REVIEW
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DNA Sensing in the Innate Immune Response

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The innate immune system recognizes conserved pathogen-associated molecular patterns and produces inflammatory cytokines that direct downstream immune responses. The inappropriate localization of DNA within the cell cytosol or endosomal compartments indicates that a cell may either be infected by a DNA virus or bacterium, or has problems with its own nuclear integrity. This DNA is sensed by certain receptors that mediate cytokine production and, in some cases, initiate an inflammatory and lytic form of cell death called pyroptosis. Dysregulation of these DNA-sensing pathways is thought to contribute to autoimmune diseases and the development of cancer. In this review, we will discuss the DNA sensors Toll-like receptor 9 (TLR9), cyclic GMP-AMP synthase (cGAS), stimulator of interferon genes (STING), absent in melanoma 2 (AIM2), and interferon gamma-inducible 16 (IFI16), their ligands, and their physiological significance. We will also examine the less-well-understood DEAH- and DEAD-box helicases DHX9, DHX36, DDX41, and RNA polymerase III, each of which may play an important role in DNA-mediated innate immunity.

infection; inflammasome; interferon; autophagy; DNA sensing; cell death; innate immunity; cGAS

Introduction

The innate immune system is the first line of defense against invading pathogens and sterile insults. Innate immune cells are equipped with pattern-recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). This sensing is crucial for an appropriate innate immune response, which is required for the direct elimination of microbes or for the subsequent mounting of an adaptive immune response. Based on their localization, PRRs can be membrane-bound or cytosolic. Types of membrane-bound receptors include the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). Types of cytosolic receptors include the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), and the absent in melanoma 2 (AIM2)-like receptors (ALRs). The diversity of PAMPs mirrors the broad range of pathogen families, including bacteria, viruses, fungi, protozoa, and helminths, and the innate immune system has evolved to sense the conserved PAMPs from these microbes. Many of the PAMPs are distinct from host-derived molecules, but some are conserved between pathogens and their host. Broadly, the PAMPs consist of pathogen cell wall components, including lipopolysaccharide, lipoteichoic acid, peptidoglycan, and glycans like β-1,3-glucan, conserved bacterial proteins such as flagellin and the type III secretion system (T3SS), and ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). Because DNA is the carrier of genetic information for a wide range of life forms, including microbes, the innate immune system has evolved proteins that can sense and discriminate between self and foreign DNA. Innate DNA sensors can be divided functionally into DNA sensors mediating type I interferon (IFN) production and those mediating inflammasome activation.

In this review, we will explore the crucial role of DNA sensing during microbial infection and autoinflammatory diseases.

Toll-Like Receptor 9: The Founding dsDNA Sensor Identified

Toll-like receptor 9 (TLR9), the founding member of the innate immune sensors for DNA, is composed of

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a leucine-rich repeat (LRR) domain and a Toll/IL-1 receptor (TIR) domain. TLR9 was discovered first in mouse macrophages and later in human cells (FIGURE 1) (10, 41). As is the case for most other TLR family members, the TIR domain of TLR9 is required for the recruitment of the signaling adaptor protein MyD88. Distinctly, TLR4 utilizes both the MyD88 and TIR domain-containing adaptor inducing IFN-β (TRIF) adaptor proteins, whereas TLR3 signals solely through TRIF. Similar to TLR3 and TLR7, TLR9 is an endosomal receptor that senses nucleic acids, but TLR9 is the only one of these that recognizes DNA. TLR9 recognizes the unmethylated cytosine-phosphate-

