Bacterial origins of human cell-autonomous innate immune mechanisms

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Abstract | The cell-autonomous innate immune system enables animal cells to resist viral infection. This system comprises an array of sensors that, after detecting viral molecules, activate the expression of antiviral proteins and the interferon response. The repertoire of immune sensors and antiviral proteins has long been considered to be derived from extensive evolutionary innovation in vertebrates, but new data challenge this dogma. Recent studies show that central components of the cell-autonomous innate immune system have ancient evolutionary roots in prokaryotic genes that protect bacteria from phages. These include the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, Toll/IL-1 receptor (TIR) domain-containing pathogen receptors, the viperin family of antiviral proteins, SAMHD1-like nucleotide-depletion enzymes, gasdermin proteins and key components of the RNA interference pathway. This Perspective details current knowledge of the elements of antiviral immunity that are conserved from bacteria to humans, and presents possible evolutionary scenarios to explain the observed conservation.

The human immune system uses a large repertoire of molecular mechanisms to protect against intracellular pathogens. Although specialized immune cells have crucial roles in carrying out a successful defence, the initial recognition and mitigation of infection often occur within non-immune cells. Most mammalian cell lineages mount cell-autonomous innate immune mechanisms that are independent from the operation of professional immune cells[1]. These cell-autonomous mechanisms enable cells to recognize that they are infected and then activate the expression of a wide variety of antibacterial and antiviral factors to curb the infection[1,2] (BOX 1).

Whereas many immune mechanisms have long been considered an evolutionary innovation of metazoans[3], recent evidence suggests that key components of the cell-autonomous innate immune system have evolved from bacterial genes[4–9]. Similarly to animals and plants, bacterial cells also use cell-autonomous immune mechanisms that allow them to survive phage infections[10,11]. The most widely studied bacterial immune mechanisms are CRISPR–Cas and restriction–modification systems, but many additional defence systems have been described in bacteria (BOX 2). Multiple studies from the past few years have provided evidence that cell-autonomous innate immune mechanisms in eukaryotes, including the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, the RNA interference (RNAi) pathway and antiviral effectors such as viperin, have all evolved from bacterial anti-phage defence genes[4,5,8,9]. In this Perspective, we review current evidence of similarities between the eukaryotic and prokaryotic immune systems. We propose an evolutionary scenario to explain the observed homologies, and discuss the implications for further discoveries of new defence principles in both humans and bacteria.

Linking bacterial and human immunity

In recent years there has been a large number of reports showing that immune mechanisms that were originally identified in animals have parallels in the bacterial immune system. These discoveries were facilitated by mining regions in bacterial genomes known as 'defence islands', in which many previously unknown defence systems were shown to reside (BOX 2). Here, we describe the parallels between bacterial and eukaryotic immunity, and discuss evidence for the evolutionary origin of eukaryotic immune mechanisms in bacteria.

The cGAS–STING pathway in animals and bacteria. The cGAS–STING pathway has a central antiviral role in the cell-autonomous innate immune system[12]. cGAS is a sensor for cytosolic double-stranded DNA (dsDNA), the presence of which is perceived as a sign of viral infection in eukaryotes[13,14]. Once activated by dsDNA, cGAS produces the unique signalling molecule 2′,3′-cyclic GMP–AMP (cGAMP)[12,15–18], which binds and activates the protein STING. STING, in turn, recruits additional factors and drives a signal transduction cascade that results in the transcription of interferon and additional antiviral factors[19,20] (FIG. 1a). Multiple DNA viruses, including herpes simplex virus, adenoviruses and papillomaviruses, activate the cGAS–STING pathway during infection of mammalian cells[21], and defects in this pathway are associated with increased susceptibility to viral infection[22] as well as autoimmune disorders resulting from aberrant over-activation of the pathway[23]. The cGAS–STING pathway is highly conserved among animals ranging from invertebrates such as cnidaria, molluscs and insects to humans[24–27].

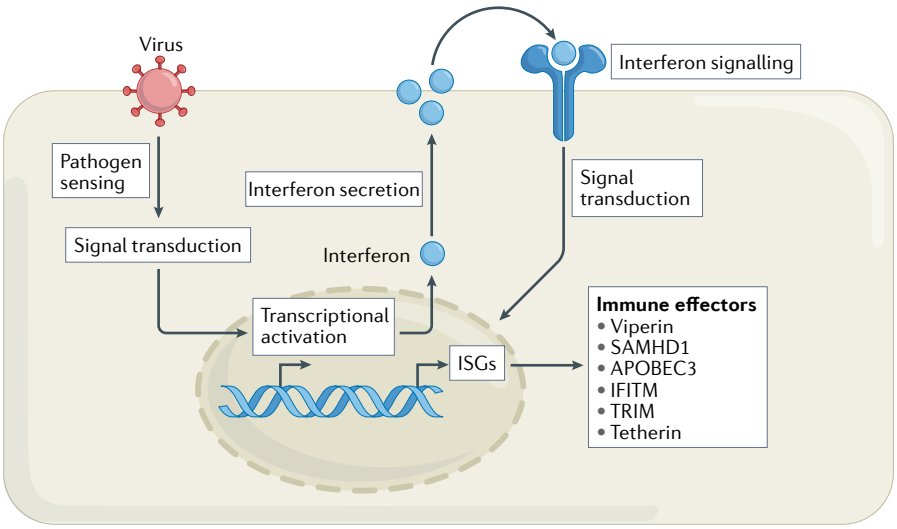
Bacteria have also been found to encode cGAS-like proteins capable of generating 3′,3′-cyclic GMP–AMP[28,29]. The comparison of crystal structures of the human and bacterial cGAS proteins shows clear conservation of the protein architecture, pointing to a common ancestor for the animal and bacterial cGAS proteins[30–32]. It was recently shown that bacterial cGAS has a central role in defence against phage[4]. Once the bacterial cGAS senses phage infection, it produces cGAMP that binds and activates an effector protein[33,34]. The effector protein then directly kills the infected cell before the phage is able to replicate, thus preventing the release of mature phages and protecting the bacterial population from phage propagation[4,35–37] (BOX 3).

