Role of the HIN Domain in Regulation of Innate Immune Responses

Neil Shaw,^a Zhi-Jie Liu^{a,b}

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China^a; Institute of Molecular and Clinical Medicine, Kunming Medical University, Kunming, China^b

The oligonucleotide/oligosaccharide binding (OB) fold is employed by proteins to bind nucleic acids during replication, transcription, and translation. Recently, a variation of the OB fold consisting of a tandem pair of OB folds named the HIN (hematopoietic expression, interferon-inducible nature, and nuclear localization) domain was shown to play essential roles in the regulation of innate immune responses originating from binding of nucleic acids in the cytoplasm or the nucleus of the cell. Although the two OB folds of the HIN domain are linked via a long linker region, conserved hydrophobic contacts between the two OB folds hold them together firmly, resulting in a single compact domain. This overall topology of the HIN domain seems to be highly conserved, and proteins containing the HIN domain have been grouped in the PYHIN family. Structures of the recently solved HIN domains reveal that these domains exhibit either absent in melanoma2 (Aim2) HIN-like or p202 HINa-like modes of DNA binding. These two modes of DNA binding seem to result in different responses and as a consequence confer distinct roles on the proteins. This review summarizes our current understanding of the structure and function of the HIN domains in context with the innate immune responses.

Proteins need to bind nucleic acids for carrying out essential functions of the human body like replication, transcription, and translation. This is accomplished by the use of folds or motifs within a protein that specifically bind to nucleic acids. One of the most remarkable features of these motifs is the ability to recognize specific features within a sequence of the nucleic acid. For example, the ability to recognize and discriminate nucleic acids based on presence of a 2' OH group on the ribose sugar exemplifies the extent of specificity and precision associated with these motifs in binding nucleic acids. Such specificity is essential for maintaining fidelity during processes like transcription and replication, in order to transmit genetic information from parent to progeny. In addition to discriminating between DNA and RNA, proteins can bind specific sequences of nucleotides within nucleic acids. For example, proteins containing CXXC (Cys-X-X-Cys) domains, like CFP1 (CXXC-type zinc finger-containing protein 1), are known to bind unmethylated CpG nucleotide sequences specifically (1). The CXXC domain contains two C-4-type zinc fingers and binds in the major groove of DNA. Recognition of the CpG motifs by the CXXC domain of CFP1 is accomplished by donating six base-specific hydrogen bonds by an inbuilt I199-R200-Q201 tripeptide. Recognition leads to recruitment and assembly of the chromatin-modifying complexes (1). Further, some proteins can recognize posttranscriptional modifications of nucleic acids as signposts for assembly of complexes or cues for discrimination between self and foreign nucleic acids. For example, the retinoic acid-inducible gene I (RIG-I) protein can specifically recognize 5' triphosphate caps of RNAs and recruit adaptor protein mitochondrial antiviral signaling protein (MAVS) (2–11). MAVS recruits tank binding kinase 1 (TBK1), which phosphorylates interferon regulatory factor 3 (IRF3), resulting in production of type I interferons (IFNs). Similarly, Toll-like receptor 9 (TLR9) specifically binds unmethylated CpG DNA, which is found in microbes, and initiates production of proinflammatory cytokines (12, 13).

In contrast to sequence-specific binding, some tasks require proteins to bind DNA in a non-sequence-specific manner. De-

tails on one such group of closely related proteins that bind DNA nonspecifically have started to emerge (14–23). Based on *in vivo* studies, a role for these proteins in the activation and regulation of cellular signaling, culminating in the production of IFNs and cytokines, upon recognition of foreign DNA has been postulated. These proteins bind DNA using a 200-amino-acid domain referred to as the HIN domain (13, 24). HIN is an acronym referring to the protein's hematopoietic expression, interferon-inducible nature, and nuclear localization. In addition to the HIN domain, most of these proteins contain a pyrin domain (PYD) and have been grouped in the PYHIN family. They are also referred to as absent in melanoma 2-like receptors (ALRs). To date, four homologs from humans (absent in melanoma 2 [AIM2], gamma interferon-inducible protein Ifi-16 [IFI16], myeloid cell nuclear differentiation antigen [MND1], and interferon-inducible protein X [IFI16]) and 13 homologs from mice belonging to the PYHIN family have been identified (25, 26). Recent studies on ALRs have expanded our knowledge about the function of these receptors and refined our understanding of the nature of ALRs. For example, although all ALRs are inducible by IFNs, their expression is not limited to hematopoietic cells. In addition to peripheral blood leukocytes, AIM2 has been detected in keratinocytes, where it is known to assemble a functional inflammasome upon sensing DNA (27). Further, although ALRs have been detected in the nucleus, many of them (for example, AIM2, p202, and IFI16) have been detected in the cytoplasm as well (26). More recently, ALRs were shown to translocate from the nucleus to the cytoplasm in association with either the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) or stimulator of interferon genes (STING)

Published ahead of print 28 October 2013

Address correspondence to Zhi-Jie Liu, zjliu@ibp.ac.cn.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/MCB.00857-13

(26). Consequently, ALRs were shown to specifically activate either the ASC-dependent inflammasome or the STING-TBK1-IRF3 pathway (26). Overexpression studies seem to indicate a cytoplasmic localization for the HIN proteins. Details of cellular expression and localization of ALRs have been covered in two excellent previous reviews (14, 28). Therefore, in this review, only recent new insights pertaining to this aspect of ALRs are mentioned, where appropriate.

All known human ALRs and most of the murine ALRs (except for Pyr-A and Pyr-RV1, which contain PYD but no HIN domain) carry out their functions by binding DNA via the HIN domain. Recently reported crystal structures of the HIN domains of AIM2, IFI16, and p202 reveal that the 200 amino acids of the HIN domain fold into two tandem oligonucleotide/oligosaccharide binding (OB) folds (29) that are held together firmly via a conserved hydrophobic interface (21, 23). Comparison of the structures reveals that although the overall topology of the HIN domain is highly conserved, the location of the DNA binding interface could change from one protein to another. At least two different modes of DNA binding by the HIN domains have been unveiled by the crystal structures (20, 23). These modes of DNA binding have implications for the function of the protein. This review summarizes our current understanding of the structure and function of the HIN domains in the context of innate immune responses and highlights unexplained structural features that provoke further investigation of these domains to uncover exciting new roles for these domains.

THE TWIN OB FOLD

The overall structure of the HIN domain consists of a tandem pair of OB folds linked via a long linker (Fig. 1A). Two or more short α -helices are embedded in the loop making up the linker. Interestingly, the OB folds are held together primarily by extensive interactions between the two OB folds and not via the linker (Fig. 1B). Most of these interactions are hydrophobic (21, 30). A number of aromatic amino acids—for example, a conserved pair of histidines and a phenylalanine—are engaged in π -cation interactions at the interface of the two OB folds. In addition, in the HINa domains of p202 and IFI16, a π - π stacking interaction between the aromatic rings of two phenylalanines is observed (Fig. 1C). Stretches of interfacing amino acids, such as MFHATVAT, are conserved among the HIN domains (Fig. 1D). Mapping of the sequence on the structures of HIN domains reveals that this stretch of amino acids is completely buried in the protein and is therefore not likely to play any role in intermolecular protein-protein interactions. Analysis of the interface between the OB folds of the HIN domain of AIM2, the HINa domain of p202, and the HIN domains of IFI16 by the PISA web server (30) reveals that the interface is interaction specific, implying that the protein is likely to exhibit these interactions when present in its canonical form. Further, the interactions between the OB folds are unlikely to be disrupted *in vivo*. In fact, bacterial expression of individual OB folds results in insoluble protein (21). Therefore, the tandem OB folds of the HIN domain might have evolved to function as a single unit. This is further illustrated by the fact that the structures of the HIN domains from AIM2, IFI16, and p202 are highly conserved in topology and show an identical topological arrangement of the tandem OB folds interacting via a conserved hydrophobic interface (20, 23) (Fig. 1E).

The canonical OB fold contains five strands that fold into two

sheets (29). Strand β 1 is shared between the two sheets. Loops connecting the five strands show variable length and are known to play important roles in the binding of the ligands. Since the OB fold was described well before the structure of any HIN domain became available, the structural elements of the HIN domain are labeled based on the OB fold convention (29). The proximal OB fold of the HIN domain is named OB1, while the distal OB fold is called OB2 (Fig. 1A). There are some differences between the canonical OB fold and the OB folds of the HIN domain (Fig. 1F) (20, 21). In particular, the canonical β 1, β 4, and β 5 strands of OB1 are usually observed split into two short β' (noncanonical) and β (canonical) strands. Similarly, the canonical β 5 of OB2 is likely to be replaced by two smaller β strands (Fig. 1F). Analysis of the crystal structures of the HIN domains reveals that the β 2 strand of OB1 is consistently the longest strand and that the region connecting strand β 1 with β 2 protrudes out of the protein. Superimposition of the structures of the HIN domains reveals that this region along with loops L_{34} and L_{45} of OB2 show the largest root mean square (RMS) deviations (Fig. 1E). The $C\alpha$ atoms of the HIN domains of IFI16 and the HINa domain of p202 overlap with an RMS deviation of 1 Å along the entire length of the structures (23). On the other hand, the $C\alpha$ atoms of the HIN domain of AIM2 show a slightly larger RMS deviation of 1.4 Å in superimposition studies. Thus, similar to the canonical OB fold, the twin OB folds of the HIN domain also show some variations in the structural elements.