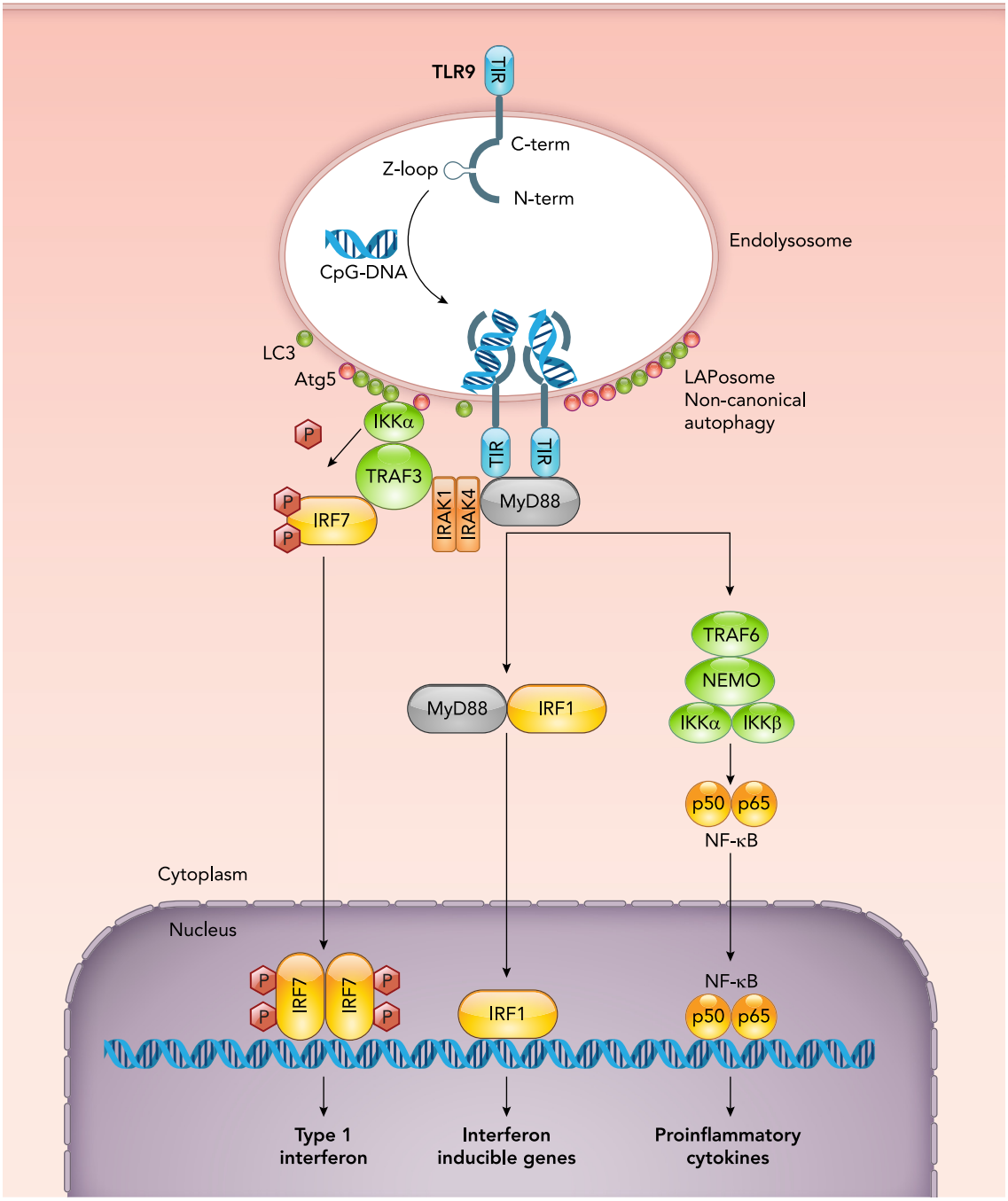

FIGURE 1. **Toll-like receptor 9, a founding dsDNA sensor mediating type I IFN signaling**
The Toll-like receptor 9 (TLR9) receptor is cleaved inside the endolysosome, where the LRR domain composed of NH_2- (N-term) and COOH-terminal (C-term) ectodomains is required for CpG-DNA binding, receptor oligomerization, and signal transduction. The TIR domain of dimerized TLR9 recruits the MyD88 adaptor protein. This recruitment induces IRAK4/IRAK1/TRAF3/IKKα complex formation, and the LC3-TLR9 interaction serves as an anchor to activate IRF7 and type I IFN expression. A second pathway downstream of TLR9 leads to a MyD88-IRF1 interaction that facilitates the nuclear translocation of IRF1 and expression of IFN-inducible genes. Finally, TLR9 activation with CpG-DNA activates the NF-κB pathway and proinflammatory cytokine release.

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guanosine (CpG) form of DNA, which is commonly found in bacterial, viral, fungal, and parasite genomes. TLR9 is crucial during bacterial meningitis and infections by Epstein-Barr virus, human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, cytomegalovirus (CMV), *Toxoplasma*, *Plasmodium* species, and *Aspergillus fumigatus* (15, 34, 52, 95, 99, 113). Unlike vertebrate DNA, where CpG dinucleotides are usually highly methylated, microbial DNA is hypomethylated. Human TLR9 is mainly expressed in dendritic cell subtype 2 (DC2) and B cells, whereas mouse TLR9 is expressed in DC2, DC1, and B cells and macrophages (41, 43, 53, 106). Indeed, unmethylated CpG-DNA triggers B-cell activation, inducing cell proliferation and immunoglobulin secretion (64, 77). CpG-DNA signals through the TLR9-MyD88 complex, activating the nuclear factor (NF)-κB pathway and IFN transcription factor 7 (IRF7) nuclear translocation and leading to inflammatory cytokine and type I IFN expression (103). Mechanistically, CpG-DNA binding leads to TLR9 dimerization and MyD88 adaptor protein recruitment to the endosomal membrane (65). The crystal structure of TLR9 has recently been solved and explains why acidification of the phagolysosome is required for CpG-DNA binding to TLR9 (85, 93). Within the endosome, a flexible Z-loop region between the ectodomains is cleaved by proteases, which is thought to be required for TLR9 oligomerization but not ligand binding (27, 85–87). Whether TLR9 processing in the endosome is universally important is still not well understood.

Earlier studies using forced plasma membrane localization of a chimeric TLR9 (made up of the TLR9 ectodomain and TLR4 transmembrane and cytosolic domains) revealed that plasma membrane-restricted TLR9 signaling fails to recognize viral DNA but is readily activated by self-DNA, highlighting how the intracellular compartmentalization of TLR9 normally restricts self-DNA recognition (9). Whether changes in the localization of TLR9 and its recognition of self-DNA are altered in autoimmune disease is an important area of future study.

A recent study has also identified an association between TLR9 and the autophagy machinery (40). CpG-DNA stimulation leads to the recruitment of the autophagy component light chain 3 (LC3) and autophagy-related 5 (ATG5) to transduce signaling through inhibitor of NF-κB kinase subunit α (IKKα), IL-1 receptor-associated kinase 1/4 (IRAK1/4), tumor necrosis factor (TNF) receptor-associated factor 3 (TRAF3), and IRF7 to drive the expression of type I IFN (40). TLR9 also was shown to signal from endosomes, independent of CpG-DNA binding, through the non-canonical LC3-associated phagocytosis (LAP) pathway. This observation suggests that TLR9 endosomal signaling may play a larger role than previously appreciated during phagocytosis (40).

A second, IRF3/7-independent pathway for TLR9-mediated IFN expression has also been described in DC1 dendritic cells and macrophages. Stimulation with CpG-DNA leads to the recruitment of MyD88 and an interaction with IRF1, increasing IRF1 nuclear translocation and the expression of IFN-stimulated genes (ISGs), and the release of inflammatory cytokines (81, 96). This pathway was recently found to mediate the expression of IRF1-dependent ISGs required for innate immune responses to *A. fumigatus*, a fungal pathogen known to actively recruit TLR9 to the phagolysosome (12, 59, 90).