Box 1 | **Cell-autonomous innate immune mechanisms**

Many cell types in the human body are capable of sensing that they are infected. The intracellular components that allow cells to sense and respond to infection are collectively known as the cell-autonomous innate immune system[1].

Sensing of viral infection within individual cells is achieved through an array of dedicated pattern recognition receptors, most notably those that sense viral nucleic acids such as cyclic GMP–AMP synthase (cGAS), AIM2, RIG-I, MDA5, OAS1 and some Toll-like receptors (TLRs)[110,136–139]. These sensors usually trigger signalling cascades that eventually result in the activation of interferon production[2] (see the figure). In addition to the secretion of interferons, this response to viruses also involves the expression of hundreds of genes (known as interferon-stimulated genes (ISGs)) that have diverse antiviral activities within the cell[2]. Antiviral genes encode enzymes such as APOBEC3 that edit and destroy viral nucleic acids[140], SAMHD1 (SAM domain and HD domain-containing protein 1) and other enzymes that deplete the cell of nutrients essential for virus replication[57], viperin proteins that produce antiviral chain terminator molecules[46], and proteins that interfere with viral entry, uncoating, assembly and budding (such as IFITM, TRIM proteins and tetherin)[2,141–143].

Membrane-bound and intracellular immune receptors can also recognize bacterial infection and activate antibacterial pathways within the cell[144]. These pathways include autophagy, by which bacterial cells are compartmentalized and destroyed by specific organelles[145], use of reactive oxygen species, which harm bacterial cells and also have a role in immune signalling[145,146], and the activation of inflammasomes that eventually lead to cell death via pyroptosis[144,147].



The phage-encoded trigger that activates the bacterial cGAS is currently unknown.

Bacterial cGAS proteins were recently found to belong to a large family of phage-defensive oligonucleotide cyclases (the cGAS/DncV-like nucleotidyltransferases (CD-NTases)) that can generate diverse cyclic oligonucleotides, including cyclic UMP–AMP, cyclic UMP–UMP, cyclic AMP–AMP–GMP and many more[31,38]. These oligonucleotide cyclases all share the structural architecture of cGAS but have different product specificities[38]. Bacterial defence systems that operate via cyclic oligonucleotide signalling were named CBASS (cyclic oligonucleotide-based anti-phage signalling systems)[4]. Such systems are found in about 13% of prokaryotic genomes, and are present in all major bacterial phyla as well as in archaea[31,34,39].

CBASS effector proteins are highly diverse. As opposed to the role of STING in eukaryotes, bacterial CBASS effectors do not activate a signal transduction cascade but, rather, directly exert cell death by degrading or perforating the bacterial inner membrane[33,40], non-specifically degrading host and phage DNA[36,37], depleting the essential molecule nicotinamide adenine dinucleotide (NAD^+)[26] and other mechanisms[34]. These effectors all have a specialized domain that, similarly to STING, binds the specific cyclic oligonucleotide produced by their cognate cyclase. Several classes of cyclic oligonucleotide-sensing domain have been characterized[35,37,41] and, in some CBASS effectors, the signal-sensing domain of the protein has clear structural homology to the animal STING protein[26]. Comparisons of the crystal structures of bacterial STING-like effectors and the STING proteins of human, oyster and anemone support an evolutionary scenario in which a primitive STING-like protein was acquired by eukaryotes early in evolution, and then underwent metazoan-specific modifications that enabled a switch from direct effector function (cell death) to the regulation of antiviral transcription[26].

Viperin originated in prokaryotes. Viperin is an interferon-induced protein that is conserved among animals and has broad antiviral activity against a wide range of viruses[42–45]. Although viperin was recognized for its antiviral activity several decades ago, its mechanism of action was not described until 2018, when it was shown that viperin catalyses the conversion of cytidine triphosphate (CTP) to 3′-deoxy-3′, 4′-didehydro-CTP (ddhCTP) in the cytosol[46]. The ddhCTP nucleotide lacks the 3′-hydroxyl group on the ribose moiety, such that incorporation of ddhCTP into the nascent viral RNA chain results in chain termination and abortion of viral RNA synthesis, thus inhibiting viral replication[46,47] (FIG. 1b). Viperin is also found in fungi[48].

A recent study discovered that viperins are not limited to eukaryotes but are also conserved in bacteria and archaea[5]. Prokaryotic viperin (pVip) proteins show clear conservation of sequence and function with human viperin, and their encoding genes are located in defence islands that are known to be populated with anti-phage defence genes[5,11,49] (BOX 2). Indeed, expression of pVip proteins in bacterial cells was shown to protect bacteria from phage infection[5]. Similarly to animal viperins, some pVip proteins produce ddhCTP, whereas others produce ddh-guanosine triphosphate (ddhGTP) or ddh-uridine triphosphate (ddhUTP), which also function as antiviral chain terminators similarly to ddhCTP[5]. Expression of pVip proteins is not toxic to bacterial cells, suggesting that as opposed to the phage polymerase, the bacterial polymerase can discriminate between normal and modified nucleotides[5], as was also suggested for viperin activity in animal cells[46]. Phylogenetic analysis of the viperin family showed that all eukaryotic viperins form a monophyletic clade within the pVip phylogenetic tree, with the closest common ancestor localizing in a clade that mostly comprises pVip proteins from archaeal species. The position of the eukaryotic viperin clade within the pVip phylogenetic tree[5], together with the structural conservation between eukaryotic viperins and pVip proteins[48,50], suggests that a single event in the ancient history of the eukaryotic lineage led to the acquisition of a eukaryotic viperin from prokaryotes, most probably from archaea[5].