HIN DOMAIN OF AIM2

Two isoforms of AIM2 are known. The only difference between the isoforms is the length. While isoform 1 of AIM2 is made up of 356 amino acids, isoform 2 is shorter by 13 amino acids at the C terminus. These amino acids fold into an N-terminal pyrin domain (PYD; amino acids [aa] 1 to 83) and a C-terminal HIN domain (aa 157 to 354) (Fig. 2A). Direct evidence for the role of AIM2-mediated responses in protection against pathogens has come from studies on AIM2-deficient mice. Mice lacking AIM2 were found to be more susceptible to infections by *Francisella tularensis* (31, 32), *Listeria monocytogenes* (33–35), and murine cytomegalovirus (mCMV) (36–38).

AIM2 senses DNA in the cytoplasm using the HIN domain and relays the message downstream via the PYD. In addition to binding DNA, AIM2 is known to participate in protein-protein interactions to carry out its diverse functions. For example, AIM2 has been shown to form heterodimers with ASC, p202, IFI16, and MNDA (16, 19, 22, 39, 40). Heterodimerization with ASC culminates into activation of procaspase 1, which ultimately leads to apoptosis via pyroptosis (40). In contrast, heterodimerization with p202 terminates the inflammatory response initiated upon sighting of DNA in the cytoplasm by AIM2 (22). AIM2 can also heterodimerize with IFI16 and MNDA, which could explain its ability to localize to the nucleus in spite of not having any known nuclear localization signals (39, 41). IFI16 is known to induce production of type I IFNs upon sensing of DNA in the cytoplasm (42). Heterodimerization with AIM2 abrogates this IFN- β production (41). Conversely, knockdown of AIM2 has been shown to increase IFN- β production in the BPH-1 prostate cancer cell line (18), confirming that heterodimerization of AIM2 with IFI16 negatively regulates IFN- β production. Further, increased expression of IFI16 in normal human prostate epithelial cells and

FIG 1 Overall structure of the HIN domain. (A) Cartoon representation of the HIN domain depicting the tandem OB folds connected by a long linker. Secondary structural elements are labeled according to the OB fold convention. (B) Stereo view of the HIN domain, with residues shown as lines. The two OB folds are in blue and magenta. They interact with each other using hydrophobic interactions. (C) Detailed analysis of the inter-OB-fold interactions within a HIN domain reveals the presence of aromatic amino acids engaged in π - π and π -cation interactions at the interface. (D) Sequence alignment of HIN domains with known crystal structures. Crystal structures of the HIN domain of AIM2 (PDB code 3RN2) (20), the HINa domain of IFI16 (PDB code 2OQ0) (21), the HINa domain of p202 (PDB code 4JBM) (23), and the HINb domain of IFI16 (PDB code 3RNU) (20) are known. Conservation scores are marked from 1 (no conservation) to 10 (absolutely conserved), with absolute conservation shown with a red star. (E) Superimposition of the C α atoms of the known crystal structures of the HIN domain. The overall topologies of the HIN domains are remarkably conserved. Deviations are observed at the L₃₄ and L₄₅ loop regions. (F) Comparison of the topology of the OB fold of a HIN domain with that of the canonical OB fold. Secondary structural elements are labeled according to the OB fold convention.

FIG 2 HIN domain of AIM2. (A) Diagrammatic representation of the domain organization of AIM2, p202, and IFI16. (B) Superimposition of murine AIM2 HIN (mAIM2 [blue]; PDB code 4JB1) (23) bound with DNA and human AIM2 HIN (hAIM2 [gray]; PDB code 3RN2) (20) bound with DNA. Although the C α atoms overlap over the entire length of the HIN domain, the DNA bound by mAIM2 is labeled along the vertical axis with respect to the DNA bound by hAIM2. (C) Surface electrostatic potential representations of the HIN domains of hAIM2 and mAIM2 show very similar surface charge distribution. (D) Structure of the hAIM2 HIN domain showing amino acids (sticks) involved in binding DNA. (E) The HIN domain of AIM2 donates hydrogen bonds to the bases of the bound DNA. The top view (left) and side view (right) of base-directed interactions of the HIN domain are shown. Hydrogen bonds are depicted as dashed lines.

human diploid fibroblasts (HDFs) results in senescence-associated cell growth arrest (18). On the other hand, knockdown of IFI16 in WI-38 HDFs delays the onset of senescence (18). Therefore, heterodimerization of AIM2 with IFI16 is likely to delay the onset of senescence. Thus, heterodimerization of AIM2 results in diverse responses depending on the type of the dimerization partner.

DNA-binding interface. Structures of the murine and human HIN domains of AIM2 bound with double-stranded DNA (dsDNA) have been reported (20, 23). Superimposition studies reveal that the overall topologies of the HIN domains from both sources are identical (Fig. 2B). These HIN domains share 58% primary sequence identity and therefore exhibit a very similar mode of DNA binding. Both the HIN domains use the region surrounding the linker connecting the two OB folds for

binding DNA. However, the axis of the DNA spiral bound by the HIN domain of hAIM2 is tilted by 35° compared to that of the DNA bound by the mHIN domain (Fig. 2B and C). A search for amino acids within 4 Å of the DNA revealed that helices α 1 and α 2 of the linker, strand β 1 and loop L₃₄ of OB1, and strands β 1, β 4, and β 5 and loop L₂₃ of OB2 contribute residues for binding DNA (Fig. 2D). Thus, AIM2 uses both the OB folds to bind dsDNA. In addition, similar to many other DNA-binding proteins, helices from the linker region are inserted into the major groove for tethering the DNA.

Nature of HIN-DNA interactions. Detailed analysis of the protein-DNA interactions indicates that they are primarily electrostatic and involve the phosphate groups of the DNA backbone and the side chains of positively charged amino acids of the HIN domain (20, 23). Although most of the interactions of the protein

FIG 3 Model for activation of AIM2-mediated signaling. AIM2 sheds autoinhibition in the presence of dsDNA in the cytoplasm. This step is subject to regulation by p22. Upon binding DNA, the PYD of AIM2 becomes available for binding PYD of ASC, which recruits procaspase 1. Molecular crowding ensures intermolecular proteolytic cleavage, producing mature caspases. p22 possibly intercalates and prevents molecular crowding and maturation of caspases. Catalytically competent caspases process pro-IL-1 β and pro-IL-18 into their mature forms, which ultimately leads to pyroptosis.

with the DNA are limited to the backbone phosphates, for any given monomer of the HIN domain of AIM2, the side chain of at least one lysine residue is observed contacting a base. For example, the side chain of K248 of murine AIM2 donates two hydrogen bonds, 2.8 Å and 2.9 Å long, to the O6 oxygen atoms of a pair of guanine residues (23) (Fig. 2E).

The role of the residues involved in binding DNA has been confirmed by site-directed mutagenesis. Using the crystal structure of the binary complex of the HIN domain of AIM2 with DNA as a guide, amino acids involved in binding DNA were identified (20). When mutated in clusters, these amino acids lost their ability to bind DNA. Such mutations were introduced into full-length AIM2, and the effect of the loss of DNA binding ability of AIM2 was evaluated in cell-based assays. HEK293T cells were transfected with AIM2 or its mutants or with ASC, procaspase-1, and pro-interleukin 1 β (pro-IL-1 β) expression constructs. Maturation of pro-IL-1 β was assessed by Western blotting using anti-IL-1 β antibody. Mutants deficient in DNA binding were impaired in maturation of IL-1 β (20). This not only validated the need for AIM2 to bind DNA for activation of signaling but also confirmed the role of amino acids implicated in binding DNA.

Activation of AIM2 signaling. At least 80 bp of dsDNA has been shown to be necessary for induction of IL-1 β production (20). In the absence of DNA, AIM2 is kept autoinhibited by intramolecular PYD-HIN interactions (Fig. 3). In the presence of DNA, this interaction is broken, making the PYD available for the recruitment and activation of downstream signaling molecules. The structure of PYD of AIM2 and the subsequent maltose binding protein (MBP) pulldown and yeast two-hybrid assays of mutants constructed based on analysis of the structure support such a model for activation of AIM2 (43). The structure further reveals that the PYD of AIM2 folds into a typical death domain. The region surrounding the negatively charged helix α 2 interacts with

the HIN domain and overlaps with the HIN domain's DNA-binding surface. This causes repression of AIM2 activity, which is released in the presence of DNA (43).

