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Physical Mechanisms of Bacterial Killing by Histones

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Abstract Antibiotic resistance is a global epidemic, becoming increasingly pressing due to its rapid spread. There is thus a critical need to develop new therapeutic approaches. In addition to searching for new antibiotics, looking into existing mechanisms of natural host defense may enable researchers to improve existing defense mechanisms, and to develop effective, synthetic drugs guided by natural principles. Histones, primarily known for their role in condensing mammalian DNA, are antimicrobial and share biochemical similarities with antimicrobial peptides (AMPs); however, the mechanism by which histones kill bacteria is largely unknown. Both AMPs and histones are similar in size, cationic, contain a high proportion of hydrophobic amino acids, and possess the ability to form alpha helices. AMPs, which mostly kill bacteria through permeabilization or disruption of the biological membrane, have recently garnered significant attention for playing a key role in host defenses. This chapter outlines the structure and function of histone proteins as they compare to AMPs and provides an overview of their role in innate immune responses, especially regarding the action of specific histones against microorganisms and their potential mechanism of action against microbial pathogens.

Abbreviations

H1	Histone H1
H2A	Histone H2A
H2B	Histone H2B
H3	Histone H3
H4	Histone H4
AMP	Antimicrobial Peptide
NETs	Neutrophil Extracellular Traps
LDs	Lipid Droplets
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
PAD4	Peptidyl arginine deiminase, type IV
MIC	Minimum inhibitory concentration
CRAMP	Cathelin-related antimicrobial peptide
NMDA	N-methyl-D-aspartate

1.1 Introduction

In 1922, Alexander Fleming discovered lysozyme from nasal mucus[1]. This was the first human antimicrobial protein to be reported; however, the discovery of penicillin in 1928[2] overshadowed this finding, and ushered the world into the "Golden Age" of antibiotics. Recently, the rise of antibiotic resistance, combined with the stagnation in discovering new, viable antimicrobial agents, has sparked renewed interest in natural host defenses. The antimicrobial activity of histones was first reported in 1942[3] and *in vitro* histone killing of bacteria was further characterized in 1958 using *Escherichia coli*[4]. However, despite originally being proposed to function as antimicrobial agents, the role of histones in condensing eukaryotic DNA became seen as their primary function and little is known about their antimicrobial role and the possible mechanisms by which they kill bacteria. The discovery that histones have a central role in innate immune responses[5] has renewed interest into understanding their antimicrobial functions.

Eukaryotic organisms possess a cell nucleus and other organelles enclosed within a membrane. Their nuclei contain genetic material, typically encoded in DNA, within a nuclear envelope. Within the nucleus, small, alkaline histone proteins are used to package the DNA into 5 nm nucleosomes that condense chromatin, the chromosomal material in eukaryotic cells that is composed protein, DNA, and a small amount of RNA. The basic structural unit of chromatin is made up of 146 DNA base pairs wrapped roughly 1.5 times around a histone core. This histone core structure is made up of eight histone components: two H2A-H2B dimers and a H3-H4 tetramer[6]. These core histones are highly conserved through evolution, containing the 'helix turn helix turn helix' central motif, named the histone fold, and an unstructured amino-terminal tail[7]. The structure of H2A, which is representative of the structure of the four core histones, is shown in Figure 1. Histones contain the positively-charged amino acids lysine and arginine, which facilitate their interactions with negatively-charged DNA. The histones are grouped into two classes: lysine-rich (H1, H2A, H2B) and arginine-rich (H3, H4)[7]. The nucleosome complex which contains the segment of DNA wrapped around the histone core, forms the repeating units of chromatin, facilitates higher order chromatin structure, and is necessary for eukaryotic survival. Histone H1 functions as a linker that binds to 20 base pairs of DNA, forming a chromatosome[8]. The structure of H1, with a long C-terminal tail, a short N-terminal tail, and a central globular domain with a winged helix domain[9], is shown in Figure 1. Linker DNA from one chromatosome binds to linker DNA from another chromatosome, further condensing the DNA into 30 nm chromatin fiber.

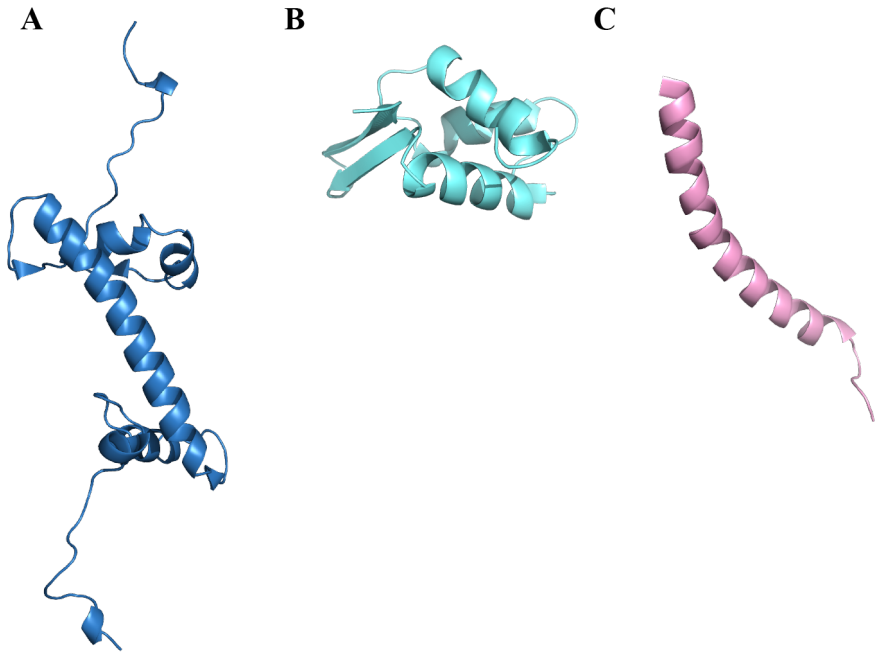

Figure 1. Structure of the core histone H2A, the linker histone H1, and the antimicrobial peptide cathelicidin LL-37. (A) The core histone H2A contains a 'helix turn helix turn helix' central motif, named the histone fold, and an unstructured amino-terminal tail (PDB ID: 1AOI)[6]. (B) The linker histone H1 contains a long C-terminal tail, a short N-terminal tail, and a central globular domain with a winged helix domain (PDB ID: 1GHC)[10]. (C) The antimicrobial peptide cathelicidin LL-37 is a linear peptide folded into an amphipathic α-helix (PDB ID: 2K6O)[11].

1.2 Innate Immune Responses for Combating Bacterial Infections

When histones were believed to be solely in the nucleus, it was hard to imagine how they might play an antimicrobial role. More recently, the observation of histones localizing outside of the cell nucleus, across multiple species, allows one to speculate that they may have roles in addition to chromosome condensation[5,12]. For instance, histones are observed inside cytoplasmic granules in human neutrophils[13]. Further, they are likely functional: H2A and H2B purified from the fetal membranes of the human placenta show dose-dependent inhibition of LPS endotoxin activity, by binding the core and lipid A portions of LPS[14]. These histones are secreted from placenta epithelial cells into the amniotic fluid, contributing to fetal host defenses. Finally, in the Asian toad *Bufo gargarizans*, H2A is synthesized in excess of the amount required for DNA packaging, and unacetylated H2A accumulates within cytoplasmic secretory granules[15]. Histones are also reported to localize to the plasma membrane, possibly for both signaling and targeted release[16].

Neutrophil extracellular traps (NETs)[5] are one of the best explored examples of histones playing a central role in combating bacterial infections. Neutrophils are the immune system's first line of defense against bacterial infections, and their prototypical function involves engulfing bacteria and other pathogens. The engulfed pathogens are subsequently killed through the fusion of the phagosome with antimicrobial, cytoplasmic granules. These granules contain an array of components that kill bacteria, including myeloperoxidase, defensins, lysozyme, proteinases (cathepsin G, elastase, and proteinase 3), bactericidal/permeability-increasing protein

(BPI), NADPH oxidase, cathelicidin LL-37, lactoferrin[17], and of course, the above-mentioned histones[13]. However, neutrophils also have a less canonical, alternate killing pathway. The presence of virulent microorganisms[18], such as *Pseudomonas aeruginosa*[19,20], *Escherichia coli*[21,22], and *Staphylococcus aureus*[23], stimulates a neutrophil immune response known as NETosis[5]. During this process, histones are citrullinated by peptidylarginine deiminase 4 (PAD4), an enzyme essential for chromatin decondensation[24]. This enzyme converts arginine residues, which are charged, into neutral citrulline residues, resulting in a more open chromatin structure. The result is the formation of NETs, which are fibrous networks that contain cation-chelating mitochondrial and nuclear DNA and antimicrobial granular proteins[5,25–30]. NETs kill and suppress the proliferation of microorganisms, though the mechanism of NET-mediated killing remains poorly understood[27]. PAD4 knockout mice have increased susceptibility to bacterial infection due to an inability to form NETs; however, these neutrophils retain the ability to kill bacteria in other ways and mice exposed to septic conditions had comparable survival to wild-type mice[31,32].

