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Epithelial antimicrobial defence of the skin and intestine

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Abstract

Surface tissues of the body such as the skin and intestinal tract are in direct contact with the external environment and are thus continuously exposed to large numbers of microorganisms. To cope with the substantial microbial exposure, epithelial surfaces produce a diverse arsenal of antimicrobial proteins that directly kill or inhibit the growth of microorganisms. In this Review, we highlight new advances in our understanding of how epithelial antimicrobial proteins protect against pathogens and contribute to microbiota–host homeostasis at the skin and gut mucosae. Further, we discuss recent insights into the regulatory mechanisms that control antimicrobial protein expression. Finally, we consider how impaired antimicrobial protein expression and function can contribute to disease.

The surfaces of the mammalian intestine, skin, respiratory tract and reproductive tract interface directly with the external environment. As a result, these epithelial tissues continuously encounter bacteria, fungi, viruses and parasites that could act as pathogens. In addition, each of these tissues is associated with indigenous communities of commensal microorganisms that comprise complex microbial ecosystems. The epithelium separating these microorganisms from mammalian internal tissues is generally only one or a few cell layers thick and represents an enormous surface area. In humans, the intestinal epithelium encompasses ~200 m² of surface area¹, with the skin contributing an additional ~2 m² surface². Thus, surface tissues are faced with the enormous challenge of defending a large surface area to maintain homeostasis with abundant communities of commensal microorganisms and to prevent pathogen invasion.

Epithelial antimicrobial proteins (AMPs) have an essential role in allowing epithelial surfaces to cope with these microbial challenges. These natural antibiotics are evolutionarily ancient innate immune effectors that are produced by almost all plants and animals³.

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Competing interests statement

The authors declare no competing financial interests.

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Mammalian AMPs are members of a diverse array of protein families, all of which function to rapidly kill or inactivate microorganisms⁴. The epithelial cells lining the gut, skin and respiratory tract produce a rich arsenal of AMPs, probably reflecting the complexity of the microbial challenges faced by these tissues and the continuous threat of microbial invasion at these sites.

In this Review, we summarize recent advances in our understanding of how AMPs function to protect mammalian body surfaces. We analyse recent insights into the regulatory networks that control AMP expression and function at these sites. Further, we discuss how AMPs function to limit pathogen colonization, shape the composition of indigenous microbial communities, and promote the physical segregation of microbiota and host. Finally, we explore how impaired antimicrobial defences can contribute to disease. Although we focus this Review on the AMPs produced by the mammalian intestine and the keratinized areas of the skin (hereafter referred to as 'skin', but excluding mucosal skin epithelia), we aim to highlight general principles that are applicable to the understanding of AMPs of other surface tissues such as the respiratory and reproductive tracts.

Antimicrobial proteins

The AMPs of the gut and skin encompass representatives of several distinct protein families. These include defensins, cathelicidins, C-type lectins (such as the regenerating islet-derived protein (REG) family), ribonucleases (RNases, such as angiogenin 4 (ANG4)) and S100 proteins (such as calprotectin (also known as S100A8–S100A9) and psoriasin (also known as S100A7)). We do not discuss each of these exhaustively here, as this topic has been well covered in previous reviews^{4,5}. Further, we have summarized the major characteristics of some of the main AMP families in gut and skin in TABLE 1. Other specialized epithelial surfaces, such as the oral and nasal mucosae, eye, lung and reproductive tract, are not discussed, but it is important to recognize that each interface has many characteristic AMPs that are uniquely necessary for that environment. Here, we briefly introduce a few key AMPs (mainly defensins, C-type lectins and cathelicidins) that are particularly relevant to our discussion below and serve to illustrate the general principle that the epithelial interface is the first line of defence of the immune system. As the antimicrobial action of cathelicidins and defensins has been widely confirmed (reviewed in REFS 3,5), and the physiological relevance of these large families of AMPs has been validated in several animal models (reviewed in REFS 3,5), our discussion of cutaneous AMPs will focus on the cathelicidins and β -defensins. Other AMPs, such as psoriasin and dermicidin, have a more limited spectrum of antimicrobial potency under *in vivo* conditions^{6,7}, and so they are not emphasized here.

The mammalian gut epithelium produces a diverse collection of AMPs, reflecting the complex microbial challenges confronted by the intestine. Among the most abundant, diverse and highly expressed AMP families in the gut are the α -defensins. These include α -defensin 5 (DEFA5; also known as HD5) and DEFA6 (also known as HD6) in humans, and cryptidins 1–16 (also known as DEFA1–16) in mice. The α -defensins are small peptides (~2–3 kDa) with a conserved three-dimensional structure that is characterized by an amphipathic arrangement of cationic and hydrophobic residues³, resulting in a positively charged surface that is spatially separated from a neighbouring hydrophobic region. This unique arrangement promotes attraction of α -defensins to the negatively charged cell surface and insertion into the lipid-rich membrane. In general, α -defensins have a broad spectrum of activity against both Gram-positive and Gram-negative bacteria, and in some cases are active against fungi, viruses and protozoa; however, particular defensin species have marked differences in their activity spectrum and expression patterns⁸.

Another key AMP family of the mammalian small intestine is the REG family of C-type lectins. REG3 lectins (such as REG3 γ in mice and hepatointestinal pancreatic/pancreatitis-associated protein (HIP/PAP; also known as REG3 α) in humans) are expressed in the small intestines of mice and humans^{9–11}, and are produced in the large intestine during pathogen infections or inflammatory conditions¹⁰. These ~15 kDa proteins have canonical C-type lectin domains that bind to the glycan chains of peptidoglycan, which is an essential component of the bacterial cell wall^{11,12}. In contrast to defensins, the bactericidal activities of REG3 γ and HIP/PAP are selective for Gram-positive bacteria¹¹. This selective activity is consistent with the accessibility of peptidoglycan on the cell surface of Gram-positive bacteria but not Gram-negative bacteria. The closely related lectin REG3 β is usually co-expressed with REG3 γ in mice. Although REG3 β has been shown to bind peptidoglycan¹², a direct demonstration of antimicrobial activity for REG3 β is still lacking.

The mammalian epidermis also deploys a diverse and potent arsenal of AMPs. Key AMPs of the skin's repertoire are the cathelicidins¹³ and β -defensins¹⁴. The single cathelicidin gene (cathelicidin antimicrobial peptide (*CAMP*) in humans) encodes a precursor protein (CAP18; also known as CAMP)¹⁵. This protein can be alternatively cleaved to generate several active AMPs, including the 37-amino-acid peptide LL37 (REF. 16) and the murine peptide cathelin-related antimicrobial peptide (CRAMP)¹⁷. Cathelicidin precursor protein and mature peptide are abundantly expressed by resident mast cells of normal skin¹⁸ and expressed at lower levels in sweat and by resting keratinocytes¹⁹. Inflamed skin greatly increases the abundance of cathelicidin through increased expression of CAP18 by keratinocytes and increased local deposition by recruited neutrophils^{20,21}. The β -defensins differ from the α -defensins in terms of the connectivity of the intramolecular disulphide bonds that establish the β -sheet structure of these peptides. In contrast to cathelicidin, approximately 90 β -defensin genes have been identified in mice and humans, with differences in activity and expression. Both cathelicidins and β -defensins are antimicrobial against a diverse range of skin pathogens, including Gram-negative and Gram-positive bacteria, fungi, viruses and parasites⁵.

Sites of expression

Epithelial cells produce the majority of AMPs in body surface tissues under steady-state conditions, although infiltrating immune cells can also contribute to AMP production during inflammation. Several distinct epithelial cell lineages comprise the intestinal epithelial surface, each of which expresses a distinct group of AMPs (FIG. 1a). The enterocyte is the most abundant epithelial cell lineage of both the small intestine and large intestine (colon). In the small intestine, enterocytes produce REG3 γ and REG3 β ^{22,23}, whereas colon enterocytes express β -defensins and cathelicidins^{24,25}. Paneth cells are located at the base of crypts of Lieberkühn and are unique to the small intestine. Many AMPs are expressed abundantly by Paneth cells, including α -defensins⁸ and ANG4, an RNase²⁶. Goblet cells constitute a third epithelial cell lineage that is present in both the small and large intestine. A major function of goblet cells is to secrete mucin glyco proteins that assemble to form a thick gel-like mucus layer that overlies the epithelium²⁷ and functions in part to concentrate secreted AMPs at or near the epithelial surface²⁸.