Receptors undergo oligomerization after binding their cognate ligands to activate downstream signaling. Unlike most known receptors that contain dimerization/oligomerization domains, AIM2 uses DNA as a platform for oligomerization, resulting in the assembly of the inflammasome (20). Upon binding DNA, the PYD-HIN interaction is broken. AIM2 recruits the adaptor protein ASC via homotypic PYD-PYD interactions (Fig. 3). ASC in turn recruits procaspase-1 through CARD-CARD interactions. Multiple molecules of AIM2 bound to DNA ensure molecular crowding, which brings the caspases into proximity for intermolecular proteolytic activation. Activated caspases cleave and mature IL-1 β and IL-18 (Fig. 3) (16, 19, 22, 38, 40).

Human AIM2 has been shown to respond to sightings of DNA via two pathways. It can exert its effect via assembly and activation of the inflammasome or induce the production of type I IFNs via the STING-TBK1-IRF3 axis (44–46). In contrast to hAIM2, mAIM2 responds to detection of dsDNA via formation of the inflammasome. Murine AIM2 is unable to activate the STING-TBK1-IRF3 pathway (26). Thus, responses originating from ALRs could be species specific, and caution needs to be exercised in extrapolating results from animal models to humans.

HIN DOMAINS OF p22

p22 is found exclusively in mice. So far, p22 homologs from humans have not been reported (26). p22 contains two HIN domains, HINa, spanning aa 46 to 243, and HINb, spanning aa 244 to 441 of the 446-amino-acid-long protein (Fig. 2A). At least two isoforms of p22, p22a and p22b, have been identified (47). These isoforms differ in seven amino acids spread across the length of the protein. Interestingly, p22a knockout mice show no

FIG 4 Structure of the HINa domain of p202. (A) Structures of the unliganded (PDB code 4JBK) (23) and liganded (PDB code 4JBM) (23) forms of HINa domain of p202 are known. Superimposition of the C α atoms of the two structures shows that the HINa domain of p202 does not undergo any major conformational changes upon DNA binding. The DNA binding site is preformed. (B) Cartoon representation of the HINa domain of p202 bound with DNA (PDB code 4JBM) (23). The secondary structural elements are labeled according to the canonical OB fold convention. DNA is shown as a blue ribbon. (C) Similar to the HIN domain of AIM2, the HINa domain of p202 also donates hydrogen bonds to the bases of the bound DNA. K53 is observed donating two hydrogen bonds (blue dashes) to the bases. (D) R224 donates hydrogen bonds to the base and sugar. Amino acids involved in interactions with DNA are shown as sticks. DNA is shown with a ribbon representation.

phenotypic defects, because p202b has been shown to be upregulated in these mice and seems to compensate for a lack of p202a (47). A pseudogene encoding a C-terminally truncated form of p202a, p202c, has also been reported. p202 can translocate to the nucleus and has been detected in the nuclear fraction. p202 has been studied extensively for its ability to interact with proteins involved in transcription and cell multiplication. For example, p202 interacts with p53 binding protein 1 (p53BP1) and prevents transcription of p53-driven expression of proteins like mouse double minute 2 (mdm2) and p21 (48). p202 is also known to associate with nuclear factor of kappa light polypeptide gene (NF- κ B; both the p50 and p65 subunits) and activator protein 1 (AP1; c-Fos and c-Jun subunits) and repress transcription (49). Thus, p202 plays an important role in regulation of cellular response to growth factors and cytokines. Incidentally, the first p202-interacting protein identified was the retinoblastoma gene product (pRB), which is essential for progression of the cell cycle from G₁ to the S phase (50). p202 is known to further influence the cell cycle via its interaction with nuclear factors like E2F-1/DP1 and E2F-4/DP4 and repress proliferation (28, 51). More recently, p202 was shown

to participate in regulation of innate immune responses originating from AIM2. This role of p202 was ascribed to its ability to bind DNA via the HIN domains (22).

DNA-binding interface of the HINa domain. The structures of unliganded and DNA-bound p202 have been reported (23). These structures of p202 reveal that the protein does not undergo large conformational changes upon binding of DNA (Fig. 4A). The DNA binding site is preformed. Amino acids from the N-terminal loop and loop L₁₂ from OB1 are within 4 Å of the dsDNA. The concave depression of the β sheet formed by strands β 1 to β 5 of OB2 anchors the DNA backbone. Specifically, amino acids from strand β 1, loop L₁₂, β 2, β 3, β 4, L₄₅, and β 5 of the OB2 fold are within 4 Å of the DNA (Fig. 4B). Thus, the OB2 fold of p202 contributes the majority of interactions for binding DNA (23).

Nature of HIN-DNA interactions. The interactions of the HINa domain of p202 with DNA are electrostatic and are mostly limited to the DNA backbone. The only exceptions are the interactions of the side chains of K53 and R224 with the bases. The side chain of K53 donates a 2.64-Å hydrogen bond to the O-2 oxygen atom of a cytosine and a 3.36-Å hydrogen bond to the N3 nitrogen

atom of an adjacent adenine (Fig. 4C). R224 donates 2 hydrogen bonds; a 2.78-Å hydrogen bond to the C-8 carbon atom of a guanine and a 2.68-Å hydrogen bond to the C-2 carbon of the sugar ring (Fig. 4D). The length and number of such base-directed interactions vary between the subunits of the HIN domain seen in the asymmetric unit, suggesting some flexibility in binding to DNA (23). Similar interactions with bases are observed when the HIN domain of AIM2 binds DNA.

The DNA-binding interface observed in the crystal structure of p202 HINa bound with DNA has been verified using site directed mutagenesis. Amino acids observed at the interface were mutated in clusters. Such mutants exhibited greatly diminished DNA binding (23). However, the effects of such mutations in full-length p202 protein *in vivo* on the physiology of the cell are unknown.

Regulation of AIM2 signaling by p202. In 2009, Roberts et al. showed that p202 from cytosolic extracts of bone marrow-derived macrophages (BMDM) bound a 44-bp dsDNA probe (22). Abrogation of this binding, for example by knockdown of p202 in BALB/c BMMs using small interfering RNA (siRNA), significantly enhanced the activation of caspases 3 and 1 upon stimulation by DNA. Conversely, under similar conditions, knockdown of AIM2 by siRNA prevented the activation of caspases 3 and 1. Thus, AIM2 seemed to activate while p202 seemed to downregulate the maturation of the caspases when BMMs were stimulated by dsDNA. Although p202 is known to heterodimerize with AIM2, the molecular basis for the role of p202 in regulation of AIM2-mediated activation of the inflammasomes was not clear until recently. The ability of p202 to mitigate AIM2-initiated responses was explained in part by the crystal structures of the HIN domain of murine AIM2 and the HINa domain of p202 bound with DNA (23, 52). Comparison of the structures revealed that although the overall topologies of the structures are similar, the location of the DNA binding site is different (Fig. 5A). Comparison of the molecular details of the mode of DNA binding by the two proteins explains the mechanism by which p202 performs its function.

Structural basis for the functional antagonism of AIM2 by p202. The structure of human AIM2's HIN domain bound with DNA provided exclusive insights into the DNA-based oligomerization and activation of signaling model for AIM2 (20). Further support for this model came in the form of the structure of murine AIM2 bound with DNA (23). The structural similarities suggested that the overall structure and function of AIM2 are conserved across species. More importantly, the structure of the HIN domain of murine AIM2 bound with DNA together with the structure of murine p202a's HINa domain bound with DNA permitted a direct comparison of the modes of DNA binding by these two proteins to explain the molecular basis for AIM2's ability to activate and p202's subsequent ability to terminate AIM2-mediated signaling. Quite unexpectedly, the structure of p202s's HINa domain bound with DNA revealed a different location for the DNA-binding site compared to that observed for the HIN domain of AIM2 (23). Superimposition of the two structures revealed that the overall topologies of the HIN domains from AIM2 and p202 are similar (Fig. 5A). While AIM2's HIN domain uses the region surrounding the linker connecting the two OB folds for binding DNA, p202a's HINa domain uses a surface opposite that of AIM2's HIN domain for binding DNA. p202a's HIN domain binds DNA primarily using loops connecting the strands of the β -sheet. Such a mode of DNA binding is commonly observed for proteins employing the canonical OB fold for binding DNA. The

FIG 5 Comparison of modes of DNA binding. (A) Superimposition of Co atoms of the HIN domain of murine AIM2 (red ribbon [DNA is in blue]; PDB code 4JBJ) (23) and the HINa domain of p202 (gray ribbon [DNA is in green]; PDB code 4JBM) (23) reveals two distinct modes of DNA binding by the HIN domains. The overall topologies of the HIN domains are remarkably similar. (B) Comparison of the surface electrostatic potential representation of HINa domain of p202 bound with DNA (left) with that of the model of HINb domain of p202 (right) overlaid with the DNA from HINa domain of p202. (C) Side view of the surface electrostatic potentials clearly shows a difference in the distribution of the surface charge between the HIN domains of p202.

use of loops permits p202a greater access for binding different regions of DNA. As a result, p202a's HINa domain makes more contact with DNA than AIM2's HIN domain. This raises the possibility of p202a sequestering DNA from AIM2 (23) (Fig. 3). Although the ability of p202a to outcompete AIM2 for DNA binding has not been demonstrated yet, the HINa domain of p202a can bind more DNA than the HIN domain of AIM2 (23).