Histones constitute a large fraction of the proteins in NETs[5]. However, initially the role of histones in NETs was unclear, as histones might simply be remnant features of the neutrophils. Nonetheless, the co-localization of histones in the NET scaffold, including with the human antimicrobial peptide cathelicidin LL-37[33,34] and HNP alpha-defensins[35], suggest that histones could have a role as an antimicrobial agent here[36,37]. Importantly, antibodies against H2A and H2B eliminate NET-mediated killing of bacteria[5]. Furthermore, purified Histone H2A kill *S. flexneri*, *S. typhimurium*, and *S. aureus* bacterial cultures in 30 minutes with concentrations as low as 2 μg/mL[5]. Combined, these findings suggest that histones likely to play an important anti-bacterial role.

1.3 Possible Side Effects of Histones and How to Modulate Them

The role of histone citrullination in NET antimicrobial activity is unclear. Antibacterial activity in antimicrobial peptides (AMPs) correlates with increasing arginine content [38], either by increasing permeabilization or improving translocation, depending on the mechanism of the AMP. PAD4-mediated citrullination of histones, which decreases the arginine content, may decrease the antimicrobial potency of histones[31]. PAD4 is tightly regulated, possibly to minimize the toxic effects of free histones in the host and to maximize antimicrobial activity, by controlling the potency of the histones within NETs[39]. Given that NETs induce inflammation and are damaging to surrounding host tissue, there is a delicate balance that needs to be met in order to fight pathogen microbes without inducing high levels of damage to the host[40].

Extracellular histone release, which can elicit toxic effects on pathogenic bacteria, can have negative side effects. Due to their ability to interact with biological membranes, extracellular histones can act as proinflammatory signals, triggering inflammatory responses and injury in the host. In contrast, AMPs are not known to serve as proinflammatory signals for the host. The presence of extracellular histones elicits the production of antibodies against histones and contributes to autoimmune and inflammatory responses in patients with systemic lupus erythematosus, neuropsychiatric lupus, and lupus nephritis[41]. Histones have a pro-inflammatory role in several diseases, including sepsis, trauma, thrombosis, stroke, atherogenesis, and systemic lupus. Histones are suspected to be mediators of mortality in sepsis, contributing to endothelial dysfunction, organ failure, and death during sepsis[42]. Extracellular histones are elevated following traumatic tissue injury and the ongoing rise of histone levels are predictive of

mortality, suggesting the role of histones in the sterile inflammatory response following trauma may parallel the role of histones in sepsis[43]. Elevated levels of circulating extracellular histones in trauma-associated lung injuries are associated with endothelial damage and coagulation activation[44].

Extracellular histones contribute as a damage-associated molecular pattern (DAMP), inducing cytotoxicity and pro-inflammatory signaling through toll-like receptor (TLR) TLR2 and TLR4[45]. Extracellular histones promote thrombin generation, which triggers thrombosis[46]. Histones bind to platelets, inducing calcium influx and platelet aggregation causing thrombocytopenia in mice within minutes[47]. Histones promote chemotaxis of human polymorphonuclear leukocytes, suggesting histones may modulate leukocyte activation[48]. Inflammation frequently causes cellular death, leading to the release of cellular components, such as chromatin components, potentially exacerbating the toxic effects of histones by causing the release of additional histones.

NETs and concentrations of H2A higher than 50 μg/mL induce the death of endothelial and lung epithelial cells[49]. While digestion of extracellular DNA decreases the ability of NETs to kill bacteria, DNA digestion does not have any effect on mediating cytotoxicity on epithelial and endothelial cells[49]. Thus, the controlled storage and release of histones upon bacterial infection appears critical. It is plausible that citrullination of histones decreases histone potency in NETs and provides a mechanism that balances antimicrobial activity and toxicity to the host.

In addition to playing an essential role in NET-mediated killing of microbes, histones have been shown to localize to cytoplasmic lipid droplets. Lipid droplets are lipid-rich organelles, found in all eukaryotic organisms, which dynamically regulate the storage and breakdown of lipids. Originally thought to serve solely as fat reservoirs, proteomic analyses have uncovered the presence various proteins, including histones[50]. In early *Drosophila melanogaster* embryos, excess H2A, H2B, and H2Av histones, a variant of H2A, are recruited and bound to lipid droplets, perhaps as a means of temporary storage to avoid toxic effects introduced by free histones[51]. In the presence of bacterial lipopolysaccharide (LPS) or lipoteichoic acid (LTA), these lipid droplet-bound histones are released from the lipid droplets and kill bacteria *in vivo*[12]. Histones bound to lipid droplets protect cells against bacteria without causing any of the harm normally associated with the presence of free histones. Purified *Drosophila* embryos lacking lipid droplet-bound histones also showed decreased survival when assaulted with bacterial species[12].

1.4 Biochemical Properties of Antimicrobial Peptides (AMPs)

Histones possess antimicrobial activity and play a critical role in the innate immune system. Histones share many biochemical similarities with AMPs, as summarized in Table 1. Like AMPs, histones are cationic, contain a high proportion of hydrophobic amino acids, and possess the ability to form alpha helices[52,53]. AMPs and individual histone proteins are comparable in size, averaging 18 kDa and 14 kDa respectively[54,55]. Additionally, both are present in NETs[35,37]. Here, we review the biochemical properties of AMPs and insights into their antimicrobial activity. However, unlike AMPs, far less is known about the antimicrobial and biochemical properties of histones.

In the first line of defense against pathogenic microbes, surface epithelial cells have a crucial role in mediating the host's innate immune response by secreting AMPs[56–58]. In addition to surface epithelial cells, these peptides are secreted by submucosal glands[59,60] and neutrophils[61,62].

AMPs are widely evolutionarily conserved and are found throughout all classes of life, including bacteria[63], plants[64], fungi[65], insects[66], aquatic species[67], birds[68], and mammals[69–71]. AMPs exhibit activity against several classes of microorganisms, including bacteria[69,72–74], fungi[65,75,76], viruses[77–79], protozoa[80], and cancerous cells[81]. In addition to their role as antimicrobial agents, AMPs direct multiple cellular processes in immune defense including cytokine release, chemotaxis, antigen presentation, angiogenesis, and wound healing[82]. These peptides have been proposed as alternative therapeutics due to their rapid-killing, high potency, and broad-spectrum of activity[53].

AMPs tend to be small, typically less than 100 amino acids[83,84]. AMPs are classified into broad groups based on secondary structure, including α-helical, β-sheet, loop, or extended[85]. An example structure of an α-helical AMP, cathelicidin LL-37, is shown in Figure 1. Most of these small peptides are cationic at physiological pH, stemming from the high proportion of the positively-charged amino acids arginine and lysine[86,87]. Despite arginine and lysine having identical charges, arginine occurs more frequently in AMPs, indicating that guanidinium groups may be more beneficial for AMP activity than amine groups[88]. This may be attributed to the ability of arginine to form multiple electrostatic interactions and hydrogen bonds with lipid heads in the membrane, which may cause membrane deformation. AMP sequences also contain a high proportion of hydrophobic residues, lending to an amphipathic structure. Both the cationic and amphipathic characteristics of these AMPs allows for interactions with the anionic lipid bilayers of bacteria. Many AMPs are unstructured in free solution and fold upon insertion into a biological membrane[89]. The ability to associate with biological membranes is a defining feature of AMPs[89,90].

The mechanism of antimicrobial action for many AMPs involves permeabilization or disruption of the microbial membrane; however, many AMPs also target DNA and protein synthesis, disrupt protein folding, or inhibit cell wall synthesis[91–93]. A proposed global mechanism of action for this class of peptides is the Shai-Matsuzaki-Huang model of spontaneous translocation[94–97]. The cationic and amphipathic properties of AMPs enable their binding to the surface of the bacterial membrane and inserting themselves, breaking lipid chain interactions and displacing cations that stabilize the membrane, such as Mg^{2+}. This alters membrane structure, causing membrane thinning and increasing membrane destabilization, in addition to increasing surface tension. At AMP concentrations above a threshold, the high level of surface tension causes permeabilization of the membrane by the formation of transient pores. This action enables additional peptides to enter the interior of the cell. If the AMP concentration is below that which will cause the membrane to fully collapse, overall membrane integrity is preserved. Virtually all AMPs, apart from insect apidaecin-type peptides, have high membrane affinity and induce a certain level of membrane perturbation[98]. An alternate mechanism for proline-rich groups of AMPs is to exploit the inner membrane protein SbmA to penetrate *E. coli*[99].