A previous study demonstrated that mice lacking TLR9 are hypersusceptible to systemic lupus erythematosus (SLE), an autoimmune disease characterized by deregulated immune responses against DNA (18). Moreover, it has been proposed that TLR9 is required for tolerance, controlling the clearance of cellular debris, and reducing the levels of endogenous inflammatory mediators (18), indicating that TLR9-mediated autophagy is protective in SLE. Indeed, mice lacking the LAP pathway are known to develop SLE-like syndromes (21, 75). Importantly, human TLR9 polymorphisms also promote SLE due to the recognition of self-DNA (23). Whether these polymorphisms result in a loss of specificity for unmethylated CpG-DNA requires further investigation. Another important mechanism by which TLR9 restricts self-reactive B-cell receptor (BCR) activation is by cooperative signaling from BCR-/TLR9-containing endosomes, which leads to apoptosis of self-reactive B cells; this process appears to be defective in patients with SLE (33, 98, 105).

cGAS: A Major Cytosolic dsDNA Sensor Mediating Type I IFN Signaling

The sensing of cytosolic dsDNA by the protein cyclic GMP-AMP synthase (cGAS) produces the secondary messenger cyclic GMP-AMP (cGAMP). cGAMP is further sensed by downstream sensor protein stimulator of IFN genes (STING), leading to the production of type I IFN. Initially discovered in 2013 after the discovery of STING, cGAS as a sensor for cytosolic dsDNA has been implicated in many diseases and in controlling infection (3, 4, 102, 109, 116).

The cGAS crystal structure with dsDNA was resolved soon after its identification as a DNA sensor upstream of type I IFN production. Surprisingly, the human and mouse cGAS are evolutionarily divergent (118). cGAS is structurally related to the

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enzyme 2′-5′-oligoadenylate synthetase 1 (OAS1), which recognizes dsRNA to mediate the production of 2-5OA; however, cGAS contains a unique zinc ribbon in its COOH-terminal domain that is necessary for the specific binding to dsDNA (20, 63, 66). The recognition of the B-form of DNA via its sugar phosphate backbone is sequence-independent, but longer stretches of DNA induce a stronger innate response, possibly due to more rapid cGAS dimer assembly (FIGURE 2) (7, 63, 66). DNA binding induces a structural switch in cGAS, called a conformation transition, which opens the catalytic pocket for the generation of cGAMP from GTP and ATP (20, 32). The binding of cGAS to DNA facilitates a liquid phase separation that promotes cGAS enzymatic activity; this is necessary for the efficient production of cGAMP and downstream innate immune responses (25). The authors proposed that cGAS droplet formation enhances the sensitivity of cGAS-DNA recognition by increasing the local concentration of the sensor and its ability to bind stretches of long DNA. Interestingly, dsRNA also induces cGAS phase separation and aggregation but does not result in the production of cGAMP. Zinc ions can further enhance the activity of cGAS by stabilizing the interaction of cGAS with negatively charged DNA following binding, enhancing the production of cGAMP and suggesting that changes in the cellular ion homeostasis may be a mechanism for regulating the binding of