By using the currently available structure-function data, a model for activation of AIM2 signaling and its termination by p202 has been proposed. According to this model, multiple mol-

ecules of AIM2's HIN domain bound in proximity to DNA demonstrate the ability of AIM2 to oligomerize using DNA as a platform. When such AIM2 bound to DNA recruits ASC and caspases, it causes crowding of the molecules (Fig. 3). Molecular crowding brings caspases close enough to execute intermolecular proteolytic cleavages, resulting in their activation (53). p202 possibly intercalates and physically obstructs the caspases from coming closer for intermolecular proteolytic cleavages (Fig. 3). Alternatively, p202a's HINa domain could bind DNA, while the HINb domain heterodimerizes with AIM2 and prevents recruitment of ASC. Lastly, p202a could simply sequester DNA from AIM2 by using a combination of different features, such as higher affinity for DNA and heterodimerization with AIM2. The structure of a quaternary complex of p202, AIM2, DNA, and other effector molecules of the inflammasome is essential for a precise understanding of the assembly of the inflammasome and its regulation by p202a.

More recently, p202 was shown to abrogate AIM2-mediated activation of procaspase 1 by heterodimerization with AIM2 (52). p202 was shown to form tetramers *in vivo* and heterodimerize with AIM2. This interaction specifically turned off the activation of procaspase 1 without affecting the ability of the cell to produce IFN- β . p202 intercalates and spatially separates AIM2 molecules, which prevents clustering of ASC. This physically obstructs intermolecular proteolytic processing of procaspase 1 (52) and terminates the AIM2-mediated responses.

Regulation of AIM2-mediated signaling by IFI16. Expression of ALRs is induced upon activation of IFN signaling and sighting of DNA (26). Specifically, type I (IFN- α and - β) and type II (IFN- γ) IFNs have been shown to upregulate the expression of AIM2 and IFI16 in peripheral blood monocytes (CD14⁺) and the THP-1 monocytic cell line (41). Upon upregulation by type I IFNs, IFI16 heterodimerizes with AIM2 in the cytoplasm and curtails activation of procaspase 1. This results in mitigation of inflammatory responses' origination from the AIM2 inflammasomes. Knockdown of IFI16 results in an increase in activation of procaspase 1, further supporting a role for IFI16 in negatively regulating expression of inflammatory cytokines. Such a downregulation forms the basis for the administration of type I IFNs during therapeutic interventions in autoimmune diseases to lower production of inflammatory cytokines (54). Thus, the innate immune responses originating from the AIM2 inflammasomes are negatively regulated at the posttranscription level by at least two different mechanisms. The first, which was elegantly demonstrated using BMDMs, showed expression of the HIN-only protein, p202, abrogating AIM2 responses (22). The second mechanism involves heterodimerization of IFI16 with AIM2 upon induction of ALRs by IFNs (41). In both these mechanisms, proteolytic activation of procaspase 1 is stalled, which affects the production of mature IL-1 β and IL-18.

Role of the HINb domain of p202. While the structure of the HINa domain of p202 in complex with DNA and the subsequent loss-of-function mutations clearly demonstrate the ability of p202 to use a DNA binding site located at a position opposite that observed for the HIN domain of AIM2, the role of the HINb domain of p202 in binding DNA is not clear. Insights obtained from homology modeling reveal that unlike other HIN domains that have a predominantly positively charged surface, the HINb domain of p202a has a large negatively charged patch on the surface (23) (Fig. 5B and C). Such a structural feature suggests a role for the HINb

domain in protein-protein interactions. In this context, HINb could either mimic PYD of AIM2 in imposing autoinhibition on p202 via intramolecular ionic interactions between its negatively charged surface and the positive potential of the HINa domain or play a role in the heterodimerization of p202a with AIM2 via intermolecular interactions with the HIN domain of AIM2 (23, 52). Further studies on mapping of domain interactions between p202a and AIM2 are likely to shed light on this possible role for the HINb domain of p202a in the function of the protein. In fact, a recent study showed that the HINb domain of p202 forms tetramers inside the cell and interacts with AIM2. Oligomerization of the HINb domain of p202 has been speculated to increase the avidity of p202 for DNA (52).

HIN DOMAINS OF IFI16

IFI16 contains two HIN domains, HINa (aa 192 to 393) and HINb (aa 515 to 710) (Fig. 2A). Both the HIN domains are known to bind DNA (55). However, they differ in their DNA-binding abilities. HINb was shown to have greater affinity for double-stranded vaccinia viral (VACV) 70-mer and double-stranded herpes simplex virus (HSV) 60-mer DNA than the HINa domain (42). However, constructs comprising both the HIN domains were shown to bind DNA with greater affinity than the individual HIN domains (42). Double-stranded DNA greater than 70 bp in length was required for optimal induction of the IFN- β promoter by IFI16 (42). In addition to the HIN domains, IFI16 also contains an N-terminal PYD (aa 6 to 84). The role of the PYD in the function of the protein is not clearly understood. IFI16 has been shown to interact with a number of transcription factors. In particular, its role in regulation of the activity of the tumor suppressor protein p53 has been investigated in detail (21). The HINa domain of IFI16 interacts with the C-terminal domain of p53 and enhances its sequence-specific binding to the p53 binding consensus DNA sequence (21). In addition, IFI16 has been shown to bind the promoter region of oncogenes like *c-MYC* and *RAS* and repress their transcription (56, 57). IFI16 has also been shown to negatively regulate the expression of human telomerase reverse transcriptase (hTERT), which is partly attributed to the downregulation of *c-MYC* (58). On the other hand, downregulation of Ikb α expression by IFI16 results in enhanced production of inflammatory cytokines via the NF- κ B pathway (59). Interestingly, IFI16 has been shown to be part of the BRCA1-associated genome surveillance complex (BASC), which is involved in repair of damaged DNA (60). IFI16 has been shown to heterodimerize with AIM2 (40) and regulate its signaling (28). More recently, IFI16 has been shown to sense pathogenic DNA in the cytoplasm as well as the nucleus (42, 61). Upon sighting DNA in the cytoplasm using the HIN domains, IFI16 activates the STING-TBK1-IRF3 signaling pathway, culminating in the production of IFNs (42). In contrast, detection of viral DNA in the nucleus results in the assembly of an AIM2-independent inflammasome, which activates procaspase 1 (61).

DNA-binding interface of HINb domain of IFI16. The crystal structures of the unliganded HINa and HINb domains of IFI16 were the first representative crystal structures for HIN domains to be reported and helped define the overall architecture of the HIN domain (21). These initial structures of the HIN domains highlighted the fact that despite low sequence identity, the HIN domains fold into highly similar topologies. The structure of the HINb domain of IFI16 bound with DNA subsequently shed light

FIG 6 Structure of the HINb domain of IFI16 bound with DNA. (A) Cartoon representation of the structure of HINb domain of IFI16 bound with DNA (PDB code 3RNU) (20). (B) Similar to HIN domain of AIM2 and HINa domain of p202, the HINb domain of IFI16 donates hydrogen bonds to the bases. Hydrogen bonds are shown as dashed blue lines. (C) HIN domains exhibit two distinct modes of DNA binding. A cartoon representation of the HINb domain of IFI16 depicts the AIM2-like mode of DNA binding in which the linker region participates in binding DNA. (D) HINa domain of p202 exhibits a mode of binding distinctly different than that of AIM2. In the p202-like mode of DNA binding, loops protruding from the β -barrel of the OB fold bind DNA. Amino acids interacting with DNA are shown as sticks.

on the mode of DNA binding (20). The structure revealed that HINb binds DNA using the positive potential of the region surrounding the linker region. This mode of binding was almost similar to that of the HIN domain of AIM2. In particular, HINb uses amino acids from loop L_{34} of OB1, helices $\alpha 1$ and $\alpha 2$ of the linker region, and loop L_{23} and strands $\beta 4$ and $\beta 5$ from OB2 to bind DNA (20) (Fig. 6A).

