial activity, butyrate treatment enhanced the antibacterial activity of chicken monocytes (Kelsy et al., 2018). Feed with 0.1% butyrate reduced the bacterial titer in the chicken cecum by a nearly 10-fold after experimental infections with *Salmonella enteritidis* (Sunkara et al., 2011).

As a secosteroid molecule, vitamin D_3 not only plays the important role in regulating calcium homeostasis but also is thought to participate in the regulation of innate and adaptive immune responses by enhancing HDP expression (Adams and Hewison, 2008; Chen et al., 2020). A study of Zhang et al. (2010) showed that vitamin D_3 could significantly promote the mRNA expression of CATH1 in the bursa of Fabricius and thymus of chickens in a dose-dependent manner in the range of 800–3,200 IU/kg, which is the potential to improve the innate ability to resist diseases. Consistently, CATH1 and CATH-B1 levels were dose-dependently increased by dietary vitamin D in the spleen of broiler chickens and that induction was further enhanced by calcium- and phosphorus-deficient diet, although CATH3 was downregulated (Rodriguez-Lecompte et al., 2016). Such studies have been described in mammals, and the vitamin D receptor elements in cathelicidin promoter were shown to be involved (Wang et al., 2004a; Cuperus et al., 2013). However, it is uncertain whether vitamin D receptor element is the only recognition and regulation site of avian cathelicidins. There are other mechanisms in the process of vitamin D_3 regulating the expression of avian HDP, which are also worthy of further study.

In addition to butyric acid and vitamin D3 described above, amino acid, microelement, and some kind of polysaccharide could also induce the expression of endogenous cathelicidins. As a nutritional strategy, dietary amino acid supplementation has been proved to be effective in regulating intestinal immune function and controlling intestinal diseases (Lallès et al., 2007). Some amino acids could affect the intestinal AMP expression by regulating the activity of key proteins in the signaling pathway of intestinal epithelial cells. Hashimoto et al. (2012) found that increasing the level of dietary tryptophan could activate the mammalian target of rapamycin signal pathway, thus increasing the mRNA expression of intestinal α-defensin genes (*Defa1* and *Defa5*). Similarly, branched-chain amino acids including isoleucine, valine, and leucine in vivo and in vitro have been demonstrated to augment the expression of porcine intestinal β-defensins through activation of the Sirt1/ERK/90RSK signaling pathway (Ren et al., 2016). Based on these, we can find that some studies were focused on the effect of amino acids as immune-enhancing formulas to promote the expression of defensins, whereas little is known about their impact on avian cathelicidins. Therefore, more experiments are needed to investigate whether amino acids such as tryptophan, lysine, and branched-chain amino acids could alleviate intestinal inflammation in avian species through cathelicidin expression.

It was found that microelements play a major role in regulating HDP expression, especially zinc. The mechanism of zinc protecting the intestinal mucosa barrier and reducing the diarrhea ratio is mediated in part by promoting the expression of HDP (Wu et al., 2019). In the Caco-2 intestinal epithelial cell line, zinc was found to induce phosphorylation of ERK and p38MAP kinases and regulate LL-37 secretion through these MAP kinases (Talukder et al., 2011). A high level of ZnO supplementation (3,000 mg/kg) in the diet significantly increased cathelicidin PR-39 peptide mRNA expression (Wang et al., 2004b). For broilers, increasing the dietary zinc and manganese content or feeding zinc and manganese in the OHCl form synergistically increased the amounts of IL-1 and cathelicidin mRNA in immune cells (Perez et al., 2007).

Some active polysaccharides in plants have broad areas of bioactivities including immune adjunction and antibacterial and antiviral activities, and so on. For example, astragalus polysaccharides have been found to induce the expression of cathelicidin LL-37 through p38MAPK/JNK and nuclear factor-κB signaling pathways in human respiratory epithelial cells, thus participating in its mediated antibacterial action (Zhao et al., 2018). To some extent, polysaccharides from microorganisms also have beneficial bioactivity in modulating pathogen-induced inflammatory responses. Shao et al. (2016) reported that the addition of 200 mg/kg yeast-β-glucans to the diet of broilers can enhance the defensins and

cathelicidins expression and reduced the higher level of *S. enteritidis* colonization and internal organs invasion in the *S. enteritidis* infected birds. In addition, it is worth noting that some studies have found that exopolysaccharides from bacteria could improve the immune function (Jones et al., 2014; Wu et al., 2019). The Se-enriched exopolysaccharides produced by *Enterobacter cloacae* Z0206 significantly increased the serum antibody titers against Newcastle disease virus and enhance the immunity of broilers (Lu et al., 2013). However, whether cathelicidin expression is one of the functions of extracellular polysaccharides is still unknown and worthy of attention.

Furthermore, some studies have shown that although probiotics could not increase the transcription of HDP genes, probiotic treatment prevents the increase of CATH1 expression induced by *S. typhimurium*, and the combination of probiotics and organic acids can increase the CATH-B1 expression of in the bursa of young broiler chickens (Akbari et al., 2008; Rodríguez-Lecompte et al., 2012).