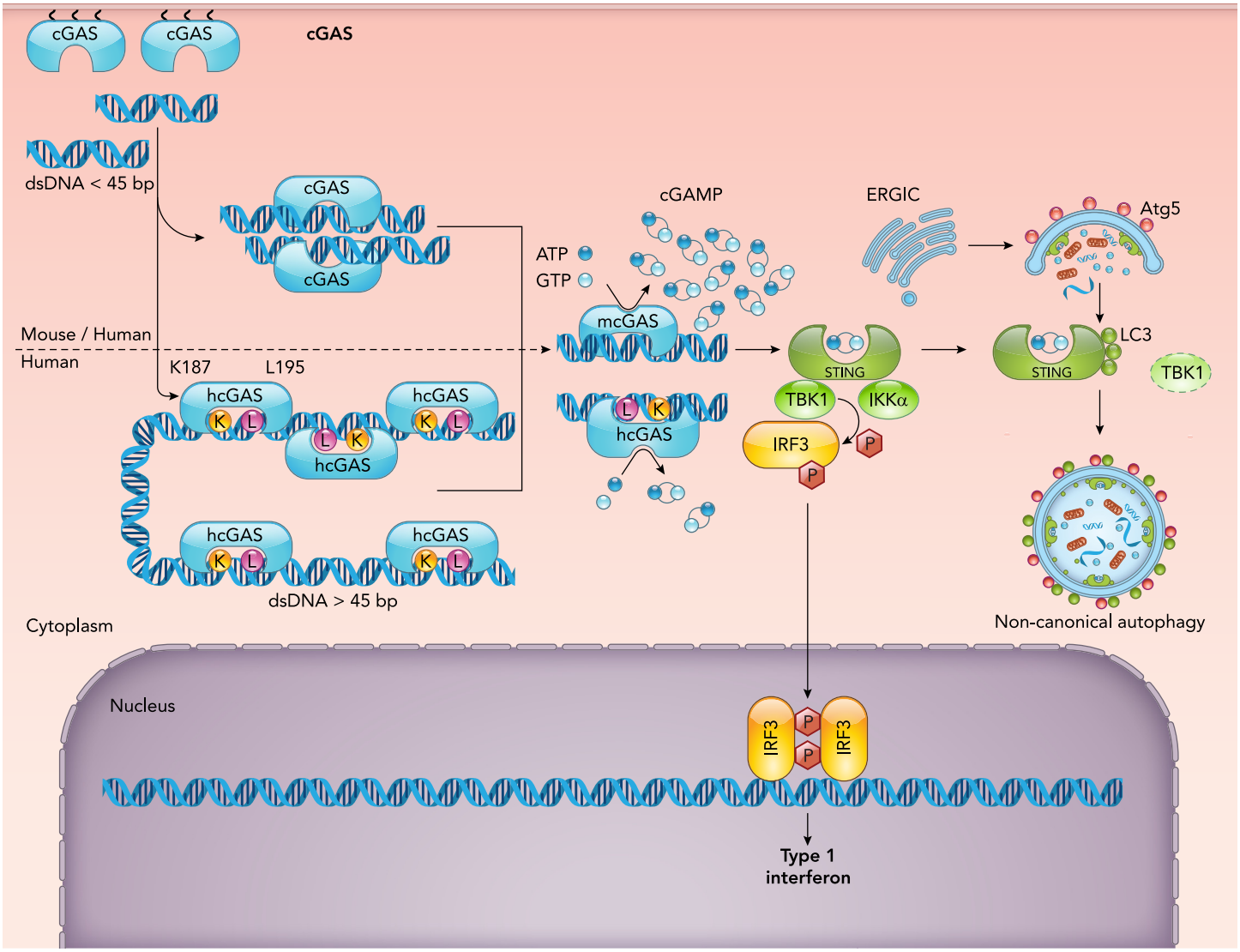

FIGURE 2. **cGAS/STING pathway, a major cytosolic dsDNA sensing pathway mediating type I IFN signaling**
cGAS is localized close to the plasma membrane. The recognition of dsDNA forms a complex with cGAS dimers binding to two dsDNA strands. cGAS-dsDNA binding induces a conformational phase transition for the catalytic synthesis of cGAMP from ATP and GTP. The human cGAS (hcGAS) has K187 and L195 mutations, which necessitate longer dsDNA for activation and cause low levels of cGAMP production in response to dsDNA. cGAMP binds to STING and recruits IKKα and TBK1 to activate IRF3 for type I IFN production. Additionally, after cGAMP binding, STING interacts with LC3 and promotes non-canonical autophagy through an ATG5-dependent mechanism after TBK1 degradation to control viral replication.

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negatively charged DNA to positively charged cGAS surfaces (25). Curiously, another recent study found that cGAS is localized to the plasma membrane via the interaction of its NH_2-terminal phosphoinositide-binding domain in phagocytic cells, suggesting that this limits the exposure of cGAS to self-DNA and influences the recognition of viral infections (8). Future studies might reveal how the localization and condensation of cGAS influence the activity of this enzyme and how it contributes to many different diseases and controlling infections.

Due to mutations in the K187 and L195 amino acids, human cGAS is less efficient than mouse or other mammalian cGAS proteins in producing cGAMP. These amino acids are required for DNA binding and complex stability (118). Similarly, these authors have shown that, in humans, the sequence differences at K187/L195 increase the stringency for long DNA fragments, mechanistically explaining why human cGAS requires longer DNA fragments than mouse cGAS does (118).

Although much of the previous work on cGAS has focused on pathogen-derived cytosolic DNA, self-DNA can also be sensed by cGAS (3). Recent findings revealed a major role for cGAS in cellular senescence and autoimmune diseases (35). In autoimmune diseases associated with defects in DNA metabolism, such as Aicardi-Goutières syndrome (AGS), cGAS has been implicated in promoting the inflammation-induced pathology (31, 35). Recent studies have also suggested a role for cGAS in promoting SLE, which is associated with increased IFN levels and cGAMP concentrations in the circulation (6). In cases of cellular senescence, cGAS appears to be activated by self-DNA released by the damaged nuclear envelope, signaling to immune cells that the cell cycle may be compromised and activating immune responses (39, 71). Whether this contributes to the process of aging is an interesting concept that needs to be explored further.

STING: A Central Adaptor Protein of Cytosolic dsDNA Sensing

Pioneering studies identified STING (previously named MITA and MPYS and encoded by *TMEM173*) as an activator of IRF3 and NF-κB, which leads to the downstream expression of type I IFN (14, 46, 47). Murine embryonic fibroblasts lacking STING are susceptible to the RNA virus vesicular stomatitis virus (VSV) and fail to produce type I IFN in response to intracellular DNA, herpes simplex virus type 1 (HSV-1), or the intracellular bacterium *Listeria monocytogenes* (46, 47). Later studies showed that STING can directly sense bacterial-derived cyclic dinucleotides (14). Although these and other studies have observed a role for the cGAS-STING pathway in response to RNA viruses, it remains unclear how these viruses activate cGAS-STING, since RNA has only been shown to bind cGAS and not to promote its functional activation (83). One hypothesis suggests that viral infection leads to the release of mitochondrial DNA, which is subsequently sensed by the canonical DNA-cGAS-STING pathway to promote an immune response (101). Alternatively, STING may interact directly with RNA sensors such as RIG-I/mitochondrial antiviral-signaling protein (MAVS), but further work is needed to clarify this cross talk between RNA- and DNA-sensing pathways (114).

Recently, STING was found to induce autophagy in response to DNA, cGAMP, or HSV-1 through an IFN-independent mechanism (37, 68). STING possesses microtubule-associated protein 1A/1B-light chain 3 (LC3)-interacting regions. LC3 is a major protein of the autophagy pathway and regulates autophagosome formation by serving as a lipidation platform for the ATG protein family. STING activation by cGAMP promotes this translocation to the endoplasmic reticulum (ER) to trigger LC3 recruitment to serve as a source of LC3 for the phagolysosome. Specifically, the ER-Golgi intermediate compartment is the membrane source for LC3 conjugation vesicles to form through non-canonical autophagy involving WIPI2- and ATG5-dependent mechanisms (FIGURE 2). STING-mediated autophagy is required to control HSV-1 replication, whereas type I IFN production is not (37).

STING is also required to control cancer progression. *Sting*-deficient mice are highly susceptible to colitis-associated colorectal cancer (CRC) due to upregulation of NF-κB and STAT3 signaling, which leads to production of proinflammatory cytokines (119). Autophagy is also critical to regulate CRC, and whether STING is required to restrict CRC through autophagy needs to be explored (110).