In the skin, the keratinocyte is the most abundant cell type in the epidermis, and several specialized keratinocyte cell types populate skin appendages such as hair follicles (FIG. 1b). Follicular keratinocytes constitutively produce cathelicidin^{20,21,29–31} and β -defensins at a higher level than other keratinocytes, but all keratinocytes produce various AMPs to defend the skin barrier. Augmenting the action of keratinocytes, secretory cells of the skin, such as those of the sweat, apocrine and sebaceous glands, each contribute additional AMPs and antimicrobial lipids to the skin surface¹⁹. Below the surface, and adding to the complexity of the cutaneous barrier, bone marrow-derived resident mast cells in the dermis also have an

important role in cutaneous defence. Mast cells normally occupy positions around blood vessels and store large amounts of cathelicidin in preformed granules. This location places the AMPs derived from mast cells in an ideal position to resist infections after skin injury and inoculation with either bacterial or viral pathogens³².

Mechanisms of action

Many AMPs target essential cell wall or cell membrane structures, which limits the ability of microorganisms to evolve resistance (BOX 1). Several AMPs are enzymes that kill bacteria by carrying out an enzymatic attack on cell wall structures. Lysozyme and phospholipase A2 (PLA2), which are both highly expressed by Paneth cells, function through such a mechanism. Lysozyme hydrolyses the glycosidic linkages between the N-acetylglucosamine and N-acetyl-muramic acid that constitute the carbohydrate backbone of cell wall peptidoglycan³³, whereas secretory PLA2 (sPLA2) kills bacteria by hydrolysing bacterial membrane phospholipids³⁴.

Many AMPs kill bacteria through non-enzymatic disruption of the bacterial membrane. Defensins comprise a major family of membrane-disrupting peptides in vertebrates. The clusters of cationic residues on most defensins interact with the bacterial membrane surface through electrostatic interactions with negatively charged phospholipid groups. This interaction is followed by the formation of defensin pores in the bacterial membrane that disrupt membrane integrity and promote lysis of the targeted microorganism³⁵. Cathelicidins also typically kill microorganisms through membrane disruption. Like the defensins, cathelicidins are usually cationic, α -helical peptides that bind to bacterial membranes through electrostatic interactions, followed by membrane insertion and disruption³⁶. Interestingly, in some mammalian species such as pig and cow, multiple cathelicidin genes are present, and some of these encode AMPs that function through mechanisms that do not involve direct membrane disruption³⁷.

The antibacterial mechanisms of several AMPs remain a mystery. ANG4 is a bactericidal RNase that is secreted by Paneth cells²⁶. Although ANG4 has weak RNase activity²⁶, there is no evidence that this is required for its bactericidal function, and its mechanism of action is currently unknown. The mechanism of action of the REG C-type lectins also remains unclear. It is known that REG3 γ recognizes its Gram-positive bacterial targets by binding to peptidoglycan^{11,12} and that the bactericidal action of REG3 γ is accompanied by disruption of cell wall integrity¹¹. It remains to be established whether REG3 γ functions through membrane disruption, enzymatic attack or another mechanism. In the skin, there are several proteins with reported antimicrobial activity that might not function through membrane disruption. These include RNase7 (REF. 38), calprotectin³⁹, psoriasin⁶ and dermcidin⁷. Calprotectin functions by metal chelation, thus regulating the availability of essential trace elements such as Zn²⁺ and Mn²⁺, and augments other host defence mechanisms for microbial killing⁴⁰. In general, the other proteins have very low direct antimicrobial potency but are abundantly expressed in epithelia, thus arguing for a substantial role in host defence. Taken together, these findings suggest that different AMP families use distinct molecular mechanisms to kill microorganisms. The use of diverse killing strategies is probably important for limiting the evolution of microbial resistance to multiple AMPs.

Immunomodulation by antimicrobial proteins

Increasing evidence indicates that some AMPs can protect tissue surfaces by alternative mechanisms that are unrelated to their capacity to directly kill microorganisms. Thus, the capacity of microorganisms to develop antimicrobial resistance (BOX 1) might be irrelevant to the overall physiological effectiveness of several types of AMP. Cathelicidin and defensin peptides are major examples of this alternative activity. These peptides can function as

potent immune regulators by signalling through chemokine receptors and by inhibiting or enhancing Toll-like receptor (TLR) signalling.

Chemotactic activity

The cathelicidins and α - and β -defensins have each been shown to have chemotactic activity and the capacity to recruit leukocytes by direct or indirect mechanisms. These actions can directly modify the inflammatory response. For example, the human cathelicidin peptide LL37 attracts neutrophils, monocytes and T cells, and in some experimental systems, this chemotactic activity is mediated by the G protein-coupled formyl peptide receptor-like 1 (FPRL1; also known as formyl peptide receptor 2)⁴¹. The mouse cathelicidin CRAMP, despite having a very different primary amino acid sequence to LL37, is also chemotactic for human cells and mouse cells in a manner dependent on FPRL1 or FPRL2 (also known as FPR3)⁴². Human α -defensins (DEFA1 (also known as neutrophil defensin 1) and neutrophil defensin 2) and β -defensins (BD3 (also known as DEFB103) and BD4 (also known as DEFB104)) have been reported to be chemotactic for monocytes and macrophages⁴³, and BD2 (also known as DEFB4A) and LL37 attract mast cells⁴⁴. The chemotactic activities of the different groups of AMPs are distinct from each other. For example, human α -defensins selectively induce the migration of human naive CD4⁺CD45RA⁺ and CD8⁺ T cells, but not CD4⁺CD45RO⁺ memory T cells⁴⁵. By contrast, β -defensins are chemotactic for immature dendritic cells and CD4⁺CD45RO⁺ memory T cells. The chemotactic effect of human defensins is inhibited by antibodies specific for CC-chemokine receptor 6 (CCR6)⁴⁶. An additional mode of action for the chemokine-like function of AMPs is through trans-activation of cell surface growth factor receptors. LL37 has been shown to activate the epidermal growth factor receptor on keratinocytes and induce migration by this mechanism⁴⁷. Studies in *Cramp*^{-/-} mice have confirmed the physiological relevance of the chemotactic effects of cathelicidins despite their low potency *in vitro*⁴⁸.

Modulation of TLR responsiveness

An exciting new aspect of our understanding of AMP function has come from studies of their interactions with TLRs. TLRs are membrane-bound pattern recognition receptors (PRRs) that are activated by conserved microbial molecular patterns, such as lipopolysaccharide (LPS) or flagellin, triggering signalling cascades that activate nuclear factor- κ B (NF- κ B), which in turn drives transcription of pro-inflammatory cytokines. The consequences of AMP interactions with TLRs are complex, with potential for either inhibiting or activating inflammatory events. Cathelicidins have been extensively studied in this regard and seem to directly interact with TLR ligands such as endotoxin or nucleic acids, including self-DNA. This modifies cellular responsiveness to these ligands by altering membrane microdomain function or the internalization of the TLR ligand^{49,50}. For example, the presence of active cathelicidin peptide (LL37) influences T helper 17 (T_H17) cell maturation by interacting with DNA to activate TLR9, which in turn leads to increased production of type I interferons⁵⁰. The consequences of these interactions imply that AMP expression during infection could kill pathogens and neutralize excessive inflammatory responses at the same time. However, inappropriate expression of some AMPs could contribute to dysregulation of inflammation and the development of autoimmune disorders, in part by modifying responsiveness to self-DNA. The pathophysiology of the human diseases rosacea and psoriasis have each been associated with the interaction of AMPs with PRRs and are discussed later in this Review.

Together, these examples underscore the fact that AMP function is complex, extending beyond direct antibacterial activity to encompass general effects on downstream immune responses.

Antimicrobial protein regulation

The expression, secretion and activity of most AMPs are tightly controlled. This is necessitated in part by the toxic effects of many of these proteins on mammalian cell membranes. In addition, the chemokine-like activities and immune-modulating effects of many AMPs probably require tight control of expression in order to avoid triggering unnecessary inflammatory responses. In the following sections, we discuss the multifaceted ways in which AMP expression and activity are regulated at body surfaces.