Despite similarities in the cationic and amphipathic nature across AMPs, there is prominent sequence diversity, allowing for some AMPs to interact with intracellular targets or affect key cellular processes, either in addition to, or instead of, membrane permeabilization. Because of their strong positive charge, most AMPs permeabilize the membrane at concentrations above the minimum inhibitory concentration (MIC) *in vitro*, indicating that membrane permeabilization is a secondary effect of most AMPs[100,101]. Peptide concentrations well above the MIC or high peptide:lipid ratios can falsely indicate a membrane lytic mechanism and mask true intracellular effects. For instance, pleurocidin-derived AMPs inhibit RNA and protein synthesis at the MIC

without affecting membrane integrity; however, at ten times the MIC, cells depolarize and membranes are disrupted[100]. Under conditions that support bacterial killing, human neutrophil peptide defensin [HNP]-1 penetrates the outer and inner membranes of *E. coli*[102]. Upon penetration, bacterial synthesis of DNA, RNA, and protein stops. Inhibition of cytokinesis has been seen with the alpha helical peptide cathelin-related AMP (CRAMP), the mouse ortholog of cathelicidin LL-37. CRAMP impairs *Salmonella typhimurium* cell division *in vitro* and in macrophage-phagocytized bacteria, resulting in long, filamentous structures[103].

Mechanisms of cell death via AMPs can be elucidated by measuring the delay between cell death, measured by an inhibition of colony formation, and membrane permeability changes. AMPs that have a lytic mechanism of action have these two events occur rapidly and concurrently, whereas non-lytic mechanisms of cell death are characterized by a delay between cell death and changes in membrane permeability. Increases in permeability as a secondary effect after bacterial death has been observed with some classic antibiotics, including ceftazidime, ciprofloxacin, and gentamicin[104,105].Various intracellular AMP mechanisms of action have been studied, but the degree to which membrane permeabilization or intracellular mechanisms have a role in cell death are often not investigated. Given the negative charge of DNA and RNA, it is not surprising that positively-charged AMPs bind to nucleic acid polymers *in vitro*.

The similar biochemical properties between AMPs, such as cathelicidin LL-37, and histone proteins have led to the conclusion that the molecules serve redundant functions in their antimicrobial activities[28]. Whether and AMPs have redundant or independent functions has not been fully explored.

Property		**Histones**				**AMPs**
	H1	**H2A**	**H2B**	**H3**	**H4**	
Molecular Weight (kDa)	22[106]	14[107]	14[107]	15[107]	11[107]	18[54]
Charge	Positive due to abundance of lysine residues	Positive due to abundance of lysine residues		Positive, due to arginine residues		Positively-charged, due to lysine and arginine residues
Structural Motifs	Winged helix motif in the globular domain, short N-terminal tail, long C-terminal tail	Histone fold domain: three α-helices connected by two loops				α-helical, β-sheet, loop, or extended
Amino Acid Composition	High proportion of positively-charged amino acids and hydrophobic amino acids	High proportion of positively-charged amino acids and hydrophobic amino acids				High proportion of positively-charged amino acids and hydrophobic amino acids
Defining Feature	Linker histone, stabilizing the chromatin fiber	Component of the histone octomer, which binds and condenses DNA				Associates with and permeabilize membranes
Sequence Diversity Among Species	Largely conserved, but less conserved than core histones	Largely conserved				Prominent sequence diversity

Table 1. Comparison of the biochemical properties of histones and AMPs.

1.5 The Role of Histones and Histone Fragments as Antimicrobial Agents

Full-length histones are antimicrobial

Full-length histones from a range of species have antimicrobial activity, including the rainbow trout, shrimp, and Atlantic salmon. Acetylated H2A is found in skin secretions of the rainbow trout *Oncorhynchus mykiss*[108]. Reconstitution of H2A within the membrane perturbs the

membrane, without forming ion channels, supporting a non-pore-forming mechanism of action. All core histone proteins, H2A, H2B, H3, H4 are found in the blood cells of the invertebrate Pacific white shrimp (*Litopenaeus vannamei*)[109]. These proteins have high sequence identity to the histones of other species, and the N-terminus of H2A has sequence identity to the antimicrobial histone peptides buforin I, parasin, and hopposin. Liver, intestine, and stomach extracts from healthy Atlantic salmon (*Salmo salar*) contain an antimicrobial protein identified as H1[110].

Histones from *Gallus gallus* and mice also have antimicrobial activity. Sequences of bactericidal proteins from mice macrophages activated by gamma interferon have similarities to H1 and H2B histone sequences[111]. H2A, H2B.V, and an H2B C-terminal fragment identified in the liver extracts of White Leghorn hens (*Gallus gallus*) and histones from chicken erythrocytes have antimicrobial activity against Gram-negative and Gram-positive bacteria[112]. Additionally, histones from chicken erythrocytes bind to cell wall components, including lipopolysaccharide (LPS) and lipoteichoic acid (LTA)[113].

Numerous reports indicate antimicrobial activity of histones in humans. H1 and its fragments are present in human terminal ileal mucosal samples and the cytoplasm of villus epithelial cells and showed antimicrobial activity against *Salmonella typhimurium*. *In vitro* culturing of villus epithelial cells from the basement membrane releases antimicrobial H1 proteins while the cells undergo programmed cell death[114]. A shotgun proteomics approach revealed the presence of core histones (H2A, H2B, H3, H4) and linker histones (H1) in human hair shafts and extracts of partially-purified histones kill *E. coli* in a radial diffusion assay[115]. The antimicrobial action of sebocytes from the SEB-1 cell line against *S. aureus* has been attributed to histone H4. Here, synergy between histones and free fatty acids in human sebum are responsible for the antimicrobial effects. As cells in the sebaceous gland secrete their cellular contents into the sebum through holocrine secretion, a secretion mode involving plasma membrane rupture and cellular death, sebocytes use histones as antimicrobial agents released as a sebum component. Analysis of the antimicrobial activity and polypeptide composition of meconium identified histones H2 and H4[116].

Histone-derived peptide fragments are also antimicrobial

Peptides that have antimicrobial activity are formed from the N-terminus cleavage of full-length histones, although this cleavage is not known to occur in humans. These are considered to be AMPs and have been extensively observed in non-mammalian species. The synthesis of inactive proteins require processing to function properly is a common tactic used to prevent off-target harmful effects to the host. Classic examples in the antimicrobial realm are antimicrobial peptides generated from trypsin-mediated cleavage of lactoferrin and neutrophil elastase-mediated cleavage of thrombin[117,118]. Endogenous proteases are implicated in the production of AMPs from lysine-rich histones. Following cleavage, AMP antimicrobial activity can be attributed to the amphipathic secondary structure with net positive charge, allowing for membrane binding, membrane permeabilization, and binding to nucleic acids[52]. Here, we provide a summary of AMPs that have sequence similarity to the N-terminus of the different histones.

Histone H1 homologs

AMPs with sequence similarity to H1 are present in Atlantic salmon, rainbow trout, and Coho salmon. In *Salmo salar*, the Atlantic salmon, a 30-residue N-terminally acetylated peptide derived from H1 is present in the skin mucus and has activity against both Gram-negative and Gram-positive bacteria. Isomerization of the proline peptide bond is crucial for activity, leading to increased structure, condensation, and rigidity of the peptide[119]. A potent antimicrobial peptide in *O. mykiss*[120] with sequence identity to the H1 induces destabilization of planar lipid bilayers. Blood and mucus antimicrobial fractions of Coho salmon (*Oncorhynchus kisutch*) has sequence identity with the N-terminus of H1. Synthetic peptides showed no antimicrobial effects, but showed synergy with the flounder peptide pleurocidin and lysozyme[121].

Histone H2A and H2B homologs

There are several known AMPs that have sequence similarity with histone H2A. Parasin I is a 19-amino acid antimicrobial peptide secreted into the epithelial mucosal layer by the catfish *Parasilurus asotus* in response to epidermal injury[122]. The AMP shows high homology to the N-terminal region of H2A and is thought to be produced by cathepsin D-directed H2A proteolysis upon injury[123]. The basic N-terminal residue is essential for membrane-binding, and the α-helical structure is necessary for membrane-permeabilizing[124].