The mode of DNA binding by the HINa domain of IFI16 is currently not known.

Nature of the protein-DNA interactions. Just as in the case of the HIN domain of AIM2 and the HINa domain of p202, the contacts of the HINb domain of IFI16 with DNA are electrostatic and involve the side chains of positively charged amino acids and the negatively charged phosphate groups of the DNA backbone (20). As observed for the structures of other HIN domains, at least one side chain of a positively charged amino acid donates a hydrogen bond to a base. For example, the NH2 nitrogen atom of R667 donates a 3.05-Å hydrogen bond to the N7 nitrogen of an adenine (Fig. 6B). However, the significance of such hydrogen bonding is not known.

The location of the DNA binding site observed in the crystal structure was verified by mutagenesis (20). Amino acids at the protein-DNA interface were mutated in clusters. Mutants deficient in DNA binding were evaluated for their ability to induce

IFN- β in cell-based assays. STING was essential for the IFI16-based activation of the IFN- β promoter, and therefore IFI16 or its mutants were cotransfected with STING. Mutants deficient in DNA binding were impaired in the induction of the IFN- β promoter. Thus, the DNA binding surface observed in the crystal structure for HINb domain of IFI16 was physiologically relevant (20). Although the structure-function studies provide detailed insights into the mode of DNA binding by the HINb domain of IFI16, the mechanisms of assembly of the inflammasome by IFI16 and the activation of the STING-TBK1-IRF3 pathway by IFI16 remain to be elucidated. Knowledge of the regions of IFI16 interacting with STING or ASC is likely to further shed light on the role of the domains in the function of the protein.

COMPARISON OF THE HIN DOMAINS

Structures of the HIN domains solved in complex with DNA from three different proteins reveals that the overall topologies of the HIN domains from different sources are remarkably similar. This is exemplified by the fact that despite differences in primary sequence, the HIN domains of AIM2, p202, and IFI16 fold into a pair of OB folds that are almost mirror images (20, 21, 23). Similar structural conservation has been observed for proteins catalyzing a diverse range of reactions using the TIM barrel or the Rossmann fold. Functional diversity is accomplished by insertion or elonga-

tion of loops, deletions, mutations, or gain of additional domains. Proteins containing HIN domains seem to use both these *modi operandi*—substitution of residues and gain of additional domains to recruit partners—for accomplishing functional diversity. For example, the primary sequence of HIN domains is different in AIM2 and p202, giving rise to differences in surface charge and resulting in differential binding to DNA. Therefore, although the proteins have similar topologies, they use different structural elements within the protein to bind DNA, resulting in different affinities for the same substrate. Such a difference in chemistry is essential for p202 to outcompete AIM2 for binding to DNA, an ability essential to its role as an AIM2 antagonist during sensing of cytoplasmic DNA (22, 23). In the absence of p202, AIM2 carries out its function by recruiting ASC using the PYD that is not present in p202 (Fig. 3).

The structures of the HIN domains have so far revealed two distinct modes of DNA binding. In the AIM2-like mode of DNA binding, the HIN domain uses the area surrounding the linker region primarily to bind DNA (Fig. 6C). In contrast, in the p202-like mode of DNA binding, the HIN domain tethers DNA using the region opposite to the linker for binding DNA (Fig. 6D). Here, loops protruding from the canonical OB fold play an important role in binding DNA. The HINb domain of IFI16 exhibits the AIM2-like mode of DNA binding (20, 23). It would be interesting to find out if HIN domains are limited to the choice of one of these two distinct modes of DNA binding or whether additional modes of DNA binding exist for HIN domains. Elucidations of the crystal structures of the HIN domains of other homologs belonging to the PYHIN family are likely to provide an answer to this question.

The mode of DNA binding by the HINb domain of IFI16 is similar to that of the HIN domain of AIM2 (20). However, IFI16 has an additional HIN domain which is lacking in AIM2. Thus, IFI16 uses the gain of a HIN domain to exert differential roles. In addition, the nature of PYD of IFI16 is different than that of the PYD of AIM2 (42). This suggests that IFI16 may not be autoinhibited in a manner similar to that seen for AIM2. Further, the mechanism of activation of IFI16 could be different than that of AIM2. Questions about how ASC is recruited or STING is activated by IFI16 remain unanswered. More recently, an enzyme called cGMP-cAMP (cyclic AMP) synthase (cGAS) was shown to bind cytoplasmic DNA and catalyze the production of cGMP-cAMP using ATP and GTP (62, 63). This endogenous secondary messenger, 2',3'-cGAMP, produced by mammalian cells, was shown to be structurally different than the cyclic nucleotides synthesized by microbes (64–68). 2',3'-cGAMP bound STING with an affinity that was ~300-fold higher than that observed for c-di-GMP and elicited a potent IFN- β response (68). Thus, cGAS senses cytoplasmic DNA and initiates type I IFN production by synthesizing 2',3'-cGAMP that activates STING. IFI16 is also known to initiate type I IFN production via the STING-TBK1-IRF3 pathway. However, it is currently not known how STING is activated upon sensing of DNA by IFI16.

The structures of the HIN domain of AIM2, the HINb domain of IFI16, and the HINa domain of p202 bound with DNA bring out the similarities between the HIN domains and highlight some differences between individual HIN domains that possibly confer distinct roles on the HIN domains in the function of the protein. For example, the HIN domain of AIM2 and the HINa domain of p202 are structurally mirror images (23). However, differences in

primary sequence confer distinct modes of DNA binding on these HIN domains. Differences in the mode of DNA binding result in different affinities for the DNA. As a consequence, upon DNA binding, AIM2 can initiate while p202 can terminate activation of inflammatory responses (22, 23). Such similarities or differences in HIN domains could be exploited to specifically modulate the downstream signaling to either mitigate an inflammatory response (for example, in autoimmune diseases) or activate a response to fight pathogens.

HIN PROTEINS AND IMMUNE DISORDERS

Several studies have implied a role for HIN proteins in the development of autoinflammatory and autoimmune disorders. Studies using New Zealand Black mice (NZM), which are genetically predisposed to development of lupus-like disease, identified the gene encoding p202 as a lupus susceptibility gene (69). Increased expression of p202a has been correlated to the development of antinuclear antibodies (70). This is because increased levels of p202 are likely to prolong the survival of cells harboring cytoplasmic DNA, leading to a chronic activation of the innate immune response that could result in the development of systemic lupus erythematosus (SLE). While type I IFNs stimulate expression of p202a, AIM2 negatively regulates type I IFN signaling (36–38). Consequently, AIM2-deficient mice show increased levels of IFN- β and p202 protein and are prone to SLE.

Physiological disturbances arising from the inflammasomes are mostly tied to the excessive activation of procaspase 1 and its subsequent processing of IL-1 β and IL-18. Deregulation of AIM2-mediated signaling perturbs cellular homeostasis, and one of the most common fallouts of the dysregulation of the AIM2 inflammasome is inflammation. This is exemplified by the formation of inflammatory skin lesions in psoriatic patients, which has been tied to increased inflammasome activity that results in excessive production of cleaved IL-1 β (27). Knockdown of AIM2 expression in keratinocytes using siRNA completely blocked the production of mature IL-1 β in response to transfection with poly(dA · dT), confirming a role for AIM2 in psoriasis. The physiological trigger for the inflammation was identified as endogenous cytosolic DNA (27). However, the question of how self DNA enters the cytosol of keratinocytes of psoriatic patients remains unanswered. Further evidence for the role of self DNA in the etiology of the disease was provided by cotransfecting DNA with a cationic peptide called cathelicidin LL-37. Such an application mitigated the inflammatory response by binding DNA and interfering with the assembly of the inflammasome (27). Taken together, these results indicate a role for the AIM2 inflammasome in the etiology of chronic inflammatory psoriasis. In addition to its role in psoriasis, elevated levels of cleaved IL-1 β have been implicated in the pathogenesis of other autoinflammatory diseases like pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) (71), cryopyrin-associated periodic syndromes (CAPS) (72), familial Mediterranean fever (FMF) (73), gout, and type 2 diabetes mellitus (74, 75). Although the source of unabated IL-1 β in these disorders is a malfunctioning NOD-like receptor (NLRP) inflammasome, excessive IL-1 β originating from AIM2 inflammasomes is also likely to induce onset of autoinflammatory diseases with similar manifestations.

In addition to p202 and AIM2, IFI16 has also been proposed to play a role in chronic inflammation. Autoantibodies against IFI16 have been detected in the serum of patients suffering from systemic lupus erythematosus, systemic sclerosis, Sjogren's syn-

drome, rheumatoid arthritis, and scleroderma/polymyositis overlap syndrome (76). Further studies are expected to uncover the exact role of IFI16 in the pathogenesis of autoimmune diseases.