Transcriptional regulation by bacteria

Germ-free animals are an indispensable experimental tool for determining which host functions are genetically encoded and which require interactions with microorganisms⁵¹. Studies of germ-free mice have revealed that some intestinal AMPs are expressed independently of the microbiota, whereas others require bacterial signals for their expression (FIG. 2a). For example, expression of most intestinal α -defensins requires the WNT pathway transcription factor TCF4 (REF. 52) but is independent of the microbiota⁵³. Similarly, expression of lysozyme, sPLA2 and certain members of the β -defensin family does not require microbial signals^{24,26,54}.

Other intestinal AMPs require microbial cues for their full expression. Members of the cryptdin-related sequence family of peptides are related to the α -defensins and have increased levels of expression in conventionally raised mice compared with germ-free mice⁵³. Similarly, key members of the human β -defensin family, including BD2, are expressed under the control of bacterial signals²⁴. For example, bacterial flagellin has been shown to be highly relevant for the induction of BD2 expression by keratinocytes⁵⁵. Finally, the expression of both ANG4 and REG3 γ is essentially absent in germ-free mice and is upregulated on colonization with a conventional microbiota^{11,26}.

Host PRRs control the expression of some of these bacterially regulated AMPs. For example, stimulation of TLRs is required for REG3 γ mRNA expression by intestinal epithelial cells. Studies in mice lacking myeloid differentiation primary response protein 88 (MYD88), an adaptor molecule that is common to several TLRs, have revealed that REG3 γ and REG3 β are expressed under the control of TLRs *in vivo*^{22,23,56,57}. Further, the MYD88 dependence is intrinsic to epithelial cells, suggesting that epithelial cells, including enterocytes and Paneth cells, directly sense bacteria through TLRs and upregulate expression of REG3 γ and REG3 β in response^{22,23,56}. Expression of these AMPs seems to be triggered by any of several TLRs, as mice deficient in individual TLRs do not have defects in REG3 γ or REG3 β expression²². This is consistent with the fact that both LPS^{22,56} and flagellin⁵⁸ (which bind TLR4 and TLR5, respectively) are sufficient to trigger REG3 γ expression. Microbial cues can also induce AMPs in a non-PRR-dependent manner; for example, production of the short-chain fatty acid butyrate in the colon through microbial fermentation of dietary fibres has been shown to be an important regulator of AMP production⁵⁹.

Intestinal epithelial cell expression of REG3 γ also requires signals from at least one immune cell lineage. Innate lymphoid cells (ILCs) reside in the lamina propria and phenotypically resemble natural killer cells⁶⁰. ILCs produce large quantities of the cytokine interleukin-22 (IL-22), which binds to IL-22 receptors on epithelial cells to modulate epithelial cell function⁶¹. ILCs from germ-free mice produce low levels of IL-22 (REF. 62), suggesting that intestinal bacteria drive IL-22 expression by these cells. Interestingly, ILC-derived IL-22 seems to be required for epithelial cell expression of REG3 γ mRNA⁶². Thus, REG3 γ expression is dependent on both epithelial cell-intrinsic TLR signalling through MYD88 and IL-22 produced by ILCs. It seems possible to reconcile these disparate

observations by proposing that IL-22 functions as an environmental cue that allows epithelial cells to become competent to express REG3 γ . Epithelial cells must then receive an additional direct bacterial signal through TLRs in order to express REG3 γ . Further studies will be required to unravel this complex regulatory network.

The expression of other intestinal AMPs is regulated by nucleotide oligomerization domain 2 (NOD2) (FIG. 2a). NOD2 is an intracellular PRR that is expressed by Paneth cells and macrophages in the small intestine⁶³. NOD2 functions in the intracellular recognition of muramyl dipeptide (MDP) — a peptidoglycan motif that is common to Gram-positive and Gram-negative bacteria — triggering signalling cascades that activate the transcription factor NF- κ B⁶⁴. MDP has been shown to stimulate the bactericidal activity of intestinal crypts harbouring Paneth cells⁶⁵. Also, *Nod2*^{-/-} mice have pronounced alterations in the composition of their small intestinal microbiota⁶⁵, as well as increased susceptibility to oral challenge with the gastrointestinal pathogen *Listeria monocytogenes*⁶⁶. These results indicate that NOD2-stimulated antimicrobial defences are important for shaping the microbiota and protecting the epithelial barrier from pathogenic invasion.

Developmental regulation

All mammals are sterile during prenatal life and acquire indigenous microbial communities immediately after birth. Profound shifts in the composition of intestinal microbial communities occur during early postnatal life, particularly during weaning when young mammals transition from milk to solid food. Over time, the gut ecosystem stabilizes and by adulthood consists of an established climax community dominated by obligate anaerobes⁶⁷. Several intestinal AMPs have developmentally regulated expression during neonatal and early postnatal life, suggesting that they might be crucial for maintaining immune homeostasis during key developmental transitions.

Both ANG4 and REG3 γ are strongly induced in the small intestine during early postnatal life. In conventionally raised mice, the level of ANG4 expression increases approximately 20-fold during weaning (approximately day 17–21 in mice) and remains at adult levels thereafter²⁶. The level of expression of REG3 γ in mice increases by a remarkable 3,000-fold during the same period¹¹. These findings suggest that ANG4 and REG3 γ function in part to maintain mucosal homeostasis during weaning in the face of the associated change in microbial ecology and the withdrawal of the passive immune protection afforded by the mother's milk. By contrast, cathelicidin expression undergoes an inverse switch, as it is abundantly expressed during early neonatal life but begins declining just before weaning⁶⁸. By adulthood, cathelicidin expression is extinguished in the small intestine but remains high in the colon. The expression of cathelicidin in the colon provides resistance to bacterial pathogens in mice⁶⁹, but the physiological relevance of decreased cathelicidin expression with maturation of the small intestine is unclear.

Regulation during epithelial damage

A major function of AMPs is to respond rapidly to epithelial disruption and establish a temporary protective shield against infection. Early observations of inducible AMP expression with injury or infection were made in insects and amphibians^{70,71}, and it was later recognized that a similar process takes place early in mammalian wound repair or infection^{13,21}. The physical barrier of the epidermis and the antimicrobial barrier are intimately linked, with each influencing the function of the other⁷². Human BD3 can be induced by growth factors that are also important for wound repair. For example, after skin wounding, keratinocyte expression of this AMP is increased by activation of the epidermal growth factor receptor⁷³. A particularly surprising observation came with the recognition that the human cathelicidin gene *CAMP* is under transcriptional control of a vitamin D

response element (VDRE)^{74,75}. Following skin injury or infection, 25-OH vitamin D3 is hydroxylated by the enzyme cytochrome p450, 27B1 (CYP27B1) to 1,25-OH vitamin D3, and this is stimulated locally by activation of TLR2 or local cytokines such as tumour necrosis factor (TNF) or type I interferons^{76,77} (FIG. 2b). This local enzymatic event enables rapid induction of *CAMP* expression through binding of 1,25-OH vitamin D3 to the VDRE. These observations suggest that AMP expression could be influenced by dietary vitamin D⁷⁸ or vitamin D generated by exposure of the skin to sunlight⁷⁹. This implies that the nutritional environment is probably a source of important signals that control AMP expression.

Post-translational regulation

A major problem faced by host cells that secrete membrane-toxic AMPs is the potential detrimental effects of these proteins on mammalian cell membranes⁸⁰. As a result, the activities of many AMPs must be tightly regulated during storage in membrane-bound secretory granules. α -defensins are stored in Paneth cell granules as inactive pro-peptides. Mouse α -defensins are processed at their amino termini by matrix metalloproteinase 7 (MMP7) to produce mature bactericidally active peptides⁸¹. In humans, trypsin cleaves α -defensins to their mature forms⁸². REG3 γ also requires N-terminal proteolytic processing to yield a bactericidally active protein. In both mice and humans, this processing is carried out by trypsin following secretion into the gut lumen⁸³. The requirement for an inhibitory N-terminal peptide during secretory granule storage indicates that REG3 γ might have membrane-disrupting activity, providing a potential clue about the mechanism of its bactericidal activity.

Different processing mechanisms generate the active forms of other AMPs. Similarly to defensins and REG3 γ , cathelicidins are synthesized as pro-peptides. However, serine proteases are responsible for the processing of cathelicidins. In neutrophils, the pro-peptide is removed by protease 3 (REF. 84), whereas processing is carried out by kallikreins in keratinocytes⁸⁵. Similarly, β -defensins are expressed as pro-peptides, but the processing mechanism remains to be established⁸⁶.