PERSPECTIVE AND CONCLUSION

Host defense peptides are important effectors of animal immune function, which can activate T cells through chemotaxis induction or direct activation of full-time antigen presenting cells and participate in the regulation of animal-specific immune response. In recent years, HDPs have become a research hotspot in many fields. On the one hand, researchers study the biological function, expression, and distribution characteristics and regulation mechanism of AMP in animals at the molecular level, and on the other hand, it is actively developing the application of HDP in the fields of medical treatment, food and health care, animal husbandry, and veterinary medicine.

It has been found that compared with other families, such as defensins, insect-derived and frog skin–derived HDP, cathelicidin families have stronger antibacterial activity and lower MIC values (Feng et al., 2011; Wang et al., 2011). Besides these in vitro tests, among the avian species, only the antibacterial efficacy of chicken CATH1 in vivo was evaluated. Intraperitoneal injection of 10 mg/kg of CATH1 analog (fowlicidin-1 (6–26)) increased the survival rate of mice with a lethal dose of methicillin-resistant *S. aureus*–induced neutropenia by 50%, while reducing bacterial titers in the peritoneal fluid and spleen of mice (Bommineni et al., 2010). In addition, Bommineni et al. (2010) further found that due to the ability of fowlicidin-1(6–26) to induce neutrophil chemotaxis and macrophage activation, 50% of mice could be protected by taking fowlicidin-1(6–26) 4 d before *S. aureus* infection, and all mice could survive if the peptide was received 1–2 d before infection (Bommineni et al., 2014). Taken together, as a novel antimicrobial, fowlicidin-1(6–26) has a good application prospect in treatment and prevention. Furthermore, the bactericidal effect of cathelicidin family HDPs was rapid, and both chicken fowlicidins (CATH1 and 2) showed rapid killing of *E. coli* with the maximum killing occurring at 30 min at MIC_{90} concentrations (Xiao et al., 2006). More importantly, some cathelicidins have very strong activity against amounts of clinically isolated drug-resistant strains, even super drug–resistant bacteria (Guang et al., 2012). Quail Cc-CATH2 and 3 displayed broad and potent antimicrobial activity against most of the 41 strains of bacteria and fungi tested, especially the clinically isolated drug-resistant strains, such as *S. aureus* and *P. aeruginosa* (Feng et al., 2011). At the same time, Cc-CATH2 and 3 showed considerable reduction of cytotoxic activity and hemolytic activity compared with other avian cathelicidins (Feng et al., 2011). Therefore, they are expected to become a class of important new antimicrobial agents, providing new means and ways to solve the increasingly serious problem of drug resistance of strains.

Host defense peptides can affect the development of adaptive immune response by regulating the migration, maturation, and activation of dendritic cells and T and B lymphocytes (Yang et al., 2004; Nicholls et al., 2010; Hancock et al., 2012; Zhang and Sunkara, 2014). As an important member of HDPs, cathelicidins can stimulate Th1 immunoreaction without LPS, thus they could be used as immunomodulators, vaccine adjuvants, or additives to strengthen the adaptive immune response (Van Harten et al., 2018). The adjuvanticity of chicken CATH1 has been experimentally verified. When mice were immunized with ovalbumin and chicken CATH1, the mice challenged with ovalbumin produced higher titers of IgG1 and IgG2a (Bommineni et al., 2014). The reason for this phenomenon may be that CATH1 might induce the expression of costimulatory molecule CD86 on the surface of macrophages more effectively than LL-37; CATH1 may be more effective in facilitating antigen presentation and adaptive immunity, so CATH1 could be a very useful adjuvant or a component of adjuvant complexes (Kindrachuk et al., 2009; Zhang and Sunkara. 2014; Bommineni et al., 2014).

Cathelicidin-related HDP have become effective substances in the innate immune system. Inferred from some other important biological functions of cathelicidins, avian cathelicidins should also have biological functions including wound repair, antitumor property, proangiogenesis, and so on. Therefore, to study the specific application of avian cathelicidin, several important topics will have to be addressed in the future. The basic identification and relevant function analysis of cathelicidins from different birds were carried out, and the role and mechanism of avian cathelicidins in disease were evaluated by in vivo or in vitro experiments, followed by clinical trial evaluation and targeted development of new antimicrobial drugs.

Conclusion

In the present review, we have described recent work on the structure, evolution, biological activity mechanisms, and expression regulation of avian cathelicidins. All these characteristics of avian cathelicidins show their

potential as a substitute for antibiotics. However, various studies have shown that although these small bioactive peptides have health effects, they are still limited in animal trials and human clinical evidence. Therefore, we hope that this review could provide a reference for the health benefit assessment and further application of avian cathelicidins.

DISCLOSURES

The authors declare no conflict of interest.

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SUPPLEMENTARY DATA

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