In human populations, STING alleles with differential responses to cGAMPs have been observed. The HAQ allele (R71H-G230A-R293Q), common in East Asians, is the second most common globally and is likely a loss-of-function allele (89). Gain-of-function mutations in STING contribute to an autoinflammatory disease called STING-associated vasculopathy with onset in infancy (SAVI) (89). More studies looking into human STING may reveal new ways to treat IFN-driven diseases and develop a better understanding of how the different STING variants contribute to various therapies that rely on this pathway.

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AIM2 and IFI16 (or IFI204 in Mice): dsDNA Sensors Mediating Inflammasome Complex and Type I IFN Signaling

AIM2 and IFI16 (or the IFI204 mouse ortholog) belong to the ALR family. Although the ALR family is composed of 4 genes in the human genome and 14 in the mouse genome (13, 22), AIM2 and IFI16 are the two most well-studied ALR proteins. In fact, the role of AIM2 in inflammasome activation in response to dsDNA has greatly increased interest in this protein (29, 42, 92). The inflammasomes are multimeric protein platforms that lead to caspase-1 activation, maturation and release of IL-1β/IL-18, and lytic cell death called pyroptosis as a result of gasdermin D cleavage. The AIM2 protein is composed of two domains, one hematopoietic IFN-inducible nuclear protein (HIN) domain and one pyrin domain (PYD), whereas IFI16 has two HIN domains, HIN-A and HIN-B, and one PYD (FIGURE 3). The crystal structure of the IFI16 HIN domain shows that two oligonucleotide/oligosaccharide binding (OB) fold domains are linked together (67, 111). The AIM2 HIN domain recognizes cytosolic DNA, inducing recruitment of the adapter protein apoptosis-associated speck-like protein containing a caspase recruitment team (ASC) through PYD-PYD interactions; this leads to caspase-1 recruitment through caspase recruitment domain (CARD)-CARD interactions and inflammasome activation. The structure of the HIN domain bound to DNA was resolved and confirmed that the HIN domain binds to the B form of DNA (50). This study also showed that the HIN-DNA interaction was DNA sequence-independent and that electrostatic interactions between the sugar backbone of dsDNA and the positively charged HIN domain promote the binding (50). How AIM2 is negatively regulated to prevent spontaneous activation is still poorly understood. One study proposed a model in which the AIM2 PYD and HIN domain interact via a charge-charge interaction and form a complex that switches off the receptor in the absence of DNA and blocks the interaction with ASC and spontaneous activation of the AIM2 inflammasome (50). However, a recent study proposed that the PYD-HIN domain interaction does not occur, suggesting the process may not be regulated by autoinhibition. The optimal dsDNA size for protein oligomerization is ~300 base pairs, and this may be one of the primary regulatory mechanisms for AIM2 inflammasome activation (50, 80).

Inflammasomes control host defense responses and autoimmune diseases. Indeed, the AIM2 receptor is essential during infection with bacterial pathogens such as *Francisella tularensis*, *L. monocytogenes*, *Streptococcus pneumoniae*, *M. tuberculosis*, and *Staphylococcus aureus*, fungal pathogens such as *A. fumigatus*, and DNA viruses such as vaccinia virus and CMV (28, 29, 38, 51, 58, 91, 94, 108). During infection with intracellular bacteria, ISGs such as guanylate-binding proteins (GBPs) and immunity-related GTPase family member b10 (IRGB10) regulated by IRF1 are required to release bacterial DNA for sensing by AIM2 (72, 73, 78). Conversely, HSV-1, a DNA virus, is known to induce activation of the NLRP3 inflammasome but not the AIM2 inflammasome (91). The HSV-1 protein VP22 inhibits AIM2 inflammasome oligomerization and activation during infection by interacting with the HIN domain of AIM2 to block oligomerization (76). This mechanism reveals a potential off switch for AIM2 inflammasome activation. However, how the interaction between the HIN domain and VP22 suppresses AIM2 activation requires further study. Similarly, during human CMV (HCMV) infection, the pUL83 viral protein interacts with AIM2 to inhibit AIM2 inflammasome activation, but the domain of interaction is still unknown (45).

The role of IFI16 in inflammasome activation is controversial. Previously, IFI16 was shown to induce inflammasome activation in human cells in response to Kaposi sarcoma-associated herpesvirus (KSHV) infection (60). Mechanistically, IFI16 seems to recognize the dsDNA of KSHV directly in the nucleus to form an inflammasome complex which then translocates to the cytoplasm. Additionally, IFI16 is required for HIV infection-mediated pyroptosis through caspase-1 activation in CD4 T cells (24, 79). Aside from their role in inflammasomes, human IFI16 and murine IFI204 have also been shown to regulate type I IFN expression in response to infection or p53 activation in response to ionizing radiation (19, 30, 112).

IFI16 recognition of HSV-1 viral DNA has been proposed to mediate IFI16-STING-mediated recruitment of TANK-binding kinase (TBK1) and DEAD-box polypeptide 3 (DDX3), activation of the IRF3 and NF-κB pathways, and production of IFN-β (104). In keratinocytes, DNA binding by IFI16 induces colocalization with TBK1 and STING in TNF-α- or IL-1β-treated cells, further supporting that IFI16-STING may be a distinct DNA-sensing pathway (16). Additionally, the inhibitory effects of HSV-1 on AIM2 activation and cGAS-mediated dsDNA detection could explain why IFI16-STING signaling may be important during HSV-1 infection. The HSV-1 VP22 protein not only inhibits AIM2 activation but also appears to inhibit cGAS-mediated recognition of viral DNA (44). In addition to VP22, the HSV-1 strain UL41 has been shown to further counteract cGAS via production of a viral

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RNase that degrades cGAS mRNA (100). Recently, the role of all other AIM2-like receptors, including IFI204, in type I IFN production was ruled out in mouse models lacking all the ALR sensors during dsDNA transfection and in the Aircardi-Goutieres syndrome model (36). The authors also showed that human cells lacking IFI16 have no defect in type I IFN during HCMV infection. But other studies suggest that IFI16 confers resistance to HSV-2, KSHV, or HIV (24, 26, 60, 79). The role of all these receptors should be reexamined to identify new functions in other pathogen models as well as in autoimmune disease models. Indeed, IFI204 was recently found to mediate autophagy and IFN-β release during *Mycobacterium bovis* infection (19).