Buforin I is a 39-amino acid AMP isolated from the Asian toad *Bufo bufo gargarizans*, composed of the N-terminal parasin and buforin II. Upon pepsin-mediated proteolysis of the Tyr^{39}-Ala^{40} H2A bond in the cytoplasm of gastric gland cells, buforin I is secreted into the gastric lumen where it adheres to the stomach mucosal surface and forms a protective antimicrobial coating[15]. In contrast, unacetylated H2A is located in the cytoplasm of gastric gland cells, suggesting a portion of cytoplasmic unacetylated H2A is secreted into the lumen and undergoes pepsin processing, while another portion of H2A is acetylated and targeted for nuclear translocation.

Buforin II (BF2) is a 21-amino acid peptide derived from endoproteinase Lys-C treatment of buforin I, which displays increased antimicrobial activity compared to buforin I and adopts a helix-hinge-helix structure in 50% trifluoroenthanol[125,126]. Both buforin I and buforin II share sequence identity to the N-terminus of H2A[127]. Circular dichroism measurements of equipotent Trp-substituted peptides indicate that BF2 binds selectively to liposomes composed of acidic phospholipids and has weak membrane permeabilization activity when compared to magainin 2, a membrane-permeabilizing *Xenopus laevis* antimicrobial peptide[128]. Instead, BF2 is efficiently translocated across lipid bilayers, supporting an intracellular mechanism of bacterial death by nucleic acid binding. The Pro^{11} residue is structurally responsible for introducing a kink in the α helix and disturbing the helical structure[129]. To translocate the lipid bilayer, BF2 forms a toroidal pore that is destabilized by the electrostatic repulsion that accompanies five basic amino acids in close proximity, promoting translocation of the peptide across the bacterial cell membrane. In membranes, amidated BF2 adopts a poorly helical structure in membranes, mimicking the composition of *E. coli*, and binds to duplex DNA causing condensation[130].

The α-helical structure, which directs cell-penetration, has been shown to be critical in determining antimicrobial efficacy[131]. The helix-hinge-helix domain enables BF2 to enter bacterial cells without inducing membrane disruption, where the AMP binds to intracellular nucleic acids and inhibit cellular functioning[132]. Although *in vitro* binding of BF2 to nucleic acid has been shown, it is unknown whether this interaction is directed or a result of opposite charged interactions. Further characterization of the nucleic acid binding property of BF2 indicates that

the R^2 and R^{20} side chains of BF2 form interactions with DNA that are stronger than non-specific electrostatic interactions, and that the substitution of the basic residues with alanine decreases the antimicrobial activity of BF2[133].

Hipposin is a potent 51-residue antimicrobial peptide isolated from the skin mucus of Atlantic halibut *Hippoglossus hippoglossus L*[134]. This AMP has 98% sequence similarity to the N-terminal of histone 2A from rainbow trout, and has sequence similarities to both parasin and BF2. The AMP was shown to kill bacteria through membrane permeabilization, as evidenced by increased propidium iodide fluorescence intracellularly following peptide exposure and localization of AlexaFluor conjugates around the cellular membrane[135]. The localization of fluorescence around the cell membrane, with low fluorescence intracellularly, is similar to the fluorescence pattern depicted by parasin, another histone-derived peptide that causes permeabilization[124]. The N-terminal parasin domain of hipposin is necessary for membrane permeabilization, as peptides lacking the parasin domain show translocation of the membrane, without permeabilization. The C-terminal domain of hipposin, HipC, is cell-penetrating, but shows no measurable antimicrobial activity.

A combination of molecular dynamics (MD) simulations and DNA binding affinity experiments provide support for BF2 forming specific interactions with DNA[133]. Additionally, through the use of BF2 variants, the affinity of the peptide for DNA has been correlated with increased antimicrobial activity. Additional MD simulations, along with electrostatic analysis and nucleic acid binding experiments, on buforin II and DesHDAP1, a designed histone-derived AMP thought to share a similar structure and mechanism of action with buforin II, support a sequence-independent method of AMP binding to DNA[136]. Instead of peptide binding with sequence specificity, peptide-phosphate interactions are thought to be the predominant basis of AMP binding to DNA. As such, arginine residues are shown to have greater antimicrobial activity than lysine residues, possibly due to increases interactions with DNA; however, higher arginine composition could also influence AMP-membrane interactions.

H3 and H4 homologs

No known natural fragments with similarity to H3 have been identified. Histograninn, a slightly modified C-terminal 15 amino acid fragment showing similarities to the C-terminal end of H4 has been identified from bovine adrenal medulla[137]. The fragment, which is synthesized from a separate mRNA variant, is an antagonist of N-methyl-D-aspartate (NMDA) receptor activity. Histograninn has antimicrobial activity against Gram-negative and Gram-positive bacteria and is thought to function through inhibition of ATP-dependent DNA gyrase, a mechanism similar to quinolone antibiotics[138].

1.6 Insights into the Mechanism of Histone-Mediated Killing of Bacteria

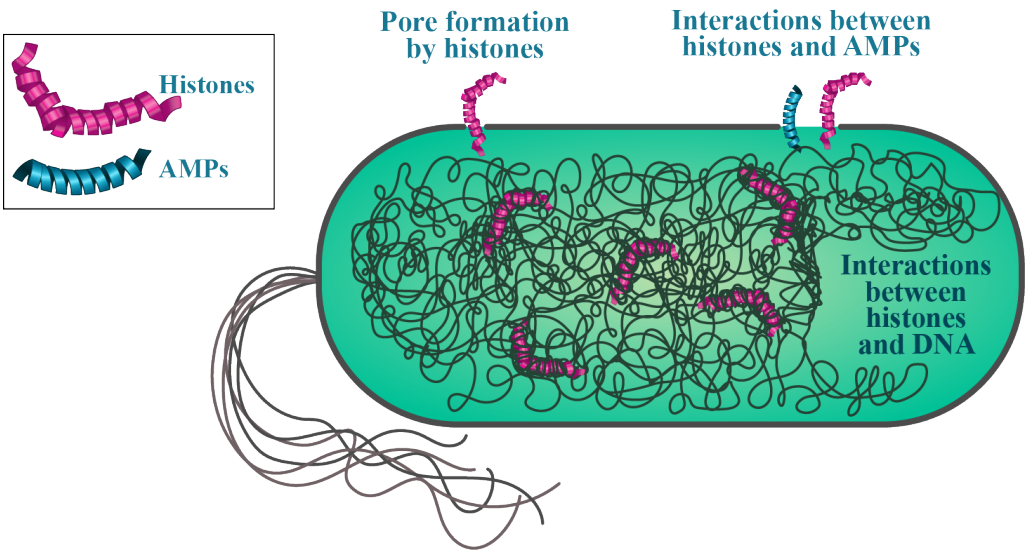

Figure 2. Potential mechanisms of bacterial killing by histones. Histones have high affinity for LPS and histone-derived peptides induce membrane permeabilization. Full-length histones thus may bind to bacterial membranes, disrupting the membrane and forming pores. The high affinity of histones for phosphodiester bonds, which enables histones to bind and condense DNA, suggests that part of the antimicrobial activity of histones may involve interactions between histones and microbial DNA. Since histones alone show weak antimicrobial activity *in vivo*, the antimicrobial activity of histones may be dependent upon interactions with AMPs or other antimicrobial agents.

The findings from histone-derived AMPs suggest that part of the antimicrobial activity of histones is achieved through membrane permeabilization, as depicted in Figure 2. The linker histone H1 and four core histones from calf thymus bind LPS present on the outer membrane. All histones except H4 have affinities for LPS that are greater than that of the antibiotic polymyxin B[139]. The strong affinity of histones for phosphodiester bonds enables histone binding to DNA and facilitates proper chromatin structure formation. However, this affinity may extend to the phosphodiester bonds in phospholipids, facilitating the integration of histones into membranes. The strong positive charge of histones from *Plasmodium falciparum*, a unicellular protozoan parasite that causes malaria in humans, increases membrane permeability in human endothelial cells and induces IL-8 production at concentrations higher than 50 μg/mL[140]. The negatively-charged glycoaminoglycans (GAGs) heparan sulfate and hyaluronan protect CHO cells from histone-induced cytotoxicity, supporting the notion that glycocalyx, the negatively-charged polysaccharide network that protects cells from bacteria, may further mitigate the effects of histones by preventing membrane insertion[141]. The strong positive charge of histones may induce permeability in membranes across of a broad range of organisms including bacteria. Divalent cations, such as Mg^{2+}, function as cationic bridges between adjacent phosphates on LPS. Histones may compete with divalent cations, compromising LPS cross-bridges, and destroying the outer membrane integrity.