The distinctive reducing environment of the intestinal lumen might provide another mechanism of post-translational regulation that protects host cells during storage of AMPs. Under oxidizing conditions, such as those present inside host cells, the human AMP BD1 has weak antimicrobial activity. However, under reducing conditions that mimic those encountered in the intestinal lumen, human BD1 undergoes marked structural changes, including reduction of its disulphide bridges and alterations in its secondary structure, that result in the unmasking of a potent antimicrobial activity⁸⁷.

Regulated secretion

The secretion of AMPs into the gut lumen is also controlled by bacterial signals. As noted above, Paneth cells produce most of the AMPs in the small intestine, including α -defensins, ANG4 and lysozyme. Paneth cells secrete their granule contents in response to exposure to live bacteria or to bacterial molecules such as LPS⁸⁸. Thus, AMP release is precisely regulated in response to potential bacterial threats, although the mechanisms of bacterial sensing that control AMP secretion by Paneth cells are not yet clear. In skin, the eccrine, apocrine and sebaceous cells carry out a similar role by releasing AMPs onto the skin surface. The sensors that recognize bacteria and mediate this regulated secretion also remain unknown.

Together, these findings show that a complex network of developmental, microbial and nutritional signals controls AMP expression and secretion. It will be important to sustain

efforts to unravel these complex regulatory networks, which may allow the development of new therapeutic strategies aimed at enhancing endogenous AMP production.

Antimicrobial protein function *in vivo*

It has long been accepted that the diverse AMP arsenals of the intestine and skin evolved to manage the large numbers of microorganisms that are encountered at these body surfaces. Recent findings in genetically engineered mouse models have offered new insights into how epithelial AMPs function *in vivo*, showing that these proteins not only protect against pathogen colonization but also determine microbiota composition and limit access of the microbiota to host tissues (FIG. 3).

Protection against pathogens

One of the first studies to reveal the importance of intestinal AMPs in pathogen protection *in vivo* involved the analysis of mice deficient in MMP7, which is required for the generation of bactericidally active α -defensins in mice⁸¹. *In vivo* studies of *Mmp7*^{-/-} mice showed increased susceptibility to oral challenge with the intestinal pathogen *Salmonella enterica* subsp. *enterica* serovar Typhimurium, revealing a crucial role of MMP7 in regulating immunity to enteric bacteria⁸¹. A more direct demonstration of the *in vivo* function of α -defensins was achieved with the development of a transgenic mouse overexpressing human DEFA5 in Paneth cells. DEFA5-expressing mice had greater resistance to oral challenge with *S. Typhimurium* than wild-type mice, pointing to an essential role for α -defensins in limiting colonization by gastrointestinal pathogens⁸⁹. The development of a cathelicidin-deficient mouse was the first direct demonstration of the importance of AMPs in defending against skin infections, and this has now been confirmed with several diverse organisms, including group A streptococcus, vaccinia virus and *Leishmania* spp.⁹⁰⁻⁹². The importance of cathelicidin for defending against infection has also been established in other organ systems, such as urinary tract infection by *Escherichia coli*⁹³ and *Pseudomonas aeruginosa* keratitis of the eye⁹⁴. Finally, antibody-mediated inactivation of REG3 γ showed that this antimicrobial lectin is important for controlling colonization of the intestinal tract by Gram-positive opportunistic pathogens, such as *Listeria monocytogenes*⁵⁶ and vancomycin-resistant *Enterococcus faecalis*⁹⁵.

Controlling the microbiota

A recent elegant study⁹⁶ showed that α -defensins regulate the composition of the intestinal bacterial community (BOX 2). The study used 16S ribosomal RNA gene sequencing to characterize the intestinal bacterial communities in *Mmp7*^{-/-} and *DEFA5*-transgenic mice. Although no differences in the total numbers of colonizing bacteria were observed, there were substantial α -defensin-dependent changes in the composition of the microbial communities compared with wild-type mice. Further, the defensin-deficient *Mmp7*^{-/-} mice and the defensin-complemented *DEFA5*-transgenic mice had reciprocal differences in community composition. These changes included alterations in the proportions of Firmicutes, the major Gram-positive phylum, and Bacteroidetes, the major Gram-negative phylum, of the mouse intestine. The *DEFA5*-transgenic mice also had a loss of segmented filamentous bacteria (SFB) relative to wild-type mice. Consistent with the fact that SFB increase the frequencies of intestinal T_H17 cells⁹⁷, the *DEFA5*-transgenic mice had lower frequencies of lamina propria T_H17 cells compared with wild-type mice. This indicates that α -defensins have an important role in shaping intestinal microbiota composition and thus controlling the level of immune stimulation.

Limiting bacterial–epithelial cell contact

A key mechanism by which the mammalian intestine maintains homeostasis with its associated bacterial communities is to minimize contact between the bacteria and the intestinal epithelium. This is accomplished in part by enhancing the physical barrier through the production and assembly of a thick mucus layer at the epithelial surface. Visualization of spatial relationships between bacteria and the intestinal surface shows that the inner mucus layer remains relatively free of bacteria, whereas the outer mucus layer retains large numbers of bacteria^{23,27}. Biochemical analysis of AMP localization has shown that most AMP activity is confined to the mucus layer and is essentially absent from the luminal content²⁸. Thus, it seems likely that, in addition to functioning as a physical barrier, the mucus layer limits bacterial penetration by forming a diffusion barrier that concentrates AMPs near the epithelial cell surface.

Recent studies have shown that REG3 γ interacts with the mucus layer to help maintain a relatively bacteria-free zone adjacent to the intestinal epithelial cell surface. Consistent with the fact that the bactericidal activity of REG3 γ specifically targets Gram-positive bacteria, *Reg3g*^{-/-} mice had increased colonization of the small intestinal epithelial surface by Gram-positive bacterial groups, including SFB²³. Interestingly, these differences did not extend to the luminal bacterial communities, which were similar between *Reg3g*^{-/-} mice and their wild-type littermates. This suggests that the antibacterial effects of REG3 γ are confined to the inner mucus layer and do not extend into the lumen of the intestine. The mucosal niche-specific activity of REG3 γ could be owing to restricted diffusion of REG3 γ through the mucus barrier, the result of specific binding interactions between REG3 γ and mucus glycoproteins, or because of a requirement for environmental conditions for REG3 γ activity that are specific to the mucosal surface niche.

Antimicrobial proteins of the microbiota

An important component of the surface antimicrobial shield of the skin is produced by the resident commensal microorganisms themselves. Gram-positive bacteria such as *Lactococcus*, *Streptococcus* and *Streptomyces* spp. produce factors, known as bacteriocins, that inhibit the growth of other bacterial strains and species that could compete for nutrients and other resources. *Staphylococcus epidermidis*, the dominant commensal bacterium found in the skin microflora, produces several AMPs. For example, epidermin, pep5 and epilancin K7 are structurally unique as they contain the thioether amino acids lanthionine and/or methylanthionine, and as such are also known as lantibiotics. *S. epidermidis* also produces peptides known as phenol-soluble modulins- γ (PSM γ) and PSM δ that function as AMPs⁹⁸. These peptides cause membrane leakage in target bacteria, which indicates that they function in a manner similar to that of host-derived AMPs such as defensins and cathelicidins. PSMs are functional *in vivo*, as nanomolar concentrations decreased the survival of group A streptococcus on normal human skin but did not affect the survival of *S. epidermidis* from which the peptide was derived⁹⁸. Another important example of the protective action of *S. epidermidis in vivo* was observed on the surface of the nasal cavity. In this study, nasal colonization by *Staphylococcus aureus* was inhibited in individuals who were colonized with specific strains of *S. epidermidis* that produced a serine protease with the capacity to destroy biofilms formed by *S. aureus*⁹⁹. In addition, a thiolactone-containing peptide produced by *S. epidermidis* blocks the *S. aureus agr* quorum sensing system that controls the production of various virulence factors¹⁰⁰. Thus, the selective activity of AMPs produced by commensal organisms might be an important part of a normal host defence strategy against pathogen colonization, and microbe-derived antimicrobials probably function together with host-derived proteins to establish the composition of the surface microbiota.

Antimicrobial proteins and disease

Given the crucial contributions of AMPs to maintaining homeostasis with microorganisms at body surfaces, it is not surprising that dysregulation of AMP production and function can be associated with disease. Abnormal AMP production is associated with both intestinal inflammatory diseases and common disorders of the human skin.