UNEXPLAINED STRUCTURAL INSIGHTS

Although short oligonucleotides of DNA were used for crystallization, the HIN domains of AIM2, p202, and IFI16 seem to have lined up the DNA end to end, giving the appearance of a long contiguous stretch of DNA. Such an ability of a protein has been reported previously for Ku proteins and has been shown to be essential for the nonhomologous end joining (NHEJ) repair mechanism of fixing double-stranded breaks of DNA resulting from damage caused by ionizing radiation or during V(D)J recombination in lymphocytes. More specifically, Ku proteins bind double-stranded breaks and bridge DNA ends such that the ends are accessible to DNA ligase for sealing the breaks (77). In contrast, the DNA ends seem to be inaccessible in the HIN-DNA complexes, suggesting that the purpose of bridging DNA fragments here could be different. All the structures of the binary complexes of the HIN domains with DNA solved so far reveal this ability of HIN domains to line up DNA end to end. Such an ability of the HIN domain of AIM2 probably increases the local concentration of the protein and ensures molecular crowding that is essential for activation of downstream signaling (20). Alternatively, it suggests a role for the protein in DNA repair. However, AIM2 has been shown to sense dsDNA in the cytosol only and to assemble the inflammasome. For participation in DNA repair, AIM2 would need to be able to sense DNA in the nucleus, an ability that has not been demonstrated for AIM2. However, AIM2 has been shown to localize to the nucleus when untreated or IFN- γ -treated 293 human cells were transfected with pCDNA3.1-AIM2-V5 vector transiently expressing AIM2 (39). Surprisingly, murine AIM2 localizes to the cytoplasm when expressed alone, highlighting species-specific differences in the ALRs (26). In contrast, IFI16 and p202 have been detected in the nucleus and the cytoplasm in humans and mice (39, 78). Specifically, IFI16 was shown to associate with BRCA1 in the nucleus when HCC1937 breast cancer cells were irradiated with ionization radiation (60). The PYD of IFI16 associated with the region from aa 502 to 802 of BRCA1 and IFI16 was shown to be part of the BRCA1-associated genome surveillance complex (BASC) involved in repairing damaged DNA. These results coupled with the structural observations open up the possibility that IFI16 participates in DNA repair. The recently reported participation of DNA-dependent protein kinase (DNA-PK) and Ku70, proteins primarily implicated in repair of damaged DNA, in the induction of IFN- λ 1 production via the IRF1/IRF7 pathway upon transfection of DNA in HEK293 cells, fibroblasts, murine macrophages, and dendritic cells (DCs) (79, 80) further suggests the need to investigate a role for the HIN proteins in DNA damage repair. It is tempting to speculate that the hydrogen bonds donated by lysines of the HIN domain to bases are part of a repair process initiated for fixing DNA damaged by ionization radiation. If we consider the example of DNA damage via the addition of an electron to pyrimidine, protons would transfer to these electron adducts possibly from a lysine by a proton shuttle via a guanine. The holes generated by radiation would localize on guanine and would result in loss of a proton from N1 to the hydrogen-bonded cytosine. The H bond donated by

lysine to O6 or N2 of guanine observed in the structures of p202 HINa or AIM2 HIN bound with DNA, respectively, would actually promote this deprotonation and would tend to localize the hole to this guanine, slowing down any further transfer of the hole. Since N1 protonation is necessary for the reaction of one electron-oxidized guanine with water to form 8-hydroxy guanine, this may indeed slow the rate of this damage. Therefore, the lysine could potentially stabilize the damage for repair by electron donors such as a serine of the HIN domain, glutathione, or other sulfhydryls. Thus, the base-directed hydrogen bonds of the HIN domain could potentially perturb the prototropic equilibria that play an important role in determining the lifetime of the radical and the subsequent reaction of the radical. Examination of the structures of HIN domains (IFI16 and p202) bound with DNA reveals that at least one serine residue is hydrogen bonded to the backbone phosphates (20, 23). In addition, a number of lysines donating hydrogen bonds to the DNA backbone and bases are observed. A decrease in pK_a of the lysines involved in binding DNA, as indicated by a PropKa analysis (<http://propka.ki.ku.dk>), suggests that these lysines are capable of donating protons under physiological conditions. Therefore, it would be interesting to find out whether the exchange of charges between the HIN domains and DNA plays a role in binding/unbinding of protein from DNA or is part of a repair mechanism for damaged DNA.

Lastly, some flexibility in binding of DNA to the HIN domains has been observed. The HIN domains can tilt and perhaps slide on the DNA, using the electrostatic interactions between the charged residues and the DNA (20). Such flexibility in binding DNA is likely to assist the HINa domain of IFI16 in enhancing the sequence-specific binding of the C-terminal domain of the transcription factor p53 (21). Taken together, the ability of the HIN domains to bind short stretches of DNA and align them end to end to form a long contiguous stretch of dsDNA and the base-directed hydrogen bonds of the side chain of a lysine residue unveiled by the crystal structures hint at additional exciting roles and mechanisms of function of HIN domains, which await discovery.

CONCLUSIONS

The recently available structures of the HIN domain from three different proteins have helped define the twin OB fold and have shed light on the modes of DNA binding by these domains. In spite of low sequence identity, the overall topologies of the HIN domain from different proteins are remarkably similar. So far, two distinctly different modes of DNA binding have been described for the HIN domains. These distinct modes of DNA binding seem to confer different roles on the protein in the regulation of innate immune responses. Additional structures of HIN domains from other proteins are likely to answer the question of whether HIN domains are limited to the choice of these two modes of DNA binding and whether these differences in modes of DNA binding confer different roles on the proteins. In addition, unexplained structural insights should provoke further research on HIN domains that could unveil new exciting roles for these proteins.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant 31330019) and the Ministry of Science and Technology of China (grant 2014CB910400).

We have no financial conflicts of interest.