Virus restriction through nucleotide depletion. SAMHD1 (SAM domain and HD domain-containing protein 1) is another interferon-induced antiviral protein that can block viral infections, specifically in non-dividing cells[51–54]. SAMHD1 was originally identified in cDNA libraries of human dendritic cells as an orthologue of the mouse interferon-γ-induced protein MG11 (REF.[55]), and was later discovered to be a restriction factor for HIV-1 in dendritic cells and myeloid cells[53,54]. SAMHD1 restricts HIV-1 and other retroviruses by depleting the deoxynucleoside triphosphate (dNTP) pool[56,57]. Its dNTPase activity removes the triphosphate from dNTPs to generate phosphate-free deoxynucleosides and inorganic triphosphate molecules[57]. This degradation of dNTPs renders the cell devoid of the building blocks needed for viral DNA replication[56] (FIG. 1c). Interestingly, some strains of HIV overcome SAMHD1-mediated antiviral defence by encoding a protein that marks SAMHD1 for degradation[53,54].

Bacteria also encode enzymes with dNTPase activities[58,59]. A recent study suggests that some of these enzymes, specifically those with dGTPase activity, protect bacteria against phage infection. These enzymes are activated during infection, possibly in response to phage-mediated inhibition of host transcription, and degrade dGTP into phosphate-free deoxyguanosine and inorganic triphosphate, thereby reducing the levels of dGTP in the cell and preventing phage genome replication[9] (FIG. 1c). Bacterial dGTPases show no sequence conservation with SAMHD1, but a comparison of the protein structures showed that they have a similar active site architecture[60,61]. Although it is unclear whether SAMHD1 and bacterial dGTPases have a common evolutionary ancestry, depletion of the nucleotide pool is an antiviral strategy that is shared in humans and bacteria.

In addition to antiviral dGTPase, recent studies suggest that some bacteria encode other enzymes that deplete the nucleotide pool to prevent phage replication. Specifically, it was shown that during phage infection, bacterial dCTP deaminases convert dCTPs into deoxyuracil molecules that are inaccessible for phage DNA replication[9,62]. Similarly to bacterial dGTPases, bacterial dCTP deaminases are also activated in response to inhibition of host transcription by phage, and were shown to completely eliminate dCTP from infected cells[9,62] (FIG. 1c).

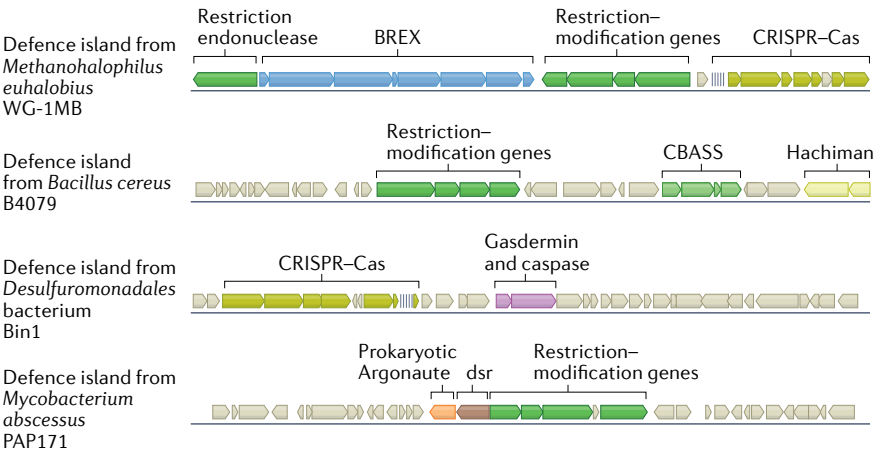
Gasdermins in animals, fungi and bacteria. Gasdermins have crucial roles in inflammasome-mediated immune responses[63–66]. The inflammasome is an intracellular signalling complex of the innate immune system that, when activated, promotes pyroptosis, a form of inflammatory cell death that involves plasma membrane perforation and cytokine release[67]. Upon sensing signs of pathogen infection, the inflammasome cleaves pro-caspases to become active caspases, and these then process gasdermin, as well as the precursors of IL-1β and IL-18, into their mature and active forms[63,65,68]. Gasdermin is normally inactive in the cell owing to an inhibitory carboxy-terminal domain that physically sequesters its lipophilic amino-terminal domain[68,69]. Inflammasome-activated caspases cleave off the inhibitory C-terminal domain of gasdermin, which releases the active N-terminal domain to oligomerize and form large pores in the membrane[68,70]. Gasdermin-mediated pore formation promotes cell death, during which active IL-1β and IL-18 are released from the cell through the gasdermin pores[64,66,71]. Gasdermins have been mostly studied in mammals, but have also been identified in primitive eukaryotes such as corals[72] and fungi[73].