Other work suggests that the antimicrobial mechanism of histones occurs following entry into the bacterium and that the target is cytoplasmic (Figure 2). An active fragment of H2B from *R. schlegelli* is thought to be generated via digestion by the bacterial outer membrane protease T (OmpT)[111]. This fragment of H2B can penetrate the cell membrane of OmpT-expressing *E. coli*, but not *ompT*-deleted *E. coli*, accumulate in the cytoplasm, and inhibit cell function, presumably

by binding to nucleic acids. In the absence of OmpT, H2B is unable to penetrate the membrane, and remains localized on the exterior of the bacteria[142]. Consistent with requirement for H2B translocation into the cell, the MIC values for H2B, H3, and H4 significantly increase in the absence of OmpT[143]. The antimicrobial effects observed at higher concentrations of histones may be due to the secondary effect of histones on increasing membrane permeation, and not the primary mechanism by which lysine-rich histones kill bacteria. In addition, lysine-rich (H1, H2A, H2B) and arginine-rich (H3, H4) histones likely kill bacteria using distinct mechanisms. While H2B penetrates *E. coli* membranes and enters the cytoplasmic space, H3 and H4 remain localized on the cell surface, causing membrane blebbing[143].

1.7 Conclusion and Future Developments

Despite being originally proposed to function as antimicrobial agents [144,145], and having an essential role in mammalian innate immune responses[5], little is known about how histones function as antimicrobial agents. Complicating matters is that studies on the antimicrobial activity of histones typically utilize low-ionic solutions and buffers that are not physiologically relevant[5,12,50,142,143,146–156]. In physiologically relevant conditions, histones are far less effective at killing bacteria[12,53], and require high, unphysiological concentrations of histones (120 μg/mL) [12,143,157]. Furthermore, some studies use concentrations of histones well above the MIC[143,157]. As noted above, peptide concentrations in excess of the MIC may render bacteria susceptible to secondary mechanisms of histones through membrane permeabilization[100,101].

It is plausible that since that histones show weak antimicrobial activity *in vivo*, the antimicrobial activity of histones is dependent upon interactions with other immune system mechanisms or components (Figure 2)[158]. Synergy between antibacterial peptides released from activated neutrophils has been reported previously. In the absence of salt, defensins show antibacterial activity in a dose-dependent manner; however, antimicrobial activity is lost in the presence of salt[159]. Defensins exhibit synergy with cathelicidins in the killing of *E. coli* and *S. aureus*[159] . There have also been reports of histone H1 fragments having synergistic antimicrobial effects with lysozyme, lysozyme-containing extracts from *O. kisutch*, and pleurocidin against *Vibrio anguillarum* and *Aeromonas salmonicida*[121]. Future experiments will need to focus on mechanistic details of histones and will need to consider their role in the context of the immune system as a whole, not as a sole antimicrobial agent.

References

1. Fleming, A. On a Remarkable Bacteriolytic Element Found in Tissues and Secretions. *Proceedings of the Royal Society B: Biological Sciences* **93**, 306–317 (1922).

2. Fleming, A. Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae by Alexander Fleming, Reprinted from the British Journal of Experimental Pathology 10:226-236, 1929. *Rev. Infect. Dis.* **2**, 129–139 (1980).
3. Miller, B. F., Abrams, R., Dorfman, A. & Klein, M. ANTIBACTERIAL PROPERTIES OF PROTAMINE AND HISTONE. *Science* **96**, 428–430 (1942).
4. Hirsch, J. G. BACTERICIDAL ACTION OF HISTONE. *Journal of Experimental Medicine* **108**, 925–944 (1958).
5. Brinkmann, V. Neutrophil Extracellular Traps Kill Bacteria. *Science* **303**, 1532–1535 (2004).
6. Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**, 251–260 (1997).
7. DeLange, R. J. & Smith, E. L. Histones: Structure and Function. *Annual Review of Biochemistry* **40**, 279–314 (1971).
8. Allan, J., Hartman, P. G., Crane-Robinson, C. & Aviles, F. X. The structure of histone H1 and its location in chromatin. *Nature* **288**, 675–679 (1980).
9. Zhou, B.-R. *et al.* Structural insights into the histone H1-nucleosome complex. *Proceedings of the National Academy of Sciences* **110**, 19390–19395 (2013).
10. Cerf, C. *et al.* Homo- and Heteronuclear Two-Dimensional NMR Studies of the Globular Domain of Histone H1: Full Assignment, Tertiary Structure, and Comparison with the Globular Domain of Histone H5. *Biochemistry* **33**, 11079–11086 (1994).
11. Wang, G. Structures of Human Host Defense Cathelicidin LL-37 and Its Smallest Antimicrobial Peptide KR-12 in Lipid Micelles. *J. Biol. Chem.* **283**, 32637–32643 (2008).

12. Anand, P. *et al.* A novel role for lipid droplets in the organismal antibacterial response. *eLife* **1**, (2012).
13. Lominadze, G. *et al.* Proteomic Analysis of Human Neutrophil Granules. *Molecular & Cellular Proteomics* **4**, 1503–1521 (2005).
14. Kim, H. S. *et al.* Endotoxin-Neutralizing Antimicrobial Proteins of the Human Placenta. *The Journal of Immunology* **168**, 2356–2364 (2002).
15. Kim, H. S. *et al.* Pepsin-Mediated Processing of the Cytoplasmic Histone H2A to Strong Antimicrobial Peptide Buforin I. *The Journal of Immunology* **165**, 3268–3274 (2000).
16. Watson, K. *et al.* Extra-nuclear location of histones in activated human peripheral blood lymphocytes and cultured T-cells. *Biochem. Pharmacol.* **50**, 299–309 (1995).
17. Segal, A. W. HOW NEUTROPHILS KILL MICROBES. *Annual Review of Immunology* **23**, 197–223 (2005).
18. Delgado-Rizo, V. *et al.* Neutrophil Extracellular Traps and Its Implications in Inflammation: An Overview. *Frontiers in Immunology* **8**, (2017).
19. Dwyer, M. *et al.* Cystic Fibrosis Sputum DNA Has NETosis Characteristics and Neutrophil Extracellular Trap Release Is Regulated by Macrophage Migration-Inhibitory Factor. *Journal of Innate Immunity* **6**, 765–779 (2014).
20. Floyd, M. *et al.* Swimming Motility Mediates the Formation of Neutrophil Extracellular Traps Induced by Flagellated Pseudomonas aeruginosa. *PLOS Pathogens* **12**, e1005987 (2016).
21. Kambas, K. *et al.* Autophagy Mediates the Delivery of Thrombogenic Tissue Factor to Neutrophil Extracellular Traps in Human Sepsis. *PLoS ONE* **7**, e45427 (2012).

22. Yu, Y. *et al.* Characterization of Early-Phase Neutrophil Extracellular Traps in Urinary Tract Infections. *PLOS Pathogens* **13**, e1006151 (2017).
23. Pilsczek, F. H. *et al.* A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. *J. Immunol.* **185**, 7413–7425 (2010).
24. Lewis, H. D. *et al.* Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat. Chem. Biol.* **11**, 189–191 (2015).
25. Keshari, R. S. *et al.* Cytokines induced neutrophil extracellular traps formation: implication for the inflammatory disease condition. *PLoS ONE* **7**, e48111 (2012).
26. Fuchs, T. A. *et al.* Novel cell death program leads to neutrophil extracellular traps. *J. Cell Biol.* **176**, 231–241 (2007).
27. Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology* **18**, 134–147 (2017).
28. Brinkmann, V. & Zychlinsky, A. Neutrophil extracellular traps: Is immunity the second function of chromatin? *The Journal of Cell Biology* **198**, 773–783 (2012).
29. Yipp, B. G. *et al.* Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat. Med.* **18**, 1386–1393 (2012).
30. Halverson, T. W. R., Wilton, M., Poon, K. K. H., Petri, B. & Lewenza, S. DNA Is an Antimicrobial Component of Neutrophil Extracellular Traps. *PLOS Pathogens* **11**, e1004593 (2015).
31. Li, P. *et al.* PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J. Exp. Med.* **207**, 1853–1862 (2010).
32. Martinod, K. *et al.* PAD4-deficiency does not affect bacteremia in polymicrobial sepsis and ameliorates endotoxemic shock. *Blood* **125**, 1948–1956 (2015).