Intestinal disease

Inflammatory bowel disease (IBD) is a chronic inflammation of the intestine that affects 0.1% of the population in North America and northern Europe. Although the fundamental causes of IBD remain poorly understood, it is clear that dysregulated control of host–microbial interactions is involved in initiating and perpetuating IBD¹⁰¹. This idea is supported by the fact that patients with IBD frequently have increased numbers of epithelial surface-associated bacteria¹⁰², suggesting a breakdown in the immune mechanisms that normally limit direct contact between the microbiota and the intestinal mucosal surface.

In support of this, several IBD risk alleles are associated with altered intestinal epithelial AMP production (FIG. 4a). For example, genetic variants in the promoter region of the WNT pathway transcription factor gene *TCF4*, which controls α -defensin expression⁵², are associated with ileal Crohn's disease¹⁰³. The importance of the WNT pathway in maintaining α -defensin expression is further supported by a recent study that linked early onset ileal Crohn's disease to polymorphisms in low density lipoprotein receptor-related protein 6 (LRP6), an essential component of the WNT signalling pathway¹⁰⁴. Polymorphisms in the *NOD2* gene are also associated with ileal Crohn's disease^{105,106}. In these patients, severe intestinal inflammation is coincident with decreased α -defensin expression¹⁰⁷. Thus, mutations in *TCF4*, *LRP6* and *NOD2* could lead to decreased α -defensin production with consequent inflammation. A fourth Crohn's disease risk allele is autophagy related 16-like 1 (*ATG16L1*), which encodes a key component of the autophagy pathway that functions in part to regulate cellular membrane recycling and homeostasis¹⁰⁸. Mice with a hypomorphic *Atg16l1* allele have impaired exocytosis of Paneth cell secretory granules, resulting in decreased AMP release¹⁰⁸. This suggests that the human *ATG16L1* risk allele might impair the ability of Paneth cells to secrete AMPs, thereby increasing the likelihood of bacterial penetration and intestinal inflammation. Finally, hypomorphic alleles of the gene encoding the transcription factor X-box-binding protein 1 (XBP1) are linked to IBD in humans¹⁰⁹. XBP1 is essential for the development of secretory cells, and thus mice lacking XBP1 have severe Paneth cell and goblet cell dysfunction that is coincident with spontaneous intestinal inflammation¹⁰⁹. Thus, Paneth and goblet cell abnormalities could account for the increased incidence of IBD in individuals harbouring *XBP1* variants.

These examples illustrate how genetic defects leading to decreased AMP production can be associated with intestinal inflammation and disease. However, it is important to note that in mice, defects in AMP production alone generally do not result in intestinal inflammation^{23,110}. This suggests that in humans, IBD may require multiple genetic lesions that target other immune mechanisms in addition to AMPs.

Skin disease

Common human skin disorders such as atopic dermatitis, rosacea and psoriasis have been linked in part to the abnormal production of AMPs. In healthy individuals, cathelicidins and β -defensins are typically expressed at low levels in non-inflamed skin, but their expression is rapidly increased upon injury or inflammation (FIG. 4b). For example, infection with *S. aureus* has been shown to increase AMP production in the skin^{111,112}. However, in patients with atopic dermatitis, the increased expression of AMPs during skin inflammation is

partially suppressed^{113–116}. Inhibition of AMP expression in atopic dermatitis seems to be partially due to excess production of the T_H2 cell cytokines IL-4 and IL-13, which in turn induce the expression of suppressor of cytokine signalling 1 (SOCS1) and SOCS3 through signal transducer and activator of transcription 6 (STAT6)¹¹⁷. This relative defect in AMP induction correlates with the increased susceptibility of patients with atopic dermatitis to infection. By contrast, patients with psoriasis and rosacea are not more prone to infection but rather show inappropriate inflammatory reactions of the skin. In these patients, the skin surface contains excessive amounts of cathelicidin AMPs (whether produced by keratinocytes or infiltrating granulocytes) that may contribute to the excessive inflammation of the skin by mechanisms involving the inflammatory properties of cathelicidin^{48,50}. In each of these disease associations, the defect in AMP expression is not inherent to the AMP genes themselves, but rather seems to be due to abnormalities in the specific systems that regulate both transcription and post-translational processing of AMPs^{117,118}. For example, in rosacea, excess cathelicidin production may be influenced by abnormal TLR2 expression or polymorphisms in the vitamin D receptor that lead to increased transcription^{118,119}. These recent observations implicating AMPs in skin disease pathogenesis have provided new opportunities for disease therapy.

Conclusions and future prospects

Body surface tissues continuously face complex microbial challenges that include maintaining homeostasis with indigenous microorganisms and limiting exposure to pathogens. The intestine and the skin each confront these challenges by producing a complex arsenal of AMPs that directly kill microorganisms and modulate immunity. It is becoming clear that endogenous AMPs are essential not only for protecting these sites from pathogenic microbial invasion but also for shaping the composition and location of indigenous microbial communities. However, many questions remain. For example, the complex regulatory networks that control the expression of several host-derived AMPs still need to be fully defined. In addition, future work should focus on gaining a precise understanding of how individual AMPs affect microbiota composition in the skin and the intestine. Finally, it will be crucial to gain a better understanding of how dysregulation of AMP expression and function contributes to diseases such as atopic dermatitis, psoriasis and IBD.

A major threat to human health is the increasing number of bacteria that have evolved resistance to available small-molecule antimicrobial therapeutics. This is in contrast to the AMPs produced by mammalian surface tissues, which for the most part have retained their antimicrobial efficacy over evolutionary timescales. Thus, it will be important to sustain efforts to understand the mechanistic basis of host-derived AMP function. Such efforts are likely to yield promising strategies for developing new antimicrobial therapeutic agents to combat disease. However, the therapeutic delivery of AMPs may be problematic owing to costs of production, potency and stability. It will thus also be important to continue to unravel the regulatory networks that dictate AMP expression. A better understanding of these regulatory networks should support strategies to augment endogenous production of AMPs during acute infections or during chronic inflammatory disease.

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Glossary

Commensal microorganisms	The microorganisms that are present in normal, healthy individuals. These microorganisms live in the gastrointestinal tract and at other body sites, and generally engage in mutually beneficial relationships with their hosts.
C-type lectins	A large family of receptors that have carbohydrate recognition domains. The designation ‘C-type’ is based on the structure of the carbohydrate recognition domain. Several epithelial antimicrobial proteins, including regenerating islet-derived protein 3 γ (REG3 γ) and hepatointestinal pancreatic/pancreatitis-associated protein (HIP/PAP), are members of the C-type lectin family.
Ribonucleases	(RNases). Enzymes that catalyse the breakdown of RNA. Several antimicrobial proteins (for example, RNase7 and angiogenin 4) have RNase activity, although the significance of this for the antibacterial activity of these proteins is not known.
Cryptdins	Mouse α -defensins are frequently designated as ‘cryptdins’, which stands for ‘crypt α -defensins’.
Peptidoglycan	A polymer of sugars, crosslinked by short peptides, that is a crucial component of the bacterial cell wall.
Enterocytes	Absorptive columnar epithelial cells that are the major epithelial lineage of the intestine.
Paneth cells	A specialized epithelial cell lineage that produces most of the antimicrobial proteins in the small intestine.
Crypts of Lieberkühn	Invaginations of the small intestinal surface that contain both Paneth cells and intestinal stem cells.
Bacterial resistance to antimicrobial proteins	The rapid development of bacterial resistance to commercially produced antibiotics has raised the question of how endogenous antimicrobial proteins (AMPs) have maintained their efficacy over mammalian evolutionary timescales. A partial answer to this question may lie in the fact that both skin and intestine deploy a diverse array of AMPs, making the development of combined resistance relatively infrequent. In addition, the targeting of essential cell wall or cell membrane structures might account for the continued effectiveness of endogenous AMPs, as bacteria cannot alter these structures without compromising fitness. However, several examples of the development of microbial resistance to mammalian AMPs have been described. Some successful human pathogens such as <i>Staphylococcus aureus</i> , <i>Salmonella enterica</i> and <i>Legionella pneumophila</i> modify their normally anionic cell walls with cationic substitutions to repulse cationic AMPs such as defensins ^{120–122} . Furthermore, many other systems have been described for the evasion of epithelial AMPs. These include the capacity of <i>S. aureus</i> and group A streptococcus to inactivate AMPs by proteolysis ^{123,124} , the efflux pumps that protect <i>Neisseria gonorrhoeae</i> from AMPs by active transport ¹²⁵ and the ability of <i>Shigella</i> spp. to evade cathelicidin and human β -defensin 1 (BD1) by inhibiting the enteric synthesis of these

defence molecules¹²⁶. Thus, although the AMPs are formidable inhibitors of microbial growth and survival, microorganisms have effectively used multiple mechanisms to evade their action. The interested reader is directed to two excellent reviews devoted to this subject^{127,128}.