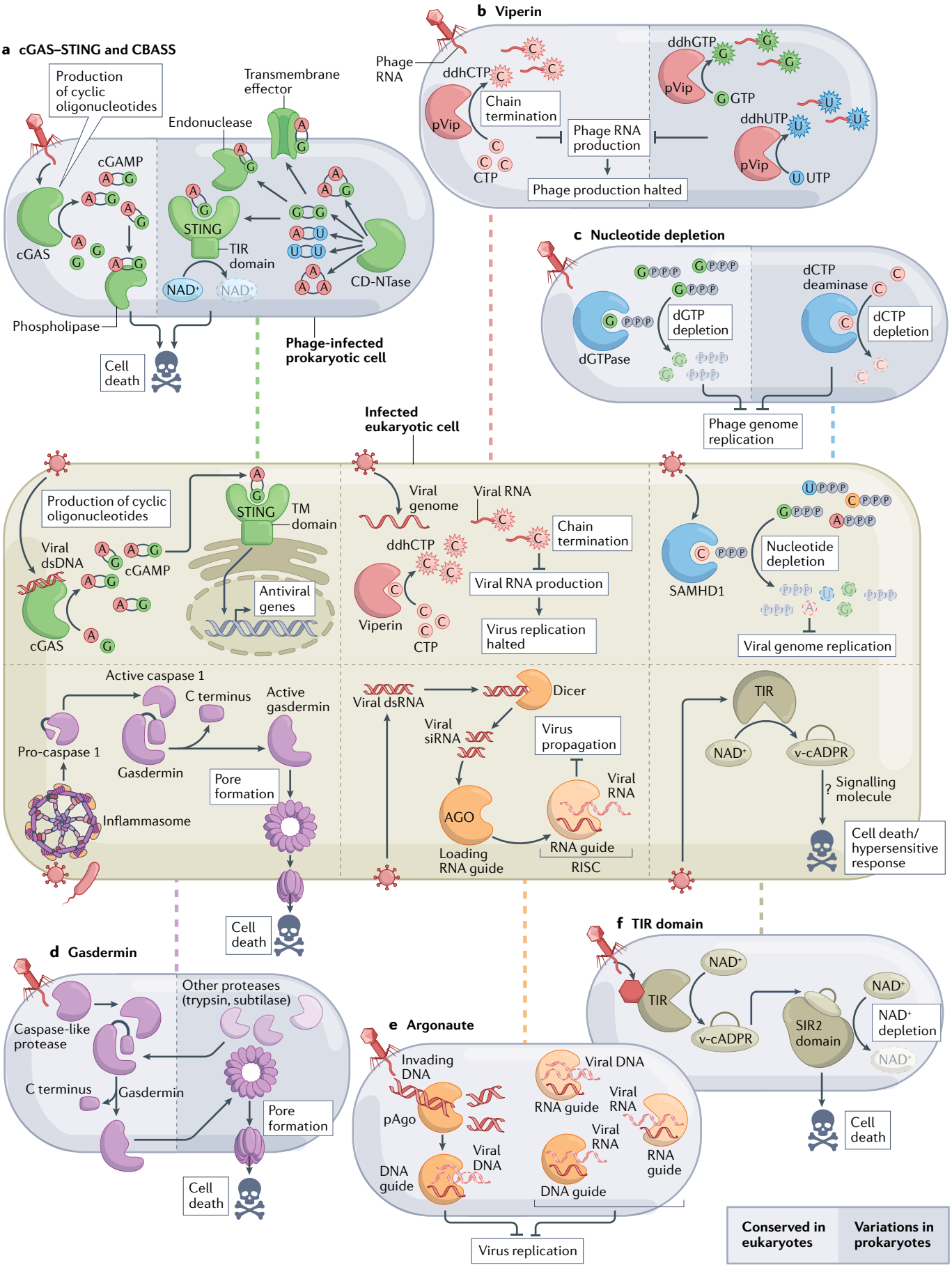
A recent study identified gasdermins in bacteria and archaea and showed that these have high levels of structural homology to the mammalian proteins[6]. Notably, similarly to eukaryotic gasdermins, prokaryotic gasdermins are mostly processed by bacterial caspase-like proteases that remove a C-terminal inhibitory peptide from gasdermin and activate it to oligomerize and form large membrane pores (FIG. 1d). An operon derived from the bacterium *Lysobacter enzymogenes*, which encodes gasdermin, two associated proteases and an ATPase, was shown to defend against multiple phages when heterologously expressed in *Escherichia coli*[6]. Phage defence depended on an intact gasdermin protein as well as one of the proteases, and involved premature cell death of the infected bacterium[6]. Therefore, bacterial gasdermins are not only structurally similar

Box 2 | **The immune system of bacteria**

The most abundant viruses on Earth are those that infect bacteria, known as phages[148,149]. To survive frequent infections by phages, bacteria have evolved an elaborate set of defence mechanisms[10,150]. Historically, studies of bacterial defence mechanisms have mainly focused on the restriction–modification systems present in about three quarters of bacterial genomes[151], which cleave phage DNA while modifying the bacterial DNA to prevent self-cleavage[152]. About 15 years ago, it was realized that about half of all bacterial genomes also encode a sophisticated adaptive immune mechanism known as CRISPR–Cas[153], which retains a memory of past infections through the acquisition of phage DNA 'snippets' and uses this memory to mitigate further infections[154].

There has recently been a renaissance in the study of the bacterial immune system, and more than 50 previously unknown bacterial defence systems have been discovered in the past few years[6,9,11,118,155–158]. These discoveries were facilitated by the realization that bacterial defence systems cluster non-randomly in bacterial genomes and form 'defence islands'[11,49] (see the figure). These genomic islands were found to contain numerous uncharacterized genes encoding new defence systems. Many of the bacterial defence systems that show homology with human immune mechanisms were discovered through analysis of these defence islands[4,7,9,118]. Notably, the mechanisms of action of the vast majority of recently discovered bacterial defence systems — for example, Zorya, Gabija, Hachiman, Wadjet, Septu[11] and BREX[159] — are still unknown. CBASS, cyclic oligonucleotide-based anti-phage signalling systems; dsr, defence-associated sirtuins.