33. von Kockritz-Blickwede, M. *et al.* Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. *Blood* **111**, 3070–3080 (2008).
34. Chow, O. A. *et al.* Statins Enhance Formation of Phagocyte Extracellular Traps. *Cell Host & Microbe* **8**, 445–454 (2010).
35. Ganz, T. Defensins: antimicrobial peptides of innate immunity. *Nature Reviews Immunology* **3**, 710–720 (2003).
36. Brinkmann, V. & Zychlinsky, A. Beneficial suicide: why neutrophils die to make NETs. *Nature Reviews Microbiology* **5**, 577–582 (2007).
37. Kawasaki, H. & Iwamuro, S. Potential roles of histones in host defense as antimicrobial agents. *Infect Disord Drug Targets* **8**, 195–205 (2008).
38. Cutrona, K. J., Kaufman, B. A., Figueroa, D. M. & Elmore, D. E. Role of arginine and lysine in the antimicrobial mechanism of histone-derived antimicrobial peptides. *FEBS Letters* **589**, 3915–3920 (2015).
39. Neeli, I. & Radic, M. Opposition between PKC isoforms regulates histone deimination and neutrophil extracellular chromatin release. *Front Immunol* **4**, 38 (2013).
40. Cheng, O. Z. & Palaniyar, N. NET balancing: a problem in inflammatory lung diseases. *Front Immunol* **4**, 1 (2013).
41. Sun, X., Shi, J., Han, L., Su, Y. & Li, Z. Anti-histones antibodies in systemic lupus erythematosus: prevalence and frequency in neuropsychiatric lupus. *Journal of Clinical Laboratory Analysis* **22**, 271–277 (2008).
42. Xu, J. *et al.* Extracellular histones are major mediators of death in sepsis. *Nat. Med.* **15**, 1318–1321 (2009).

43. Kutcher, M. E. *et al.* Extracellular histone release in response to traumatic injury: implications for a compensatory role of activated protein C. *J Trauma Acute Care Surg* **73**, 1389–1394 (2012).
44. Abrams, S. T. *et al.* Circulating Histones Are Mediators of Trauma-associated Lung Injury. *American Journal of Respiratory and Critical Care Medicine* **187**, 160–169 (2013).
45. Xu, J., Zhang, X., Monestier, M., Esmon, N. L. & Esmon, C. T. Extracellular Histones Are Mediators of Death through TLR2 and TLR4 in Mouse Fatal Liver Injury. *The Journal of Immunology* **187**, 2626–2631 (2011).
46. Semeraro, F. *et al.* Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* **118**, 1952–1961 (2011).
47. Fuchs, T. A., Bhandari, A. A. & Wagner, D. D. Histones induce rapid and profound thrombocytopenia in mice. *Blood* **118**, 3708–3714 (2011).
48. Nowak, D., Piasecka, G. & Hrabec, E. Chemotactic activity of histones for human polymorphonuclear leukocytes. *Experimental Pathology* **40**, 111–116 (1990).
49. Saffarzadeh, M. *et al.* Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. *PLoS ONE* **7**, e32366 (2012).
50. Cermelli, S., Guo, Y., Gross, S. P. & Welte, M. A. The Lipid-Droplet Proteome Reveals that Droplets Are a Protein-Storage Depot. *Current Biology* **16**, 1783–1795 (2006).
51. Li, Z. *et al.* Lipid Droplets Control the Maternal Histone Supply of Drosophila Embryos. *Current Biology* **22**, 2104–2113 (2012).
52. Hancock, R. E. W. & Lehrer, R. Cationic peptides: a new source of antibiotics. *Trends in Biotechnology* **16**, 82–88 (1998).

53. Hancock, R. E. W. & Sahl, H.-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* **24**, 1551–1557 (2006).
54. Dürr, U. H. N., Sudheendra, U. S. & Ramamoorthy, A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1758**, 1408–1425 (2006).
55. Chua, E. Y. D. *et al.* 3.9 Å structure of the nucleosome core particle determined by phase-plate cryo-EM. *Nucleic Acids Res.* **44**, 8013–8019 (2016).
56. Bals, R. Epithelial antimicrobial peptides in host defense against infection. *Respiratory Research* **1**, 5 (2000).
57. Agerberth, B. *et al.* Antibacterial Components in Bronchoalveolar Lavage Fluid from Healthy Individuals and Sarcoidosis Patients. *American Journal of Respiratory and Critical Care Medicine* **160**, 283–290 (1999).
58. Bals, R., Wang, X., Zasloff, M. & Wilson, J. M. 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 (1998).
59. Dajani, R. *et al.* Lysozyme Secretion by Submucosal Glands Protects the Airway from Bacterial Infection. *American Journal of Respiratory Cell and Molecular Biology* **32**, 548–552 (2005).
60. Chen, P.-H. & Fang, S.-Y. The Expression of Human Antimicrobial Peptide LL-37 in the Human Nasal Mucosa. *American Journal of Rhinology* **18**, 381–385 (2004).
61. Wiesner, J. & Vilcinskas, A. Antimicrobial peptides: The ancient arm of the human immune system. *Virulence* **1**, 440–464 (2010).

62. Jann, N. J. *et al.* Neutrophil antimicrobial defense against *Staphylococcus aureus* is mediated by phagolysosomal but not extracellular trap-associated cathelicidin. *Journal of Leukocyte Biology* **86**, 1159–1169 (2009).
63. Hassan, M., Kjos, M., Nes, I. F., Diep, D. B. & Lotfipour, F. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. *Journal of Applied Microbiology* **113**, 723–736 (2012).
64. Castro, M. S. & Fontes, W. Plant defense and antimicrobial peptides. *Protein Pept. Lett.* **12**, 13–18 (2005).
65. Hegedüs, N. & Marx, F. Antifungal proteins: More than antimicrobials? *Fungal Biology Reviews* **26**, 132–145 (2013).
66. Lemaitre, B. & Hoffmann, J. The Host Defense of *Drosophila melanogaster*. *Annual Review of Immunology* **25**, 697–743 (2007).
67. Cole, A. M., Weis, P. & Diamond, G. Isolation and characterization of pleurocidin, an antimicrobial peptide in the skin secretions of winter flounder. *J. Biol. Chem.* **272**, 12008–12013 (1997).
68. van Dijk, A., Veldhuizen, E. J. A. & Haagsman, H. P. Avian defensins. *Vet. Immunol. Immunopathol.* **124**, 1–18 (2008).
69. Hancock, R. E. W. & Diamond, G. The role of cationic antimicrobial peptides in innate host defences. *Trends in Microbiology* **8**, 402–410 (2000).
70. Selsted, M. E. & Ouellette, A. J. Mammalian defensins in the antimicrobial immune response. *Nature Immunology* **6**, 551–557 (2005).
71. Zanetti, M. Cathelicidins, multifunctional peptides of the innate immunity. *Journal of Leukocyte Biology* **75**, 39–48 (2004).

72. Mahlapuu, M., Håkansson, J., Ringstad, L. & Björn, C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Frontiers in Cellular and Infection Microbiology* **6**, (2016).
73. Reddy, K. V. R., Yedery, R. D. & Aranha, C. Antimicrobial peptides: premises and promises. *International Journal of Antimicrobial Agents* **24**, 536–547 (2004).
74. Marr, A., Gooderham, W. & Hancock, R. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Current Opinion in Pharmacology* **6**, 468–472 (2006).
75. Cheng, S.-C., Joosten, L. A. B., Kullberg, B.-J. & Netea, M. G. Interplay between Candida albicans and the Mammalian Innate Host Defense. *Infection and Immunity* **80**, 1304–1313 (2012).
76. De Lucca, A. J. & Walsh, T. J. Antifungal Peptides: Novel Therapeutic Compounds against Emerging Pathogens. *Antimicrobial Agents and Chemotherapy* **43**, 1–11 (1999).
77. Hsieh, I.-N. & Hartshorn, K. The Role of Antimicrobial Peptides in Influenza Virus Infection and Their Potential as Antiviral and Immunomodulatory Therapy. *Pharmaceuticals* **9**, 53 (2016).
78. Bastian, A. & Schäfer, H. Human alpha-defensin 1 (HNP-1) inhibits adenoviral infection in vitro. *Regul. Pept.* **101**, 157–161 (2001).
79. Horne, W. S. *et al.* Antiviral cyclic d,l-α-peptides: Targeting a general biochemical pathway in virus infections. *Bioorganic & Medicinal Chemistry* **13**, 5145–5153 (2005).
80. Torrent, M., Pulido, D., Rivas, L. & Andreu, D. Antimicrobial peptide action on parasites. *Curr Drug Targets* **13**, 1138–1147 (2012).
81. Deslouches, B. & Di, Y. P. Antimicrobial peptides with selective antitumor mechanisms: prospect for anticancer applications. *Oncotarget* **8**, (2017).