Pattern recognition receptors	(PRRs). Host receptors (such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs)) that can sense pathogen-associated molecular patterns and initiate signalling cascades that lead to an innate immune response. These can be membrane bound (for example, TLRs) or soluble cytoplasmic receptors (for example, RIG-I, MDA5 and NLRs).
Germ-free animals	Animals that are reared in isolators, without exposure to microorganisms.
WNT pathway	A signalling pathway that controls several physiological processes, including embryogenesis and cancer development. It also controls normal biological functions in adult animals and is essential for the expression of α -defensins in the small intestine.
Conventionally raised mice	Mice that have been raised with normal exposure to microorganisms.
Innate lymphoid cells	(ILCs). A diverse family of immune cells that produce cytokines and function to coordinate immunity and inflammation in body surface tissues such as the intestine and the lung. Although their developmental origins are still unclear, they phenotypically resemble natural killer cells.
Climax community	A mature, stable community of organisms that develops through a process of ecological succession and remains in a steady state for an extended period of time.
Anaerobes	Microorganisms that grow in the absence of oxygen.
16S ribosomal RNA gene sequencing	Determination of the sequences of the variable regions of bacterial ribosomal RNA genes, which are conserved within a species but differ between species. It is frequently used as a culture-independent technique for evaluating the composition of bacterial communities.
Segmented filamentous bacteria	(SFB). A group of Gram-positive bacteria that are members of the intestinal microbiota of mice. They are characterized by their ability to adhere to the intestinal surface and are frequently immunostimulatory.
The microbiota of the intestine and skin	The intestine and the skin are each home to large communities of indigenous bacteria. For decades, our understanding of these bacterial communities was based on identifying resident organisms by culture-based methods. However, many of the bacteria that reside at these sites are refractory to culture, making it difficult to assemble a complete catalogue of organisms residing at these tissue sites. In the past few years, improved culture-independent molecular profiling methods, such as 16S ribosomal RNA gene sequence analysis, have led to a revolution in the understanding of indigenous microbial communities. Microbial communities in the

intestine and skin both have a high level of diversity at the species level but low phylum-level diversity. In all vertebrates, intestinal microbial communities are dominated by two phyla: the Firmicutes and the Bacteroidetes^{129,130}. The Firmicutes are Gram-positive bacteria that include numerous species belonging to the Clostridia class, in addition to Enterococcaceae and Lactobacillaceae families and *Lactococcus* species. The Bacteroidetes are Gram-negative bacteria comprised of several *Bacteroides* species. Prominent intestinal *Bacteroides* species include *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and *Bacteroides ovatus*¹²⁹. The remaining intestinal bacteria, comprising less than 10% of the total population, belong predominantly to the Proteobacteria and Actinobacteria phyla¹²⁹. The skin microbiota is also composed predominantly of species from the Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. However, the proportional representation of these phyla differs from the gastrointestinal tract, with Actinobacteria constituting the dominant bacterial phylum on the skin². It is also important to note that there is variability in microbiota composition, even within an individual, depending on which skin site is sampled¹³¹.

Quorum sensing

A system used by bacteria to coordinate gene expression as a function of population density.

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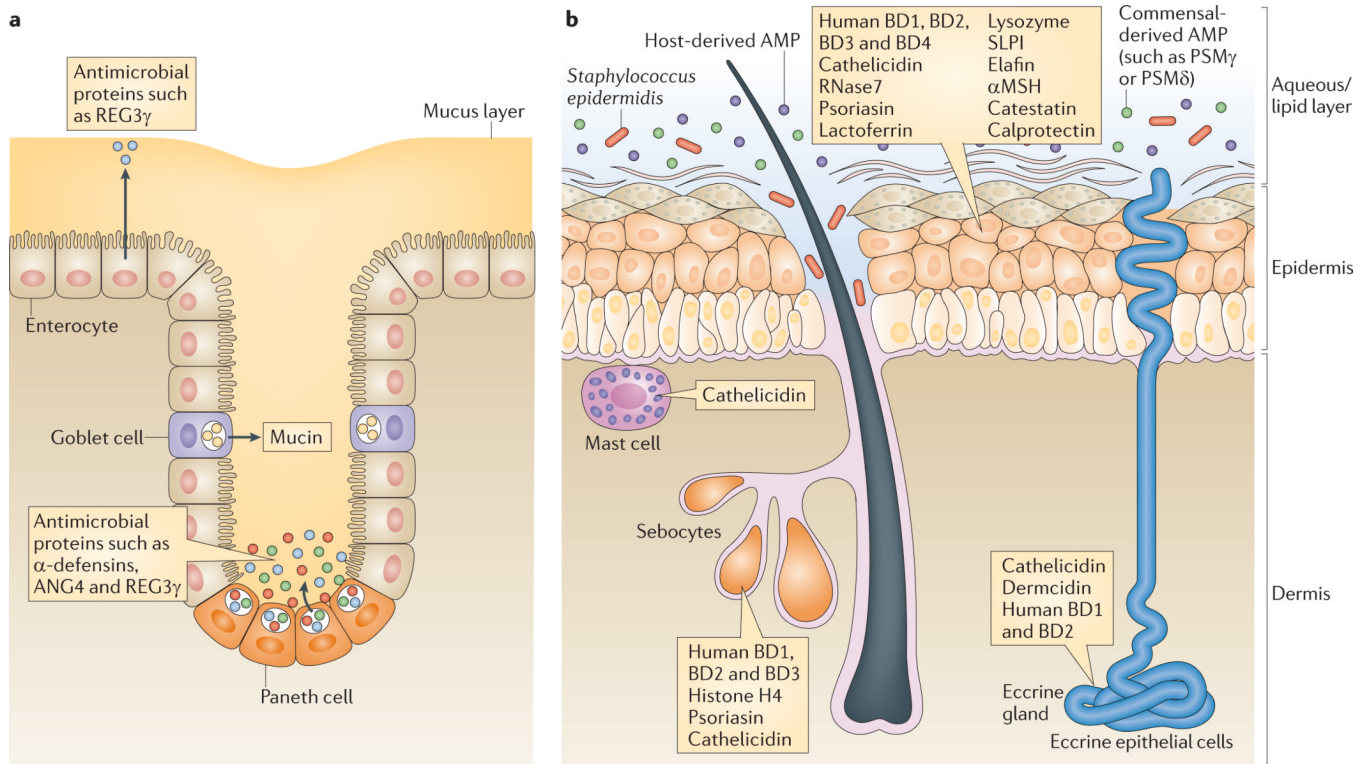


Figure 1. Epithelial barriers of the intestine and skin

a The intestinal epithelium comprises several cell lineages. Enterocytes constitute the most abundant epithelial cell type, and secrete several antimicrobial proteins (AMPs) such as regenerating islet-derived protein 3 γ (REG3 γ). Paneth cells are unique to the small intestine and secrete abundant quantities of AMPs, such as α -defensins. Finally, goblet cells secrete mucin glycoproteins that assemble to form a thick mucus layer overlying the epithelium. The mucus layer seems to have a crucial role in concentrating secreted AMPs near the epithelial surface. **b** The epithelial barrier of the skin includes keratinocytes at the surface and the hair unit, and specialized secretory organs such as sebocytes and eccrine glands. Many diverse AMPs, including cathelicidins and defensins, are produced by these cells under steady-state and/or inflammatory conditions. The aqueous and lipid components of the skin surface combine with AMPs produced by microorganisms to enhance the barrier/protective function. The aqueous/lipid layer may serve a function that is similar to that of intestinal mucus by trapping AMPs at the epithelial surface. Resident bone marrow-derived cells in the dermis, such as mast cells, provide essential additional AMPs after skin injury or in early stages of infection. α MSH, α -melanocyte-stimulating hormone; ANG4, angiogenin 4; BD, β -defensin; PSM, phenol-soluble modulins; RNase7, ribonuclease 7; SLPI, secretory leukocyte protease inhibitor (also known as ALP).