REFERENCES

- Xu C, Bian C, Lam R, Dong A, Min J. 2011. The structural basis for selective binding of non-methylated CpG islands by the CFP1 CXXC domain. *Nat. Commun.* 2:227–230. <http://dx.doi.org/10.1038/ncomms1237>.
- Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. 2009. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat. Immunol.* 10:1065–1072. <http://dx.doi.org/10.1038/ni.1779>.
- Chiu YH, Macmillan JB, Chen ZJ. 2009. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* 138:576–591. <http://dx.doi.org/10.1016/j.cell.2009.06.015>.
- Cui S, Eisenacher K, Kirchhofer A, Brzozka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP. 2008. The C-terminal regulatory domain is the RNA 5'-triphosphate sensor of RIG-I. *Mol. Cell* 29:169–179. <http://dx.doi.org/10.1016/j.molcel.2007.10.032>.
- Jiang F, Ramanathan A, Miller MT, Tang GQ, Gale M, Jr, Patel SS, Marcotrigiano J. 2011. Structural basis of RNA recognition and activation by innate immune receptor RIG-I. *Nature* 479:423–427. <http://dx.doi.org/10.1038/nature10537>.
- Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, Tsujimura T, Takeda K, Fujita T, Takeuchi O, Akira S. 2005. Cell type-specific involvement of RIG-I in antiviral response. *Immunity* 23:19–28. <http://dx.doi.org/10.1016/j.immuni.2005.04.010>.
- Kowalinski E, Lunardi T, McCarthy AA, Loubet J, Brunel J, Grigoriev B, Gerlier D, Cusack S. 2011. Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. *Cell* 147:423–435. <http://dx.doi.org/10.1016/j.cell.2011.09.039>.
- Lu C, Xu H, Ranjith-Kumar CT, Brooks MT, Hou TY, Hu F, Herr AB, Strong RK, Kao CC, Li P. 2010. The structural basis of 5' triphosphate double-stranded RNA recognition by RIG-I C-terminal domain. *Structure* 18:1032–1043. <http://dx.doi.org/10.1016/j.str.2010.05.007>.
- Luo D, Ding SC, Vela A, Kohlway A, Lindenbach BD, Pyle AM. 2011. Structural insights into RNA recognition by RIG-I. *Cell* 147:409–422. <http://dx.doi.org/10.1016/j.cell.2011.09.023>.
- Wang Y, Ludwig J, Schuberth C, Goldeck M, Schlee M, Li H, Juranek S, Sheng G, Micura R, Tuschl T, Hartmann G, Patel DJ. 2010. Structural and functional insights into 5'-ppp RNA pattern recognition by the innate immune receptor RIG-I. *Nat. Struct. Mol. Biol.* 17:781–787. <http://dx.doi.org/10.1038/nsmb.1863>.
- Yoneyama M, Fujita T. 2008. Structural mechanism of RNA recognition by the RIG-I-like receptors. *Immunity* 29:178–181. <http://dx.doi.org/10.1016/j.immuni.2008.07.009>.
- Kawai T, Akira S. 2010. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat. Immunol.* 11:373–384. <http://dx.doi.org/10.1038/ni.1863>.
- Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T, Golenbock DT. 2004. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat. Immunol.* 5:190–198. <http://dx.doi.org/10.1038/ni1028>.
- Asefa B, Klarmann KD, Copeland NG, Gilbert DJ, Jenkins NA, Keller JR. 2004. The interferon-inducible p200 family of proteins: a perspective on their roles in cell cycle regulation and differentiation. *Blood Cell. Mol. Dis.* 32:155–167. <http://dx.doi.org/10.1016/j.bcmd.2003.10.002>.
- Barbalat R, Ewald SE, Mouchess ML, Barton GM. 2011. Nucleic acid recognition by the innate immune system. *Annu. Rev. Immunol.* 29:185–214. <http://dx.doi.org/10.1146/annurev-immunol-031210-101340>.
- Burckstummer T, Baumann C, Bluml S, Dixit E, Durnberger G, Jahn H, Planyavsky M, Bilban M, Colinge J, Bennett KL, Superti-Furga G. 2009. An orthogonal proteomic-genomic screen identifies Aim2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol.* 10:266–272. <http://dx.doi.org/10.1038/ni.1702>.
- Choubey D, Li SJ, Datta B, Gutterman JU, Lengyel P. 1996. Inhibition of E2F-mediated transcription by p202. *EMBO J.* 15:5668–5678.
- Duan X, Ponomareva L, Veeranki S, Panchanathan R, Dickerson E, Choubey D. 2011. Differential roles for the interferon-inducible IFI16 and AIM2 innate immune sensors for cytosolic DNA in cellular senescence of human fibroblasts. *Mol. Can. Res.* 9:589–602. <http://dx.doi.org/10.1158/1541-7786.MCR-10-0565>.
- Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA. 2009. Aim2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458:514–518. <http://dx.doi.org/10.1038/nature07725>.
- Jin T, Perry A, Jiang J, Smith P, Curry J, Unterholzner L, Jiang Z, Horvath G, Rathinam V, Johnstone RW, Hornung V, Latz E, Bowie A, Fitzgerald KA, Xiao TS. 2012. Structures of the HIN domain:DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* 36:561–571. <http://dx.doi.org/10.1016/j.immuni.2012.02.014>.
- Liao JC, Lam R, Brazda V, Duan S, Ravichandran M, Ma J, Xiao T, Tempel W, Zuo X, Wang YX, Chirgadze NY, Arrowsmith CH. 2011. Interferon-inducible protein 16: insight into the interaction with tumor suppressor p53. *Structure* 19:418–429. <http://dx.doi.org/10.1016/j.str.2010.12.015>.
- Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, Hardy LL, Garceau V, Sweet MJ, Ross IL, Hume DA, Stacey KJ. 2009. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* 323:1057–1060. <http://dx.doi.org/10.1126/science.1169841>.
- Ru H, Ni X, Zhao L, Crowley C, Ding W, Hung L-W, Shaw N, Cheng G, Liu Z-J. 2013. Structural basis for termination of AIM2-mediated signaling by p202. *Cell Res.* 23:855–858. <http://dx.doi.org/10.1038/cr.2013.52>.
- Albrecht M, Choubey D, Lengauer T. 2005. The HIN domain of IFI-200 proteins consists of two OB folds. *Biochem. Biophys. Res. Commun.* 327:679–687. <http://dx.doi.org/10.1016/j.bbrc.2004.12.056>.
- Atianand MK, Fitzgerald KA. 2013. Molecular basis of DNA recognition in the immune system. *J. Immunol.* 190:1911–1918. <http://dx.doi.org/10.4049/jimmunol.1203162>.
- Brunette RL, Young JM, Whitley DG, Brodsky IE, Malik HS, Stetson DB. 2012. Extensive evolutionary and functional diversity among mammalian Aim2-like receptors. *J. Exp. Med.* 209:1969–1983. <http://dx.doi.org/10.1084/jem.20121960>.
- Dombrowski Y, Peric M, Koglin S, Kammerbauer C, Goss C, Anz D, Simanski M, Glaser R, Harder J, Hornung V, Gallo RL, Ruzicka T, Besch R, Schaubert J. 2011. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci. Transl. Med.* 3:82ra38. <http://dx.doi.org/10.1126/scitranslmed.3002001>.
- Johnstone RW, Trapani JA. 1999. Transcription and growth regulatory functions of the HIN-200 family of proteins. *Mol. Cell. Biol.* 19:5833–5838.
- Theobald DL, Mitton-Fry RM, Wuttke DS. 2003. Nucleic acid recognition by OB-fold proteins. *Annu. Rev. Biophys. Biomol. Struct.* 32:115–133. <http://dx.doi.org/10.1146/annurev.biophys.32.110601.142506>.
- Krissinel E, Henrick K. 2007. Inference of macromolecular assemblies from crystalline state. *J. Mol. Biol.* 372:774–797. <http://dx.doi.org/10.1016/j.jmb.2007.05.022>.
- Henry T, Brotcke A, Weiss DS, Thompson LJ, Monack DM. 2007. Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J. Exp. Med.* 204:987–994. <http://dx.doi.org/10.1084/jem.20062665>.
- Mariathasan S, Weiss DS, Dixit VM, Monack DM. 2005. Innate immunity against *Francisella tularensis* is dependent on the ASC/caspase-1 axis. *J. Exp. Med.* 202:1043–1049. <http://dx.doi.org/10.1084/jem.20050977>.
- Kim S, Bauernfeind F, Ablasser A, Hartmann G, Fitzgerald KA, Latz E, Hornung V. 2010. *Listeria monocytogenes* is sensed by the NLRP3 and AIM2 inflammasome. *Eur. J. Immunol.* 40:1545–1551. <http://dx.doi.org/10.1002/eji.201040425>.
- Sauer J-D, Witte CE, Zemansky J, Hanson B, Lauer P, Portnoy DA. 2010. *Listeria monocytogenes* triggers AIM2-mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host Microbe* 7:412–419. <http://dx.doi.org/10.1016/j.chom.2010.04.004>.
- Warren SE, Armstrong A, Hamilton MK, Mao DP, Leaf IA, Miao EA, Aderem A. 2010. Cutting edge: cytosolic bacterial DNA activates the inflammasome via AIM2. *J. Immunol.* 185:818–821. <http://dx.doi.org/10.4049/jimmunol.1000724>.
- Fernandes-Alnemri T, Yu JW, Juliana C, Solorzano L, Kang S, Wu J, Datta P, McCormick M, Huang L, McDermott E, Eisenlohr L, Landel CP, Alnemri ES. 2010. The AIM2 inflammasome is critical for innate immunity to *Francisella tularensis*. *Nat. Immunol.* 11:385–393. <http://dx.doi.org/10.1038/ni.1859>.
- Jones JW, Kayagaki N, Broz P, Henry T, Newton K, O'Rourke K, Chan S, Dong J, Qu Y, Roose-Girma M, Dixit VM, Monack DM. 2010. Absent in melanoma 2 is required for innate immune recognition of *Francisella tularensis*. *Proc. Natl. Acad. Sci. U. S. A.* 107:9771–9776. <http://dx.doi.org/10.1073/pnas.1003738107>.