82. Lai, Y. & Gallo, R. L. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends in Immunology* **30**, 131–141 (2009).
83. Jenssen, H., Hamill, P. & Hancock, R. E. W. Peptide Antimicrobial Agents. *Clinical Microbiology Reviews* **19**, 491–511 (2006).
84. Sang, Y. & Blecha, F. Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. *Anim. Health. Res. Rev.* **9**, 227–235 (2008).
85. Bahar, A. & Ren, D. Antimicrobial Peptides. *Pharmaceuticals* **6**, 1543–1575 (2013).
86. Epand, R. M. & Vogel, H. J. Diversity of antimicrobial peptides and their mechanisms of action. *Biochim. Biophys. Acta* **1462**, 11–28 (1999).
87. Lehrer, R. I. & Ganz, T. Antimicrobial peptides in mammalian and insect host defence. *Curr. Opin. Immunol.* **11**, 23–27 (1999).
88. Hristova, K. & Wimley, W. C. A look at arginine in membranes. *J. Membr. Biol.* **239**, 49–56 (2011).
89. Yeaman, M. R. Mechanisms of Antimicrobial Peptide Action and Resistance. *Pharmacological Reviews* **55**, 27–55 (2003).
90. Pasupuleti, M., Schmidtchen, A. & Malmsten, M. Antimicrobial peptides: key components of the innate immune system. *Critical Reviews in Biotechnology* **32**, 143–171 (2012).
91. Kumar, P., Kizhakkedathu, J. & Straus, S. Antimicrobial Peptides: Diversity, Mechanism of Action and Strategies to Improve the Activity and Biocompatibility In Vivo. *Biomolecules* **8**, 4 (2018).
92. Scocchi, M., Mardirossian, M., Runti, G. & Benincasa, M. Non-Membrane Permeabilizing Modes of Action of Antimicrobial Peptides on Bacteria. *Curr Top Med Chem* **16**, 76–88 (2016).

93. Le, C.-F., Fang, C.-M. & Sekaran, S. D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrob. Agents Chemother.* **61**, e02340-16, /aac/61/4/e02340-16.atom (2017).
94. Shai, Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim. Biophys. Acta* **1462**, 55–70 (1999).
95. Shai, Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* **66**, 236–248 (2002).
96. Matsuzaki, K. Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim. Biophys. Acta* **1376**, 391–400 (1998).
97. Huang, H. W. Action of Antimicrobial Peptides: Two-State Model †. *Biochemistry* **39**, 8347–8352 (2000).
98. Casteels, P. & Tempst, P. Apidaecin-type peptide antibiotics function through a non-poreforming mechanism involving stereospecificity. *Biochem. Biophys. Res. Commun.* **199**, 339–345 (1994).
99. Mattiuzzo, M. *et al.* Role of the Escherichia coli SbmA in the antimicrobial activity of proline-rich peptides. *Mol. Microbiol.* **66**, 151–163 (2007).
100. Patrzykat, A., Friedrich, C. L., Zhang, L., Mendoza, V. & Hancock, R. E. W. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in Escherichia coli. *Antimicrob. Agents Chemother.* **46**, 605–614 (2002).
101. Podda, E. *et al.* Dual mode of action of Bac7, a proline-rich antibacterial peptide. *Biochim. Biophys. Acta* **1760**, 1732–1740 (2006).

669 102. Lehrer, R. I. *et al.* Interaction of human defensins with Escherichia coli. Mechanism of
670 bactericidal activity. *Journal of Clinical Investigation* **84**, 553–561 (1989).
671 103. Rosenberger, C. M., Gallo, R. L. & Finlay, B. B. Interplay between antibacterial
672 effectors: a macrophage antimicrobial peptide impairs intracellular Salmonella replication.
673 *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2422–2427 (2004).
674 104. Walberg, M., Gaustad, P. & Steen, H. B. Rapid assessment of ceftazidime, ciprofloxacin,
675 and gentamicin susceptibility in exponentially-growing E. coli cells by means of flow
676 cytometry. *Cytometry* **27**, 169–178 (1997).
677 105. Wickens, H. J., Pinney, R. J., Mason, D. J. & Gant, V. A. Flow cytometric investigation
678 of filamentation, membrane patency, and membrane potential in Escherichia coli following
679 ciprofloxacin exposure. *Antimicrob. Agents Chemother.* **44**, 682–687 (2000).
680 106. Bäuerle, M., Doenecke, D. & Albig, W. The Requirement of H1 Histones for a
681 Heterodimeric Nuclear Import Receptor. *J. Biol. Chem.* **277**, 32480–32489 (2002).
682 107. Haas, A. L., Bright, P. M. & Jackson, V. E. Functional diversity among putative E2
683 isozymes in the mechanism of ubiquitin-histone ligation. *J. Biol. Chem.* **263**, 13268–13275
684 (1988).
685 108. Fernandes, J. M. O., Kemp, G. D., Molle, M. G. & Smith, V. J. Anti-microbial properties
686 of histone H2A from skin secretions of rainbow trout, Oncorhynchus mykiss. *Biochemical*
687 *Journal* **368**, 611–620 (2002).
688 109. Patat, S. A. *et al.* Antimicrobial activity of histones from hemocytes of the Pacific white
689 shrimp. *European Journal of Biochemistry* **271**, 4825–4833 (2004).

110. Richards, R. C., O'Neil, D. B., Thibault, P. & Ewart, K. V. Histone H1: An Antimicrobial Protein of Atlantic Salmon (Salmo salar). *Biochemical and Biophysical Research Communications* **284**, 549–555 (2001).

111. Kawasaki, H., Koyama, T., Conlon, J. M., Yamakura, F. & Iwamuro, S. Antimicrobial action of histone H2B in Escherichia coli: Evidence for membrane translocation and DNA-binding of a histone H2B fragment after proteolytic cleavage by outer membrane proteinase T. *Biochimie* **90**, 1693–1702 (2008).

112. Li, G.-H., Mine, Y., Hincke, M. T. & Nys, Y. Isolation and characterization of antimicrobial proteins and peptide from chicken liver. *Journal of Peptide Science* **13**, 368–378 (2007).

113. Rose-Martel, M. & Hincke, M. T. Antimicrobial histones from chicken erythrocytes bind bacterial cell wall lipopolysaccharides and lipoteichoic acids. *International Journal of Antimicrobial Agents* **44**, 470–472 (2014).

114. Rose, F. R. *et al.* Potential role of epithelial cell-derived histone H1 proteins in innate antimicrobial defense in the human gastrointestinal tract. *Infect. Immun.* **66**, 3255–3263 (1998).

115. Adav, S. S. *et al.* Studies on the Proteome of Human Hair - Identification of Histones and Deamidated Keratins. *Scientific Reports* **8**, (2018).

116. Kai-Larsen, Y. *et al.* Antimicrobial Components of the Neonatal Gut Affected Upon Colonization. *Pediatric Research* **61**, 530–536 (2007).

117. Kuwata, H., Yip, T.-T., Yip, C. L., Tomita, M. & Hutchens, T. W. Bactericidal Domain of Lactoferrin: Detection, Quantitation, and Characterization of Lactoferricin in Serum by

SELDI Affinity Mass Spectrometry. *Biochemical and Biophysical Research Communications* **245**, 764–773 (1998).

118. Papareddy, P. *et al.* Proteolysis of Human Thrombin Generates Novel Host Defense Peptides. *PLoS Pathogens* **6**, e1000857 (2010).

119. Luders, T., Birkemo, G. A., Nissen-Meyer, J., Andersen, O. & Nes, I. F. Proline Conformation-Dependent Antimicrobial Activity of a Proline-Rich Histone H1 N-Terminal Peptide Fragment Isolated from the Skin Mucus of Atlantic Salmon. *Antimicrobial Agents and Chemotherapy* **49**, 2399–2406 (2005).

120. Fernandes, J. Isolation and characterisation of oncorhyncin II, a histone H1-derived antimicrobial peptide from skin secretions of rainbow trout, Oncorhynchus mykiss. *Developmental & Comparative Immunology* **28**, 127–138 (2004).

121. Patrzykat, A., Zhang, L., Mendoza, V., Iwama, G. K. & Hancock, R. E. W. Synergy of Histone-Derived Peptides of Coho Salmon with Lysozyme and Flounder Pleurocidin. *Antimicrobial Agents and Chemotherapy* **45**, 1337–1342 (2001).