In addition to the role of the AIM2 and IFI16 receptors during pathogen infection, these two receptors play important roles in sterile

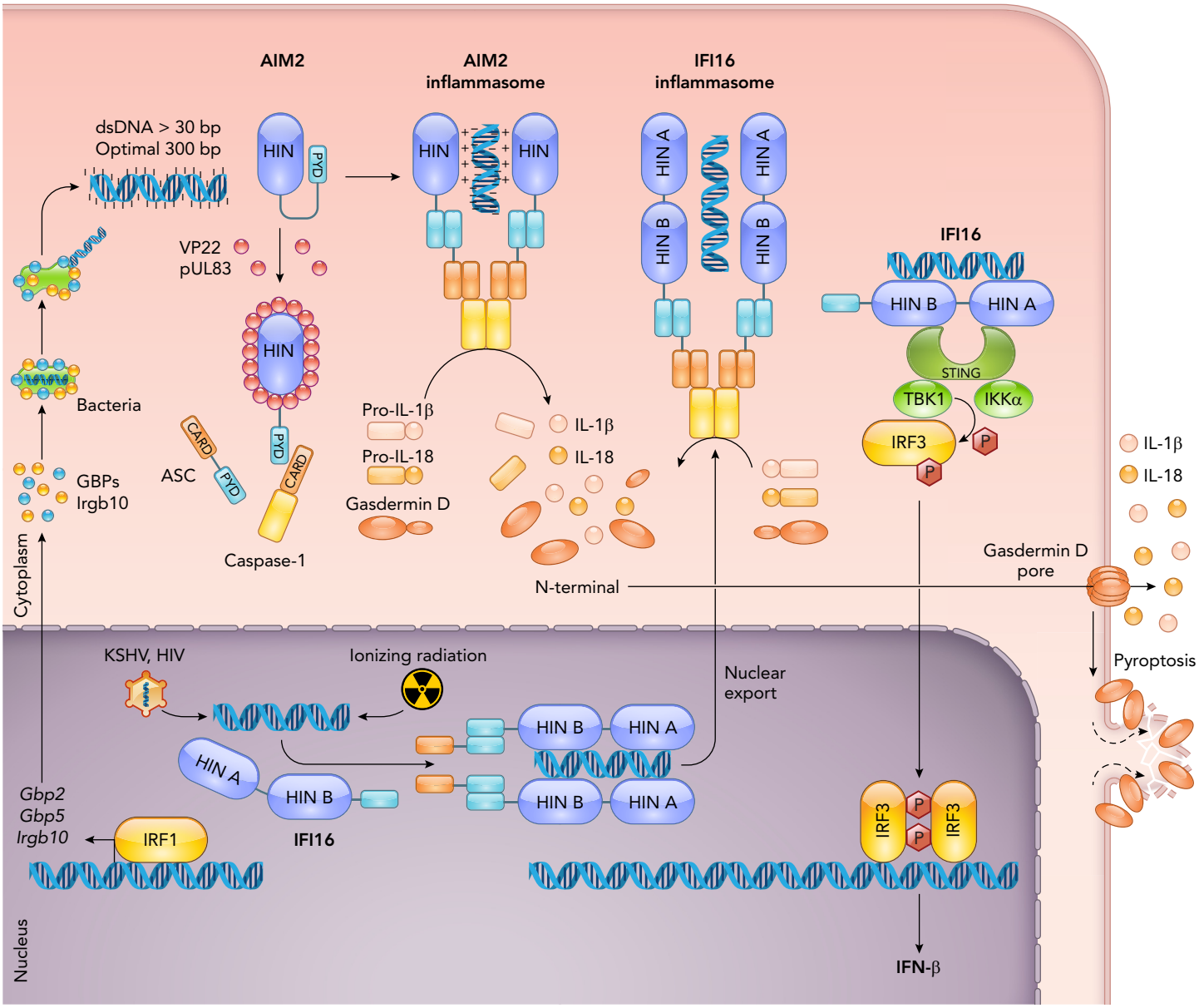

FIGURE 3. **AIM2 and IFI16 (or IFI204 in mice), dsDNA sensors mediating inflammasome complex and type I IFN signaling**
Electrostatic interactions between the AIM2 HIN domain and the dsDNA sugar backbone are essential for keeping AIM2 in a quiescent state. IRF1-dependent GBPs and IRGB10 facilitate the release of bacterial DNA into the cytosol. AIM2 inflammasome sensing of DNA triggers recruitment of the inflammasome adaptor ASC and caspase-1. Caspase-1 directly cleaves pro-IL-1β, pro-IL-18, and gasdermin D. The NH_2-terminal gasdermin D fragment forms pores in the plasma membrane and initiates pyroptosis. IL-1β and IL-18 are released through the gasdermin D pore. HSV-1 and HCMV produce the viral proteins VP22 and pUL83, respectively, which interact with the HIN domain to block AIM2 oligomerization. IFI16 is localized in the nucleus and senses viral dsDNA directly from there. DNA recognition induces IFI16 oligomerization inside the nucleus, and then the oligomer migrates to the cytoplasm to finalize inflammasome complex formation. IFI16 activation in response to viral dsDNA or ionizing radiation mediates type I IFN production after a direct interaction with STING/TBK1/IKKα and IRF3 activation.