a cGAS–STING and CBASS
Production of cyclic oligonucleotides
Transmembrane effector
Endonuclease
cGAMP
cGAS
STING
TIR domain
NAD+
CD-NTase
Phospholipase
Cell death
Phage-infected prokaryotic cell
b Viperin
Phage RNA
ddhCTP
pVip
Chain termination
Phage RNA production
CTP
Phage production halted
ddhGTP
GTP
ddhUTP
UTP
c Nucleotide depletion
dGTPase
dGTP depletion
dCTP deaminase
dCTP depletion
Phage genome replication
Infected eukaryotic cell
Production of cyclic oligonucleotides
Viral dsDNA
cGAMP
cGAS
STING
TM domain
Antiviral genes
Viral genome
Viral RNA
ddhCTP
Chain termination
Viral RNA production
Viperin
CTP
Virus replication halted
SAMHD1
Nucleotide depletion
Viral genome replication
Active caspase 1
C terminus
Active gasdermin
Pro-caspase 1
Gasdermin
Pore formation
Inflammasome
Cell death
Viral dsRNA
Dicer
Viral siRNA
Virus propagation
AGO
Loading RNA guide
Viral RNA
RNA guide
RISC
TIR
NAD+
v-cADPR
? Signalling molecule
Cell death/ hypersensitive response
d Gasdermin
Other proteases (trypsin, subtilase)
Caspase-like protease
C terminus
Gasdermin
Pore formation
Cell death
e Argonaute
Invading DNA
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DNA guide
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Virus replication
f TIR domain
NAD+
TIR
v-cADPR
SIR2 domain
NAD+ depletion
Cell death
Conserved in eukaryotes
Variations in prokaryotes

◀ Fig. 1 | **Innate immune mechanisms shared between eukaryotes and prokaryotes.** Central eukaryotic cell and six prokaryotic cells (panels **a**–**f**) show antiviral innate immune mechanisms present in both prokaryotes and eukaryotes. Prokaryotic cells also show variations that represent the diversity of anti-phage mechanisms observed in prokaryotes. **a** | Upon sensing of virus infection in both prokaryotes and eukaryotes, the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway produces 2′,3′-cyclic GMP–AMP (cGAMP) that binds to STING (stimulator of interferon genes). In eukaryotes, this elicits a signalling cascade resulting in expression of antiviral genes and production of interferons. In prokaryotes, cGAMP binds to an effector that causes premature cell death, thus preventing virus propagation. Bacterial and archaeal homologues of cGAS, known as cGAS/DncV-like nucleotidyltransferases (CD-NTases), can produce alternative cyclic oligonucleotides that bind to a diversity of effector proteins. CBASS (cyclic oligonucleotide-based anti-phage signalling systems) directly exert cell death by degrading or perforating the bacterial inner membrane, non-specifically degrading host and phage DNA, depleting the essential molecule nicotinamide adenine dinucleotide (NAD^+) and other mechanisms. **b** | In eukaryotes, viperin modifies the nucleotide cytidine triphosphate (CTP) to 3′-deoxy-3′,4′-didehydro-CTP (ddhCTP), which functions as a chain terminator and inhibits viral genome replication. Some prokaryotic viperin (pVip) proteins also modify CTP to ddhCTP, whereas others modify GTP or UTP into ddhNTP forms, which are also chain terminators. **c** | Nucleotide depletion starves viruses of deoxynucleoside triphosphates (dNTPs) that are essential for replication. In animal cells, dNTPs are depleted by the protein SAMHD1 (SAM domain and HD domain-containing protein 1). In prokaryotes, dGTPase degrades dGTP and dCTP deaminase eliminates dCTP, both resulting in depletion of these nucleotides during phage infection. **d** | Gasdermin activation, through cleavage of the inhibitory carboxy-terminal domain, leads to formation of pores in the cell membrane and, thereby, cell death. In eukaryotes, pathogen sensing triggers formation of an inflammasome, which then activates inflammatory caspases such as caspase 1 that cleave gasdermin, causing pyroptosis, a form of cell death that involves pore formation and release of cytokines. In prokaryotes, caspase-like proteases and other proteases (such as trypsin and subtilases) activate bacterial gasdermins in response to phage infection, leading to pore formation and premature cell death. **e** | Argonaute (AGO) proteins are essential for antiviral RNA interference (RNAi) in eukaryotes, whereby RNA functions as a guide to eliminate viral RNA. Short interfering RNA (siRNA) molecules are generated from viral double-stranded RNA (dsRNA) and loaded as guide RNAs onto the Argonaute-containing RNA-induced silencing complex (RISC). RISC then cleaves complementary viral RNA targets. Prokaryotic Argonaute (pAgo) proteins have a diversity of functions, and proteins from this family can use DNA or RNA as a guide to cleave DNA or RNA. **f** | Upon pathogen infection, plant Toll/IL-1 receptor (TIR) domains produce a variant of cyclic adenine diphosphate ribose (v-cADPR) that functions as a signalling molecule and is thought to activate a cascade that leads to cell death (hypersensitive response). A similar activity is observed for bacterial TIR domains that participate in the prokaryotic Thoeris system. These bacterial TIRs generate a v-cADPR isomer, which activates a sirtuin (SIR2)-domain protein, leading to NAD^+ depletion and eventual cell death. ddhGTP, ddh-guanosine triphosphate; ddhUTP, ddh-uridine triphosphate; TM, transmembrane.

to mammalian gasdermins but are also similarly activated by dedicated proteases and defend against pathogen propagation by inducing pore-mediated premature celldeath.

The RNAi pathway and prokaryotic Argonautes. RNAi pathways are a central tool in the antiviral arsenal of plants and animals[74–76]. These pathways process incoming viral RNA into short single-stranded RNA oligonucleotides ~20–30 nucleotides in length, known as short interfering RNAs (siRNAs). The siRNAs are loaded onto a protein complex that then identifies target viral RNAs through base pairing and silences these targets[77,78]. The two core components of the RNAi pathway are Dicer and Argonaute proteins. Dicer generates short RNA molecules from viral dsRNA and, then, one RNA strand of the processed molecule (the siRNA) is loaded onto the RNA-induced silencing complex (RISC) that contains Argonaute[79,80]. The complex, including the guide siRNA, then recognizes complementary RNA targets and either directly cleaves them via the endonuclease activity of Argonaute or carries out functions such as repression of translation and transcription[77,81,82]. RNAi is considered a major antiviral pathway in plants[74] and is also crucial in the defence of invertebrates against viruses[77,83]. Although the role of RNAi in vertebrate immunity has long been debated, recent findings suggest that it is an important component of the antiviral response, particularly in non-differentiated stem cells[84–86]. Human RNAi-mediated immune responses are independent of the interferon pathway[87].