122. Park, I. Y., Park, C. B., Kim, M. S. & Kim, S. C. Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, *Parasilurus asotus*. *FEBS Letters* **437**, 258–262 (1998).

123. Cho, J. H. *et al.* Cathepsin D produces antimicrobial peptide parasin I from histone H2A in the skin mucosa of fish. *The FASEB Journal* **16**, 429–431 (2002).

124. Koo, Y. S. *et al.* Structure–activity relations of parasin I, a histone H2A-derived antimicrobial peptide. *Peptides* **29**, 1102–1108 (2008).

125. Park, C. B., Kim, M. S. & Kim, S. C. A Novel Antimicrobial Peptide fromBufo bufo gargarizans. *Biochemical and Biophysical Research Communications* **218**, 408–413 (1996).

126. Yi, G.-S., Park, C. B., Kim, S. C. & Cheong, C. Solution structure of an antimicrobial peptide buforin II. *FEBS Letters* **398**, 87–90 (1996).

127. Kim, H. S., Park, C. B., Kim, M. S. & Kim, S. C. cDNA Cloning and Characterization of Buforin I, an Antimicrobial Peptide: A Cleavage Product of Histone H2A. *Biochemical and Biophysical Research Communications* **229**, 381–387 (1996).

128. Kobayashi, S., Takeshima, K., Park, C. B., Kim, S. C. & Matsuzaki, K. Interactions of the Novel Antimicrobial Peptide Buforin 2 with Lipid Bilayers: Proline as a Translocation Promoting Factor †. *Biochemistry* **39**, 8648–8654 (2000).

129. Kobayashi, S. *et al.* Membrane Translocation Mechanism of the Antimicrobial Peptide Buforin 2 †. *Biochemistry* **43**, 15610–15616 (2004).

130. Lan, Y. *et al.* Structural contributions to the intracellular targeting strategies of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1798**, 1934–1943 (2010).

131. Park, C. B., Yi, K.-S., Matsuzaki, K., Kim, M. S. & Kim, S. C. Structure-activity analysis of buforin II, a histone H2A-derived antimicrobial peptide: The proline hinge is responsible for the cell-penetrating ability of buforin II. *Proceedings of the National Academy of Sciences* **97**, 8245–8250 (2000).

132. Park, C. B., Kim, H. S. & Kim, S. C. Mechanism of Action of the Antimicrobial Peptide Buforin II: Buforin II Kills Microorganisms by Penetrating the Cell Membrane and Inhibiting Cellular Functions. *Biochemical and Biophysical Research Communications* **244**, 253–257 (1998).

133. Uyterhoeven, E. T., Butler, C. H., Ko, D. & Elmore, D. E. Investigating the nucleic acid interactions and antimicrobial mechanism of buforin II. *FEBS Letters* **582**, 1715–1718 (2008).

134. Birkemo, G. A., Lüders, T., Andersen, Ø., Nes, I. F. & Nissen-Meyer, J. Hipposin, a histone-derived antimicrobial peptide in Atlantic halibut (Hippoglossus hippoglossus L.). *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1646**, 207–215 (2003).

135. Bustillo, M. E. *et al.* Modular analysis of hipposin, a histone-derived antimicrobial peptide consisting of membrane translocating and membrane permeabilizing fragments. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1838**, 2228–2233 (2014).

136. Sim, S. *et al.* Investigating the nucleic acid interactions of histone-derived antimicrobial peptides. *FEBS Letters* **591**, 706–717 (2017).

137. Lemaire, S. *et al.* Isolation and characterization of histogranin, a natural peptide with NMDA receptor antagonist activity. *Eur. J. Pharmacol.* **245**, 247–256 (1993).

138. Lemaire, S. *et al.* Antimicrobial effects of H4-(86-100), histogranin and related compounds--possible involvement of DNA gyrase. *FEBS J.* **275**, 5286–5297 (2008).

139. Augusto, L. A. *et al.* Histones: A Novel Class of Lipopolysaccharide-Binding Molecules †. *Biochemistry* **42**, 3929–3938 (2003).

140. Gillrie, M. R. *et al.* Plasmodium falciparum Histones Induce Endothelial Proinflammatory Response and Barrier Dysfunction. *The American Journal of Pathology* **180**, 1028–1039 (2012).

141. Chaaban, H. *et al.* Inter- inhibitor protein and its associated glycosaminoglycans protect against histone-induced injury. *Blood* **125**, 2286–2296 (2015).

142. Kawasaki, H., Koyama, T., Conlon, J. M., Yamakura, F. & Iwamuro, S. Antimicrobial action of histone H2B in Escherichia coli: Evidence for membrane translocation and DNA-binding of a histone H2B fragment after proteolytic cleavage by outer membrane proteinase T. *Biochimie* **90**, 1693–1702 (2008).

143. Tagai, C., Morita, S., Shiraishi, T., Miyaji, K. & Iwamuro, S. Antimicrobial properties of arginine- and lysine-rich histones and involvement of bacterial outer membrane protease T in their differential mode of actions. *Peptides* **32**, 2003–2009 (2011).

144. Miller, B. F., Abrams, R., Dorfman, A. & Klein, M. ANTIBACTERIAL PROPERTIES OF PROTAMINE AND HISTONE. *Science* **96**, 428–430 (1942).

145. Hirsch, J. G. BACTERICIDAL ACTION OF HISTONE. *Journal of Experimental Medicine* **108**, 925–944 (1958).

146. Brinkmann, V. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).

147. Urban, C. F. *et al.* Neutrophil Extracellular Traps Contain Calprotectin, a Cytosolic Protein Complex Involved in Host Defense against Candida albicans. *PLoS Pathogens* **5**, e1000639 (2009).

148. Kahlenberg, J. M., Carmona-Rivera, C., Smith, C. K. & Kaplan, M. J. Neutrophil Extracellular Trap-Associated Protein Activation of the NLRP3 Inflammasome Is Enhanced in Lupus Macrophages. *The Journal of Immunology* **190**, 1217–1226 (2013).

149. Park, I. Y., Park, C. B., Kim, M. S. & Kim, S. C. Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, *Parasilurus asotus*. *FEBS Letters* **437**, 258–262 (1998).

150. Birkemo, G. A., Lüders, T., Andersen, Ø., Nes, I. F. & Nissen-Meyer, J. Hipposin, a histone-derived antimicrobial peptide in Atlantic halibut (Hippoglossus hippoglossus L.). *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1646**, 207–215 (2003).

151. Patat, S. A. *et al.* Antimicrobial activity of histones from hemocytes of the Pacific white shrimp. *European Journal of Biochemistry* **271**, 4825–4833 (2004).

152. Luders, T., Birkemo, G. A., Nissen-Meyer, J., Andersen, O. & Nes, I. F. Proline Conformation-Dependent Antimicrobial Activity of a Proline-Rich Histone H1 N-Terminal Peptide Fragment Isolated from the Skin Mucus of Atlantic Salmon. *Antimicrobial Agents and Chemotherapy* **49**, 2399–2406 (2005).

153. Cho, J. H., Sung, B. H. & Kim, S. C. Buforins: Histone H2A-derived antimicrobial peptides from toad stomach. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1788**, 1564–1569 (2009).

154. Dorrington, T., Villamil, L. & Gómez-chiarri, M. Upregulation in response to infection and antibacterial activity of oyster histone H4. *Fish & Shellfish Immunology* **30**, 94–101 (2011).

155. Morita, S., Tagai, C., Shiraishi, T., Miyaji, K. & Iwamuro, S. Differential mode of antimicrobial actions of arginine-rich and lysine-rich histones against Gram-positive Staphylococcus aureus. *Peptides* **48**, 75–82 (2013).

156. Hancock, R. E. W. & Sahl, H.-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* **24**, 1551–1557 (2006).

157. Morita, S., Tagai, C., Shiraishi, T., Miyaji, K. & Iwamuro, S. Differential mode of antimicrobial actions of arginine-rich and lysine-rich histones against Gram-positive Staphylococcus aureus. *Peptides* **48**, 75–82 (2013).

158. Elsbach, P., Weiss, J. & Levy, O. Integration of antimicrobial host defenses: role of the bactericidal/permeability-increasing protein. *Trends Microbiol.* **2**, 324–328 (1994).

159. Nagaoka, I., Hirota, S., Yomogida, S., Ohwada, A. & Hirata, M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. *Inflamm. Res.* **49**, 73–79 (2000).