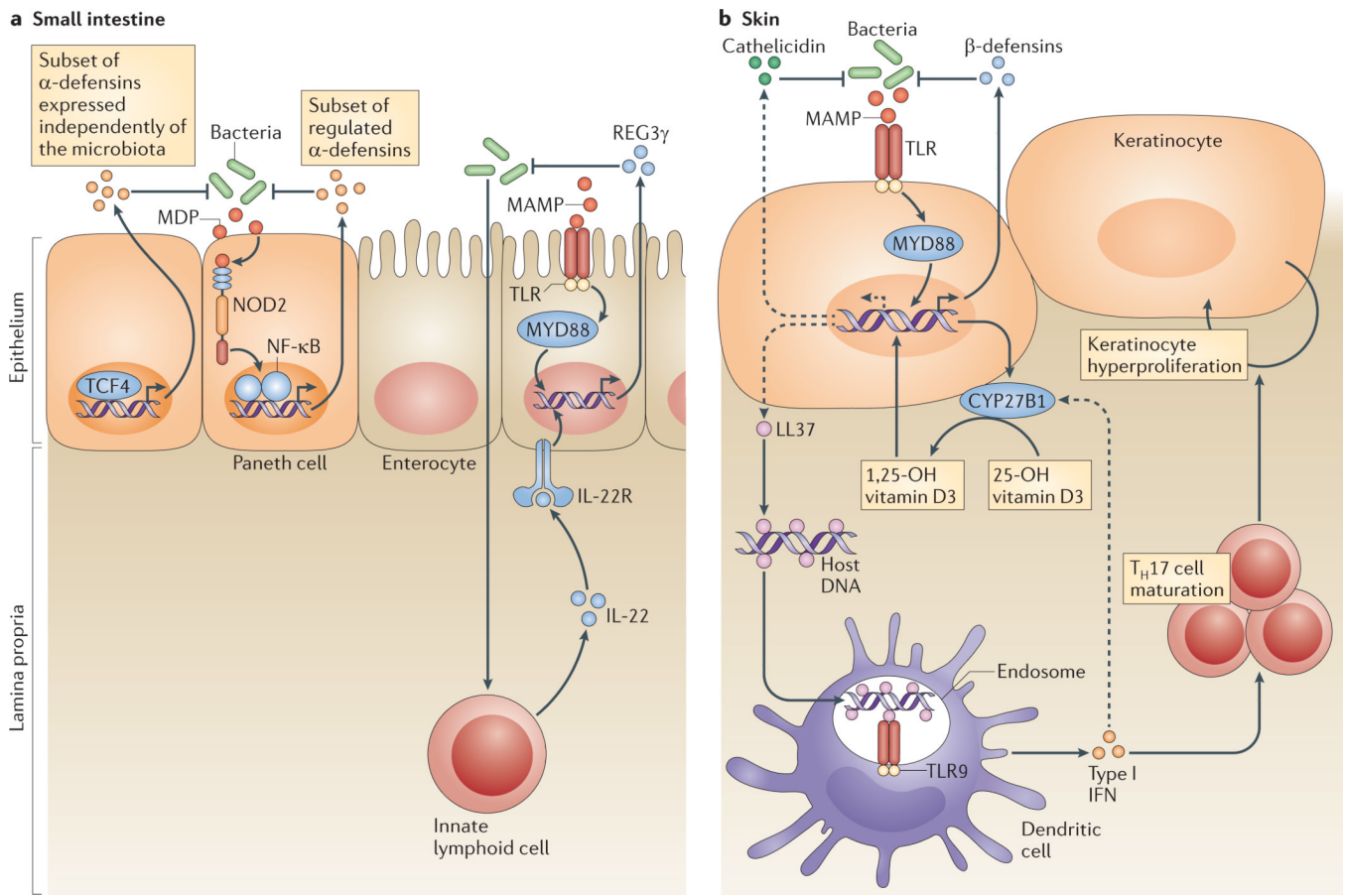


Figure 2. Regulatory mechanisms that shape antimicrobial protein expression and function
a In the small intestine, the transcriptional control of α -defensin expression depends crucially on transcription factor 4 (TCF4)⁵². The pattern recognition receptor nucleotide oligomerization domain 2 (NOD2) also controls the expression and/or secretion of antimicrobial activities in the small intestinal crypt⁶⁵. Regenerating islet-derived protein 3 γ (REG3 γ) mRNA expression in enterocytes and Paneth cells is controlled by microorganism-associated molecular patterns through Toll-like receptors (TLRs) and is dependent on the TLR signalling adaptor myeloid differentiation primary response protein 88 (MYD88)^{22,23}. REG3 γ mRNA expression also requires interleukin-22 (IL-22)-mediated signals from innate lymphoid cells⁶⁰. **b** In the skin, TLR2 activation can directly induce β -defensin mRNA expression¹³² but indirectly influences cathelicidin expression. The activation of MYD88 by TLR2 results in a direct transcriptional increase in cytochrome p450, 27B1 (CYP27B1) and β -defensin expression. The increased expression of CYP27B1 hydroxylates 25-OH vitamin D3 to 1,25-OH vitamin D3, and this then induces cathelicidin mRNA expression^{76,77}. β -defensins and cathelicidin released at the skin surface can function against microorganisms. Below the surface, the presence of cathelicidin peptide in the form of LL37 interacts directly with host DNA and can activate TLR9. TLR9-mediated activation of dendritic cells results in production of type I interferons (IFN α/β) and influences T helper 17 (T_H17) cell maturation and the production of IL-17, which can drive keratinocyte hyperproliferation⁵⁰. IL-22R, IL-22 receptor; MAMP, microbe-associated molecular pattern; MDP, muramyl dipeptide; NF- κ B, nuclear factor- κ B.

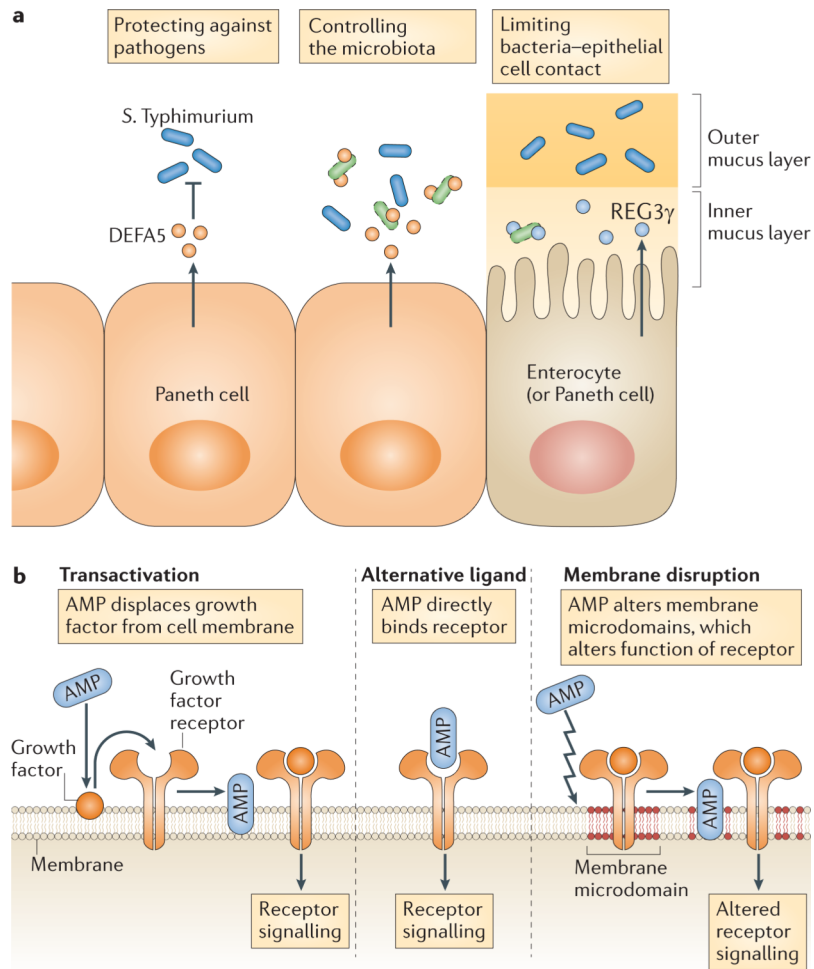


Figure 3. Functions of antimicrobial proteins in the defence of body surfaces

a Forced expression of a human α -defensin 5 (*DEFA5*) transgene in Paneth cells limits colonization by *Salmonella enterica* subsp. *enterica* serovar Typhimurium⁸⁹ and controls the composition of the microbiota in the small intestine⁹⁶. Regenerating islet-derived protein 3 γ (REG3 γ) confines bacteria to the outer mucus layer, thus limiting bacterial contact with the small intestinal epithelial surface²³. **b** Cathelicidins carry out additional immunomodulatory functions in skin defence by acting on cell membrane receptors. Depending on the cell type and condition, evidence for three potential mechanisms of action exists: transactivation by antimicrobial proteins (AMPs) releases membrane-bound growth factors; AMPs bind and directly activate receptors; or AMPs activate or inactivate receptors by disrupting membrane microdomains.