The presence of Argonaute proteins in bacteria and archaea has long been recognized, and some of the early crystal structures that were determined for this protein family were those of Argonautes of prokaryotic origin[88–91]. However, only recently has it been shown that prokaryotic Argonaute (pAgo) proteins are part of the prokaryotic immune system and defend against invading parasitic DNA, such as phages and plasmids[92–94]. Unlike their eukaryotic counterparts, most pAgo proteins that have been characterized so far have been shown to function as nucleases with specificity towards DNA rather than RNA targets[95–99] (FIG. 1e). The repertoire of functions of pAgo proteins seems to be more diverse than for Argonaute proteins found in eukaryotes. In some cases, pAgo proteins process invading DNA into short DNA sequences that are then loaded on the pAgo protein and function to identify and cleave target DNA (DNA-guided DNA silencing)[94,95,100], whereas in other cases pAgo proteins load RNA guides to recognize and cleave target DNA (RNA-guided DNA silencing)[93]. In vitro activity of some pAgo proteins against RNA has also been shown[101,102]. Several pAgo proteins were shown to first degrade invading DNA sequences non-specifically (similarly to Dicer) and then use the DNA degradation products as guide DNAs[100]. In addition, data from recent preprints (not peer reviewed) suggest that short, catalytically inactive pAgo proteins can exert anti-phage activity that results in abortive infection by triggering cell-killing effectors[103–105].

Overall, Argonaute-based innate immunity seems to have maintained its core functions and main structural domains in prokaryotes and eukaryotes. Importantly, the greater diversity of pAgo proteins compared with eukaryotic Argonaute proteins suggests that pAgo proteins first evolved and diversified in prokaryotes, and were only later acquired by eukaryotes[8,106]. Furthermore, phylogenetic analyses support the notion that the archaeal pAgo machinery may have been the direct ancestor of eukaryotic RNAi, which likely acquired additional components, such as the Dicer protein, later in evolution[8,91,107].

TIR domains are ancient immune modules. The Toll/IL-1 receptor (TIR) domain has long been recognized as an important and widespread module of innate immunity across the evolutionary tree[108,109]. TIR domains are found in animal Toll-like receptors (TLRs), which are integral membrane proteins that sense molecular features of invading pathogens[110,111]. When a TLR is activated by ligand binding, its cytoplasmic TIR domain dimerizes and then recruits downstream adaptor proteins to initiate signalling via protein–protein interactions[110,111]. In plants, TIR domains are frequently found in intracellular, non-membrane-bound receptors for pathogens, such as nucleotide-binding leucine-rich repeat proteins (NLRs) and

other TIR-containing pathogen sensors[112]. TIR domains in plant immune proteins were shown to have an enzymatic capacity and to be capable of processing NAD^+ into a variant of cyclic adenine diphosphate ribose (v-cADPR)[113,114]. Pathogen recognition by plant TIR domain proteins initiates a signalling cascade that, ultimately, leads to the 'hypersensitive response', involving death of the infected cell as well as of neighbouring cells[115]. It has been hypothesized that the v-cADPR molecules produced by plant TIR domains mediate the signal transfer from the initial pathogen recognition to the eventual immune-mediated cell death[116,117] (FIG. 1f).

Proteins with TIR domains are widespread in bacteria and were reported as being essential components of a phage defence system known as Thoeris[11]. Recently, the TIR domain proteins in the Thoeris system were shown to be responsible for recognition of invading phages[7]. It was found that — similarly to the plant TIR domains — when the bacterial TIR domain proteins recognize phage invasion, they process NAD^+ to produce an isomer of cADPR resembling the plant v-cADPR[7]. The cADPR isomer functions as an immune signalling molecule — it binds a second protein in the Thoeris system and activates it to execute cell death[7] (FIG. 1f). The observation that second messenger molecules are produced by both bacterial TIR domains of the Thoeris system and plant TIR domains, and that they are involved in mediating cell death following pathogen infection in both cases, suggests that the TIR domain is an ancient immune module that originated in bacteria.

TIR domains are also involved in non-Thoeris anti-phage defence mechanisms in bacteria[26]. These domains were found to function as effectors of the CBASS system, in which their enzymatic NADase activity serves to deplete the cell of NAD^+ and, thus, abort the infection[26]. A similar role in NAD^+ depletion was also shown for TIR domains in the Pycsar (pyrimidine cyclase system for anti-phage resistance) system[118]. Notably, a human TIR-domain protein known as SARM1 (sterile α and TIR motif containing 1), which is essential for nerve injury-activated axon degeneration, is also capable of NAD^+ processing and depletion[119]. The fact that the enzymatic activity of metabolizing NAD^+ is a common feature of TIR domains across the evolutionary tree of life, as well as the shared involvement of TIR domains in innate immunity, suggests a common ancestry of this important component of eukaryotic innate immune systems that stems from prokaryotic defence against phages.

An evolutionary scenario

The striking conservation of cell-autonomous innate immune mechanisms across prokaryotes and eukaryotes is unlikely to be a result of convergent evolution. Rather, the structural and functional conservation of prokaryotic cGAS, STING, viperin, gasdermin, Argonaute and TIR domains with their eukaryotic counterparts[4–8,26,48] points to common ancestry. We propose that these proteins first evolved in prokaryotes as anti-phage defence systems, and when the early eukaryote was formed by fusion between a bacterium and an archaeon (endosymbiosis)[120] it acquired their defence systems. In this scenario, the antiviral mechanisms that were inherited from bacteria and archaea formed the basis for the primitive immune system of the early eukaryote lineage, and later evolved into their more sophisticated roles in the contemporary cell-autonomous innate immune system (FIG. 2).