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inflammatory diseases. Similar to the TLR9 and cGAS receptors, AIM2 and IFI16 receptors are associated with SLE development. A correlation between AIM2 and IFI16 expression and the severity of SLE in patients has been identified (62, 115). Moreover, AIM2 participates in the development of an arthritis-like syndrome in a double DNaseII/IFN-α/β/ω receptor knockout mouse model (11, 48). However, it is still not clear whether the contribution of AIM2 in SLE and polyarthritis is inflammasome-dependent. AIM2 is also involved in inflammatory bowel diseases and controlling CRC through an inflammasome-independent mechanism (74, 97, 107). Intestinal stem cells lacking AIM2 have uncontrolled Wnt pathway activation, resulting in over-activation of AKT and expression of the transcription factor proto-oncogene c-MYC, which drives cell proliferation, and reduces cell death and tumorigenesis associated with polymicrobial dysbiosis (74). However, the inflammasome-independent mechanism by which AIM2 controls cell proliferation is still unknown. Besides its role in CRC, the role of AIM2 in the development of other cancers has been discussed in detail elsewhere (56, 57). Given the importance of AIM2, STING, and cGAS in regulating gastrointestinal inflammation, it is intriguing to consider whether AIM2 acts through an interaction with the cGAS-STING pathway, similar to what is observed during IFI16-STING signaling (69, 119).

Recently, the role of AIM2 in severe acute pancreatitis (AP) has been highlighted. Nucleosomes (dsDNA with histones) are released during AP and are responsible for inflammatory responses and pancreatic damage (55). In a follow-up study, nucleosomes were found to induce inflammasome activation in a receptor for advanced glycation end-products (RAGE)- and AIM2-dependent mechanisms (54). AIM2 expression also correlates with disease severity in patients with AP and transient or persistent organ failure (5). Nonetheless, understanding whether nucleosomes directly activate the AIM2 inflammasome requires further study because the histones may mask recognition of the dsDNA by the HIN domain.

The DDX and DHX Family, RNA Helicase Proteins Sensing dsDNA

Another family of sensors that regulate DNA-mediated type I IFN production are the RNA helicases. This family is composed of two subgroups: the DEAH-box helicases (DHX) and the DEAD-box helicases (DDX) (1).

DHX9 and DHX36 are known to interact with unmethylated CpG-DNA, like TLR9 does, to activate downstream signaling in the IRF7 and NF-κB pathways in response to microbial DNA. DHX9 and DHX36 recognize dsDNA with different domains and different CpG-DNA forms, which are distinguished by the composition of their backbones and nucleotide sequences. DHX9 recognizes CpG-B (ODN 2006) DNA via its DUF1605 domain, whereas DHX36 recognizes CpG-A (ODN 2216) DNA via its DEAH domain (FIGURE 4) (61). Recently, DHX9 was found to play a role in regulating the NF-κB-mediated immune response to HSV-1 infection, but this was independent of its DNA-sensing function (82).

In the DDX family, DDX41 has been identified as a dsDNA sensor during HSV-1 viral infection and following B-form DNA transfection. The DDX41 Walker A and Walker B motifs, present in the DEAD domain, are required for the interaction with dsDNA. The DNA binding promotes an interaction between DDX41 and STING, and triggers TBK1 phosphorylation to activate downstream IRF3, NF-κB, and type I IFN production (117). Surprisingly, it has been demonstrated that DDX41 can directly sense the bacterial second-messenger cyclic-di-GMP (c-di-GMP), which is structurally similar to the second messenger cGAMP and is also part of the same cyclic nucleotide family (FIGURE 4) (88). It has been previously shown that c-di-GMP directly interacts with and activates STING (14, 109, 116). Furthermore, DDX41 was shown to be required for efficient IFN-β induction in response to poly(dA:dT) and cGAMP stimulation (70). It will be interesting to explore whether other DDX family members can directly sense cGAMP and whether DDX41 participates in the cGAS pathway. Although the crystal structure of the human DDX41 DEAD domain has been resolved, this structural analysis is based only on the truncated protein (49). It is also important to consider that genetic evidence using mouse or human knockouts of the DHX and DDX family members have not yet shown a critical role for these genes in immune responses to DNA, suggesting further study is needed.

RNA Polymerase III: The Crosstalk Between dsDNA and dsRNA Sensing

The RNA polymerase III (POL III) is a DNA sensor for AT-rich DNA. Poly(dA:dT) DNA is converted to poly(dA:dU) RNA through an active mechanism by POL III (FIGURE 4). This neo-dsRNA containing a 5′-triphosphate is recognized by the RIG-I sensor and activates MAVS to mediate type I IFN production (2, 17). POL III is required during bacterial and viral infection. Recently, the role of POL III has been highlighted during varicella zoster virus (VZV) infection in children (84). Heterozygous missense

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mutations in *POLR3A* and/or *POLR3C* lead to a reduced type I IFN response to AT-rich or VZV DNA and reduced control of VZV replication. Patients with these mutations develop severe acute varicella (chickenpox) symptoms with central nervous system and lung invasion, suggesting a critical role for POL III as a DNA sensor in human immunity (84). Notably, other DNA sensors, including cGAS, DDX41, and IFI16, were not able to compensate for these POL III mutations. This is interesting because cGAS and IFI16 are thought to recognize dsDNA in a sequence-independent manner; however, they fail to recognize AT-rich dsDNA in the absence of POL III. Future work focused on DNA sensing during VZV infection may reveal new viral inhibitory proteins, and genetic approaches should help clarify the role of each of these sensors in type I IFN production.