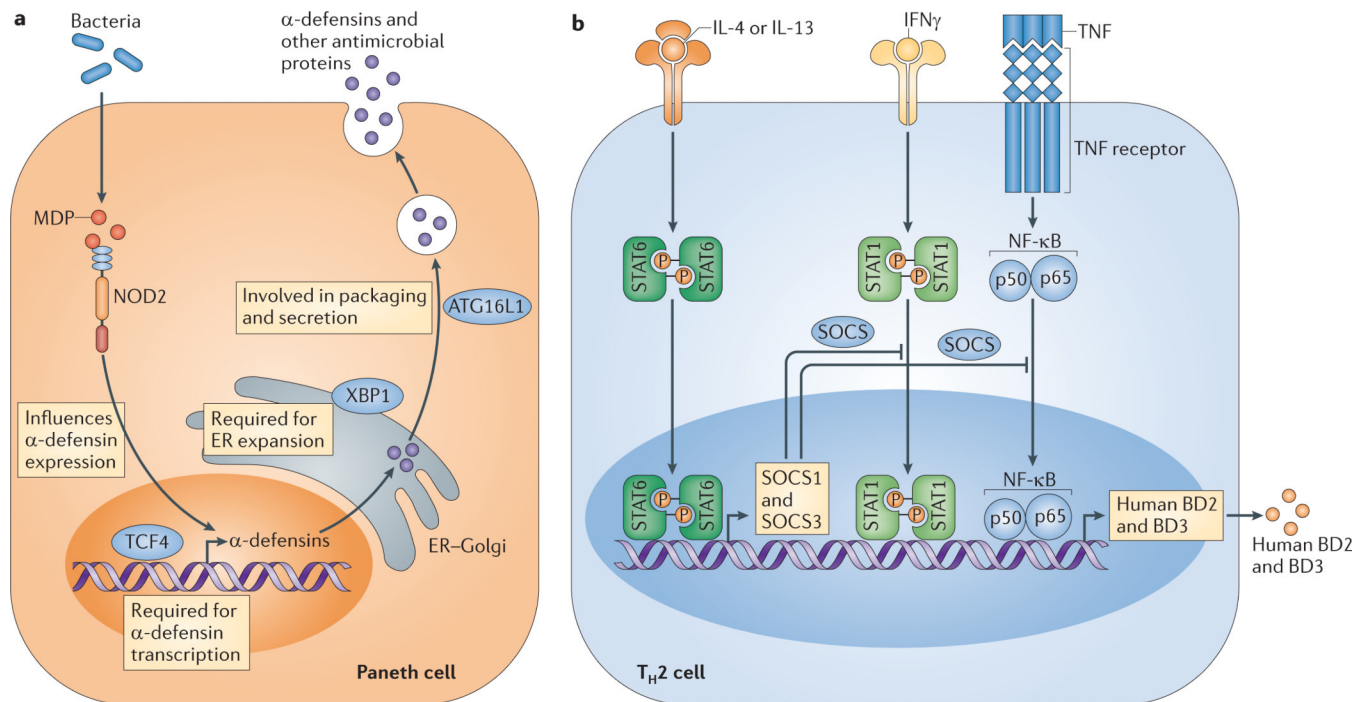


Figure 4. Dysregulation of antimicrobial proteins in disease

a Nucleotide oligomerization domain 2 (NOD2), transcription factor 4 (TCF4), X-box-binding protein 1 (XBP1) and autophagy related 16-like 1 (ATG16L1) promote antimicrobial protein (AMP) expression and secretion by Paneth cells. Polymorphisms in the corresponding genes are associated with an increased incidence of inflammatory bowel disease^{105–109}. This could be due to compromised production of AMPs that normally control the microbiota and limit bacterial contact with the intestinal epithelial surface. **b** In the skin, excess production of T helper 2 (T_H2) cell cytokines is associated with atopic dermatitis, which might be owing to inhibitory effects on the induction of β -defensins. Interleukin-4 (IL-4) and IL-13 induce the expression of suppressor of cytokine signalling 1 (SOCS1) and SOCS3 through signal transducer and activator of transcription 6 (STAT6). This inhibits the action of stimulatory signals for human β -defensin 2 (BD2) and BD3 expression that are transmitted by interferon- γ (IFN γ) through STAT1, or by tumour necrosis factor (TNF) through nuclear factor- κ B (NF- κ B). ER, endoplasmic reticulum; MDP, muramyl dipeptide.

Table 1

Major antimicrobial protein families in skin and intestine

Family	Representative proteins	Mechanism of action	Tissue sites of expression	Cellular sites of expression	Target organisms	Refs
α -defensins (cryptidins in mice)	DEFA5, DEFA6 (in humans); cryptidins 1–16 (in mice)	Membrane disruption	Small intestine	Paneth cells, neutrophils, macrophages	Gram-positive bacteria, Gram-negative bacteria, fungi, viruses, protozoa	8
β -defensins	BD1, BD2, BD3	Membrane disruption; lipid II binding (BD3)	Large intestine, skin, respiratory tract	Enterocytes, keratinocytes, respiratory tract epithelial cells	Gram-positive bacteria, Gram-negative bacteria, fungi, viruses, protozoa	86,133
Calprotectin (S100A8–S100A9)	NA	Metal chelation	Abscesses	Neutrophils	<i>Staphylococcus aureus</i>	39
Cathelicidins	LL37 (in humans); CRAMP (in mice)	Membrane disruption	Large intestine, skin, lung, urinary tract	Neutrophils, mast cells, epithelial cells	Gram-positive bacteria, Gram-negative bacteria, viruses, fungi	36
C-type lectins	HIP/PAP (in humans); REG3 γ (in mice)	Peptidoglycan recognition; killing mechanism unknown	Small intestine	Epithelial cells (Paneth cells, enterocytes)	Gram-positive bacteria	11,12,23
Galectins	GAL4, GAL8	Unknown	Intestine	Broad expression, including by epithelial cells	Bacteria bearing blood group antigens	134
Lipocalin	Lipocalin 2	Sequestration of iron-laden siderophores	Broad expression, including intestine and lung	Macrophages, epithelial cells	<i>Escherichia coli</i>	135,136
Lysozyme	NA	Enzymatic attack on bacterial cell wall peptidoglycan	Intestine, eye, and more; secretions, including tears, saliva	Intestinal Paneth cells	Gram-positive bacteria; some activity against Gram-negative bacteria	137
Peptidoglycan recognition proteins	PGLYRP1–4 in mammals	Activation of bacterial two-component systems; PGLYRP2 is an amidase that targets peptidoglycan	Liver, intestine, skin, mammary gland	Epithelial cells	Gram-positive and Gram-negative bacteria	138
Phospholipase A2	NA	Hydrolysis of bacterial membrane phospholipids	Intestine; secretions, including tears, inflammatory fluids	Paneth cells, macrophages	Gram-positive bacteria	139
Psoriasin (S100A7)	NA	Unknown	Skin	Keratinocytes	<i>Escherichia coli</i>	12
RNases	ANG4, RNase7	Unknown	Skin, intestine	Epithelial cells	Gram-positive and Gram-negative bacteria	26,38

ANG4, angroginin 4; BD, β -defensin; CRAMP, cathelin-related antimicrobial peptide; DEFA, α -defensin; GAL, galectin; HIP/PAP, hepatointestinal pancreatic/pancreatitis-associated protein; NA, not applicable; PGLYRP, peptidoglycan recognition protein; REG3 γ , regenerating islet-derived protein 3 γ ; RNase, ribonuclease.