The endosymbiotic theory is the most widely accepted model for the formation of the first eukaryotes[120]. According to this theory, eukaryotes evolved from fusion of an archaeal cell and a bacterial endosymbiont that eventually became the mitochondrion[120]. The theory further posits that genes from organelles such as mitochondria migrated to the genome in the shared nucleus[121], which provides an explanation for why eukaryotic genomes seem to be chimaeras of genes from archaeal and bacterial origins[122,123]. Indeed, phylogenetic analyses suggest that some eukaryotic immune genes, such as those encoding viperin[5] and Argonaute[8], are most closely related to genes of archaeal origin, whereas other eukaryotic immune genes, for example those encoding the cGAS–STING pathway, are more closely related to bacterial genes[26,31,34,35].

Many of the defence genes that are conserved between prokaryotes and eukaryotes show larger functional diversity in prokaryotes (FIG. 2a). For example, whereas eukaryotic cGAS proteins generate cyclic GMP–AMP molecules (with some variations in the cyclization bonds)[15–18], prokaryotic cGAS-like enzymes have been estimated to synthesize a remarkable diversity of more than 180 signals, including cyclic dinucleotide and trinucleotide molecules involving all four standard nucleobases[35,38]. Similarly, whereas the mammalian viperin protein and some pVip proteins produce the antiviral molecule ddhCTP, other pVip proteins were found to produce ddhUTP or ddhGTP and, in some cases, the same pVip protein can produce multiple ddhNTP products[5]. We hypothesize that the reduced diversity observed in the eukaryotic lineage stems from the strong evolutionary bottleneck that occurred during formation of the early eukaryote, which limited the initial set of antiviral genes to those encoded by the specific archaeon and bacterium that gave rise to eukaryotes (FIG. 2b). Horizontal gene transfer may have enabled eukaryotes to acquire genes from prokaryotes at early stages of their evolution, but at some point the development of multicellularity and sexual reproduction eventually limited gene flux via horizontal transfer from

Box 3 | **Self-inflicted cell death as a defence strategy in bacteria**

A large diversity of known bacterial defence mechanisms kill the infected cell once phage invasion has been detected[160]. This self-killing phenotype may appear counter-intuitive given that bacteria are single-celled organisms, but it is an efficient strategy for long-term protection of the bacterial population. Most bacteria naturally reside in tightly packed colonies of multiple isogenic or almost isogenic cells, and if one of these cells is infected by phage, viral replication and spread puts the entire colony at risk[160]. However, if the infected cell induces its own death before phage replication has been completed, phage spread is prevented and the colony survives[160].

Bacterial defence systems that operate through self-inflicted cell death can be activated when the first lines of defence, such as CRISPR–Cas and restriction–modification systems, have failed. Some self-killing defence systems, such as the PrrC protein[161] and specific retron systems[156], actively guard restriction enzymes and other cellular immune modules, and become activated if these immune modules are inhibited by phages[156,162]. Other self-killing bacterial defence systems, including CBASS (cyclic oligonucleotide-based anti-phage signalling system), Thoeris and Pycsar (pyrimidine cyclase system for anti-phage resistance), become activated only when the phage has reached a late stage in its replication[4,7,118], which indicates that CRISPR–Cas and restriction enzymes were not able to inhibit the earlier replication stages.

Some of the immune mechanisms that are conserved between bacteria and eukaryotes function by cell killing in both cases. For example, gasdermin-mediated immunity was shown to involve eventual cell death both in bacteria[6] and in mammals[63,64,66], and Toll/IL-1 receptor (TIR) domain-mediated signalling in bacteria and in plant cells involves death of the infected cell in both cases[7,113,114,117].