Summary and Future Perspectives

Although DNA has long been known to be a strong immunogenic component and inducer of type I IFN, the receptors for DNA binding were only identified in the past 20 years. Today, the role of DNA sensing by the innate immune system is supported by strong genetic evidence. DNA sensors participate in the response to many pathogens, including bacteria, fungi, and viruses, and mediate their

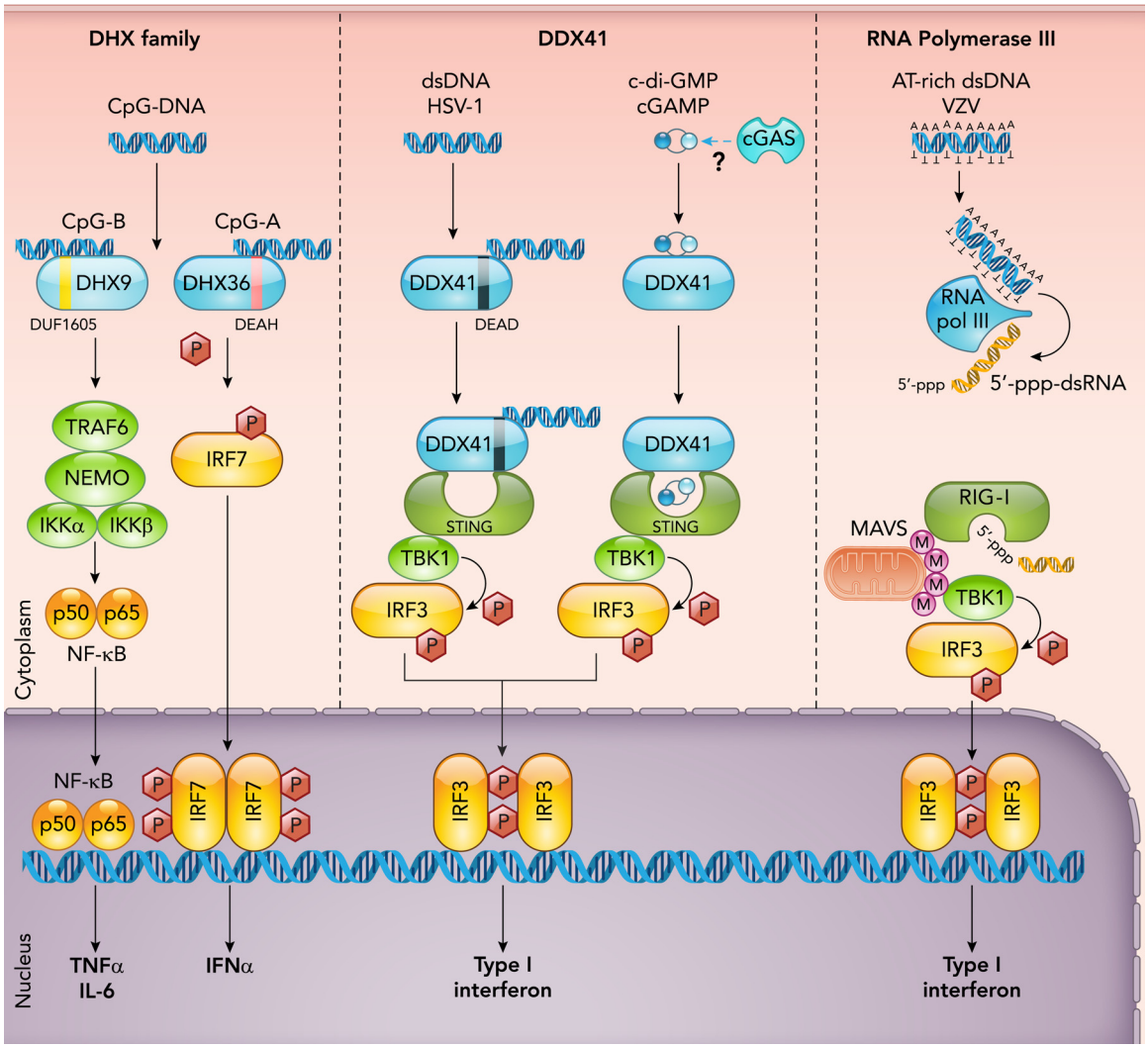

FIGURE 4. **RNA helicases, the proteins sensing dsDNA and the cross talk between dsDNA and dsRNA sensing**
DHX9 binds to CpG-B DNA through its DUF1605 domain and leads to NF-κB pathway activation and proinflammatory cytokine release. DHX36 binds to CpG-A DNA through its DEAH domain, resulting in IRF7 activation and IFN-α release. DDX41 recognizes dsDNA through its DEAD domain and interacts with STING/TBK1 to mediate IRF3 activation and type I IFN expression. DDX41 interacts directly with bacterial c-di-GMP and cGAMP from cGAS to mediate type I IFN signaling after interaction with STING. POL III binds to AT-rich dsDNA or VZV DNA to convert this DNA to 5′-triphosphate dsRNA (5′-ppp-dsRNA). 5′-ppp-dsRNA is recognized by RIG-I and interacts with MAVS to mediate type I IFN production.

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clearance. These DNA sensors also contribute to the pathogenesis of autoinflammatory diseases and cancer. Each DNA receptor has a specific function during homeostasis. Many of these sensors activate a type I IFN response to signal to the immune system; these activating sensors include TLR9, cGAS, STING, DHX9, DHX36, DDX41, IFI16, and POL III. Uniquely, the DNA sensors AIM2 and IFI16 have a key role in activating the inflammasome complex, which provides cells with a mechanism for cell-autonomous pathogen clearance. Each of the DNA sensors has a different mechanism of recognition of dsDNA, with some being sequence specific, non-sequence specific, or chemical modification specific. Differences between mouse and human DNA sensors suggest evolutionary pressures on their specificity and sensitivity, which are important for regulating innate immune responses in a host-specific manner. These differences also suggest that overlap between the specificity of each sensor is important for host defense.

The potential role of cross talk between dsDNA sensors is an important avenue to consider in future studies, since pathogens have adapted to inhibit immune pathways as part of their survival strategies. Therapeutic strategies for complex autoimmune diseases mediated by these pathways may also be generated by better understanding the cross talk between the DNA-sensing pathways. Importantly, recent work on the connection between type I IFN induction and downstream AIM2 inflammasome activation highlights a key synergy between these pathways in bacterial infections. Future studies are likely to reveal more important cross talk between these sensing pathways and their relevance to human disease. ■

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