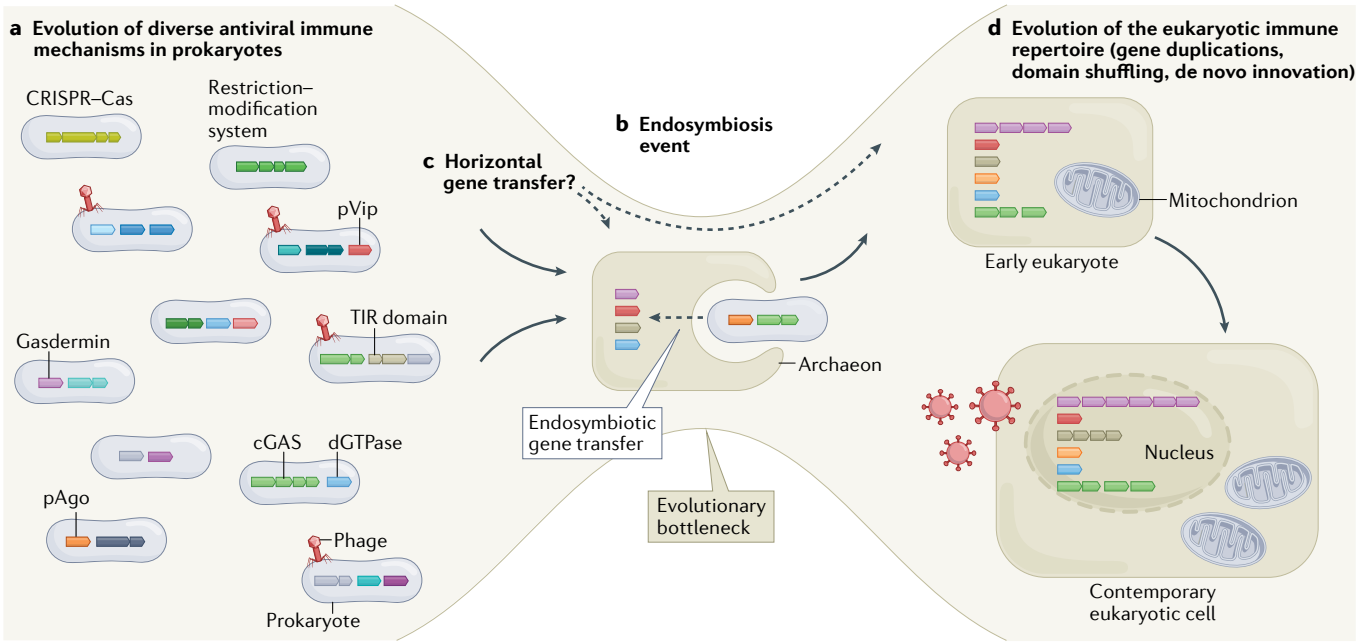

Fig. 2 | **A potential evolutionary scenario to explain the conservation of immune mechanisms between prokaryotes and eukaryotes. a** | Bacteria and archaea have a large diversity of cell-autonomous innate immune mechanisms, and each species encodes a different variety of these mechanisms[10]. **b** | Emergence of the first eukaryotic cells via an endosymbiotic event likely posed an evolutionary bottleneck, such that the immune arsenal of the early eukaryote depended on the defence systems that were present in the specific archaeal and bacterial cells that gave rise to the eukaryote. This bottleneck may have resulted in the disappearance of defence systems such as CRISPR–Cas or restriction–modification, which are notably absent from eukaryotic cells. It is also possible that these systems imposed a negative fitness cost on the early eukaryote, owing to incompatibility or autoimmunity, and thus were selected against. **c** | Transfer of genes from the bacterial endosymbiont to the nucleus that included the archaeal genome created a chimaera of genes from archaeal and bacterial origins. Up to a certain point, the ancient eukaryotic cell may also have been able to acquire additional prokaryotic immune genes through horizontal gene transfer. **d** | Processes such as domain shuffling, gene duplication and de novo functional innovations continue to diversify and enrich the immune arsenal in the eukaryotic lineage. cGAS, cyclic GMP–AMP synthase; pAgo, prokaryotic Argonaute protein; pVip, prokaryotic viperin protein; TIR, Toll/IL-1 receptor.

bacteria (FIG. 2c). Following that stage, processes such as gene duplication and domain shuffling, as well as de novo functional innovations, became more prominent in shaping the eukaryotic immune repertoire (FIG. 2d,e). For example, the human genome contains six gasdermin homologues that are thought to have evolved through recent gene duplication in the mammalian lineage[124,125], and there are usually several homologues of cGAS encoded in animal genomes[25,27,126,127]. In addition, the zinc-ribbon domain that enables cGAS to detect dsDNA was only added in the vertebrate lineage[25,128,129]; by contrast, a cGAS homologue in *Drosophila* senses dsRNA in the absence of the zinc-ribbon domain[127]. The STING domain is responsible for signal transduction and is fused to a transmembrane domain in vertebrates, whereas invertebrate metazoans encode TIR–STING variants with a predicted architecture similar to that of bacterial STING[24,26,130].

Given that so many immune mechanisms are conserved between prokaryotes and eukaryotes, it is puzzling why two very central elements of the bacterial immune system, CRISPR–Cas and restriction–modification systems, are absent in eukaryotes. It is possible that, just by chance, the two microorganisms that gave rise to the early eukaryotes did not encode these systems[106]. Another possibility is that CRISPR–Cas and restriction–modification systems caused incompatibility or autoimmunity issues following the cell fusion event that formed the first eukaryote, and were therefore selected against and eliminated from the lineage[106].

With decades of research into human innate immune mechanisms, it is remarkable that the conservation with bacterial immune counterparts has only been described recently. This is partially attributed to the rapid evolution of immune genes, which are subject to rapid and frequent selective 'sweeps' owing to the arms race between pathogens and their hosts[131–133]. Likely as a result of many such sweeps over the course of evolution, one cannot detect significant sequence similarity when directly comparing human immune genes such as those encoding cGAS, STING or gasdermin with their bacterial counterparts[4,6,26]. However, recent use of sensitive homology search tools such as HHpred[134], as well as comparisons of the crystal structures of eukaryotic and prokaryotic proteins, has revealed clear and strong homologies (for example, in the case of gasdermin[6]). Broader application of such tools and techniques, in combination with the analysis of defence islands in bacterial genomes (BOX 2), may uncover additional immune mechanisms of animals and plants that have evolutionary roots in bacteria.

Deciphering new immune mechanisms

The realization that multiple defensive proteins used by human cells have direct homologues in bacteria that function in anti-phage defence not only sheds light on the evolution of our immune system but also has implications for future mechanistic studies of human immunity. It is estimated that the cell-autonomous innate immune system can activate the expression of several hundred interferon-stimulated genes (ISGs), the purpose of many of which is to curb the infection[2]. However, the mechanisms of action of many of these ISGs are unknown[2], leaving a large knowledge gap in our understanding of the human antiviral defence arsenal. Studying

bacterial homologues of human ISGs could lead to an increased mechanistic understanding of their function, aided by the relative simplicity of the bacteria–phage experimental model systems compared with experiments using eukaryotic cells and their viruses. For example, studies of sirtuin proteins in vertebrates, which were shown to be involved in the antiviral response through an unknown mechanism[135], can be informed by recent reports on bacterial sirtuins (SIR2), which were shown to defend against phage by inducing NAD^+ depletion following phage infection (preprint data, not peer reviewed)[7,103,104]. Therefore, beyond its evolutionary implications, we believe that the conservation between prokaryotic and eukaryotic defence systems will aid in deciphering new antiviral mechanisms in humans.

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