38. Rathinam VA, Jiang Z, Waggoner SN, Sharma S, Cole LE, Waggoner L, Vanaja SK, Monks BG, Ganesan S, Latz E, Hornung V, Vogel SN, Szomolanyi-Tsuda E, Fitzgerald KA. 2010. The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11:395–402. <http://dx.doi.org/10.1038/ni.1864>.
39. Cresswell KS, Clarke CJP, Jackson JT, Darcy PK, Trapani JA, Johnstone RW. 2005. Biochemical and growth regulatory activities of the HIN-200 family member and putative tumor suppressor protein AIM2. *Biochem. Biophys. Res. Commun.* 326:417–424. <http://dx.doi.org/10.1016/j.bbrc.2004.11.048>.
40. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. 2009. Aim2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* 458:509–513. <http://dx.doi.org/10.1038/nature07710>.
41. Veeranki S, Duan X, Panchanathan R, Liu H, Choubey D. 2011. IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. *PLoS One* 6:e27040. <http://dx.doi.org/10.1371/journal.pone.0027040>.
42. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S, Sirois CM, Jin T, Latz E, Fitzgerald KA, Paludan SR, Bowie AG. 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* 11:997–1004. <http://dx.doi.org/10.1038/ni.1932>.
43. Jin T, Perry A, Smith PT, Jiang J, Xiao TS. 2013. Structure of the AIM2 pyrin domain provides insights into the mechanisms of AIM2 autoinhibition and inflammasome assembly. *J. Biol. Chem.* 288:13225–13235. <http://dx.doi.org/10.1074/jbc.M113.468033>.
44. Ishikawa H, Barber GN. 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 455:674–678. <http://dx.doi.org/10.1038/nature07317>.
45. Shaw N, Ouyang S, Liu Z-J. 2013. Binding of bacterial secondary messenger molecule c di-GMP is a STING operation. *Protein Cell* 4:117–129. <http://dx.doi.org/10.1007/s12338-012-2071-0>.
46. Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, Lei C, He X, Zhang L, Tien P, Shu HB. 2008. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29:538–550. <http://dx.doi.org/10.1016/j.immuni.2008.09.003>.
47. Wang H, Chatterjee G, Meyer JJ, Liu CJ, Manjunath NA, Bray-Ward P, Lengyel P. 1999. Characteristics of three homologous 202 genes (*IFI202a*, *IFI202b*, and *IFI202c*) from the murine interferon-activatable gene 200 cluster. *Genomics* 60:281–294. <http://dx.doi.org/10.1006/geno.1999.5923>.
48. Datta B, Li B, Choubey D, Nallur G, Lengyel P. 1996. p202, an interferon-inducible modulator of transcription, inhibits transcriptional activation by the p53 tumor suppressor protein, and a segment from the p53-binding protein 1 that binds to p202 overcomes this inhibition. *J. Biol. Chem.* 271:27544–27555. <http://dx.doi.org/10.1074/jbc.271.44.27544>.
49. Min W, Ghosh S, Lengyel P. 1996. The interferon-inducible p202 protein as a modulator of transcription: inhibition of NF-kappa β , c-FOS, and c-JUN activities. *Mol. Cell. Biol.* 16:359–368.
50. Choubey D, Lengyel P. 1995. Binding of an interferon-inducible protein (p202) to the retinoblastoma protein. *J. Biol. Chem.* 270:6134–6140. <http://dx.doi.org/10.1074/jbc.270.11.6134>.
51. Choubey D, Gutterman J. 1997. Inhibition of E2F-4/DP-1-stimulated transcription by p202. *Oncogene* 15:291–301. <http://dx.doi.org/10.1038/sj.onc.1201184>.
52. Yin Q, Sester D, Tian Y, Hsiao Y-S, Lu A, Cridland J, Sagulenko V, Thygesen S, Choubey D, Hornung V, Walz T, Stacey K, Wu H. 2013. Molecular mechanism for p202-mediated specific inhibition of AIM2 inflammasome activation. *Cell Rep.* 4:327–339. <http://dx.doi.org/10.1016/j.celrep.2013.06.024>.
53. Muruve DA, Petrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, Parks RJ, Tschopp J. 2008. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452:103–107. <http://dx.doi.org/10.1038/nature06664>.
54. Billiau A. 2006. Anti-inflammatory properties of type I interferons. *Antiviral Res.* 71:108–116. <http://dx.doi.org/10.1016/j.antiviral.2006.03.006>.
55. Yan H, Dalal K, Hon BK, Youkharibache P, Lau D, Pio F. 2008. RPA nucleic acid-binding properties of IFI16-HIN200. *Biochim. Biophys. Acta* 1784:1087–1097. <http://dx.doi.org/10.1016/j.bbapap.2008.04.004>.
56. Egistelli L, Chichiarelli S, Gauci E, Eufemi M, Schininà ME, Giorgi A, Lascu I, Turano C, Giartosio A, Cervoni L. 2009. IFI16 and NM23 bind to a common DNA fragment both in the p53 and the cMYC gene promoters. *J. Cell. Biochem.* 106:666–672. <http://dx.doi.org/10.1002/jcb.22053>.
57. Zhang Y, Howell R, Alfonso D, Yu J, Kong L, Wittig J, Liu C-J. 2007. IFI16 inhibits tumorigenicity and cell proliferation of bone and cartilage tumor cells. *Front. Biosci.* 12:4855–4863. <http://dx.doi.org/10.2741/2433>.
58. Song LL, Ponomareva L, Shen H, Duan X, Alimirah F, Choubey D. 2010. Interferon-inducible IFI16, a negative regulator of cell growth, down-regulates expression of human telomerase reverse transcriptase *hTERT* gene. *PLoS One* 5:e8569. <http://dx.doi.org/10.1371/journal.pone.0008569>.
59. Caposio P, Gugliesi F, Zannetti C, Sponza S, Mondini M, Medico E, Hiscott J, Young HA, Gribaudo G, Gariglio M, Landolfo S. 2007. A novel role of the interferon-inducible protein IFI16 as inducer of proinflammatory molecules in endothelial cells. *J. Biol. Chem.* 282:33515–33529. <http://dx.doi.org/10.1074/jbc.M701846200>.
60. Aglipay JA, Lee SW, Okada S, Fujiuchi N, Ohtsuka T, Kwak JC, Wang Y, Johnstone RW, Deng C, Qin J, Ouchi T. 2003. A member of the pyrin family, IFI16, is a novel BRCA1-associated protein involved in the p53-mediated apoptosis pathway. *Oncogene* 22:8931–8938. <http://dx.doi.org/10.1038/sj.onc.1207057>.
61. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, Chandran B. 2011. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi sarcoma-associated herpesvirus infection. *Cell Host Microbe* 9:363–375. <http://dx.doi.org/10.1016/j.chom.2011.04.008>.
62. Sun L, Wu J, Du F, Chen X, Chen ZJ. 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 339:786–791. <http://dx.doi.org/10.1126/science.1232458>.
63. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, Chen ZJ. 2013. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* 339:826–830. <http://dx.doi.org/10.1126/science.1229963>.
64. Ablasser A, Goldeck M, Cavlari T, Deimling T, Witte G, Rohl I, Hopfner K-P, Ludwig J, Hornung V. 2013. C-GAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature* 498:380–384. <http://dx.doi.org/10.1038/nature12306>.
65. Diner E, Burdette D, Wilson S, Monroe K, Kellenberger C, Hyodo M, Hayakawa Y, Hammond M, Vance R. 2013. The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human sting. *Cell Rep.* 3:1355–1361. <http://dx.doi.org/10.1016/j.celrep.2013.05.009>.
66. Gao P, Ascano M, Wu Y, Barchet W, Gaffney B, Zillinger T, Serganov A, Liu Y, Jones R, Hartmann G, Tuschl T, Patel D. 2013. Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell* 153:1094–1107. <http://dx.doi.org/10.1016/j.cell.2013.04.046>.
67. Kranzusch P, Lee A-Y, Berger J, Doudna J. 2013. Structure of human cGAS reveals a conserved family of second-messenger enzymes in innate immunity. *Cell Rep.* 3:1362–1368. <http://dx.doi.org/10.1016/j.celrep.2013.05.008>.
68. Zhang X, Shi H, Wu J, Zhang X, Sun L, Chen C, Chen Z. 2013. Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Mol. Cell* 51:226–235. <http://dx.doi.org/10.1016/j.molcel.2013.05.022>.
69. Rozzo SJ, Allard JD, Choubey D, Vyse TJ, Izui S, Peltz G, Kotzin BL. 2001. Evidence for an interferon-inducible gene, IFI202, in the susceptibility to systemic lupus. *Immunity* 15:435–443. [http://dx.doi.org/10.1016/S1074-7613\(01\)00196-0](http://dx.doi.org/10.1016/S1074-7613(01)00196-0).
70. Choubey D, Kotzin B. 2002. Interferon-inducible p202 in the susceptibility to systemic lupus. *Front. Biosci.* 7:e252–e262. <http://dx.doi.org/10.2741/choubey>.
71. Conforti-Andreoni C, Ricciardi-Castagnoli P, Mortellaro A. 2011. The inflammasomes in health and disease: from genetics to molecular mechanisms of autoinflammation and beyond. *Cell Mol. Immunol.* 8:135–145. <http://dx.doi.org/10.1038/cmi.2010.81>.
72. Hoffman H, Wanderer A. 2010. Inflammasome and IL-1 β -mediated disorders. *Curr. Allergy Asthma Rep.* 10:229–235. <http://dx.doi.org/10.1007/s11882-010-0109-z>.
73. Masters SL, Simon A, Aksentijevich I, Kastner DL. 2009. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. *Annu. Rev. Immunol.* 27:621–668. <http://dx.doi.org/10.1146/annurev.immunol.25.022106.141627>.
74. Dinarello CA. 2011. A clinical perspective of IL-1 β as the gatekeeper of inflammation. *Eur. J. Immunol.* 41:1203–1217. <http://dx.doi.org/10.1002/eji.201141550>.
75. Netea MG, Joosten LAB, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ,

- Tack CJ, van Krieken H, Kim S-H, Stalenhoef AF, van de Loo FA, Verschueren I, Pulawa L, Akira S, Eckel RH, Dinarello CA, van den Berg W, van der Meer JWM. 2006. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat. Med.* 12:650–656. <http://dx.doi.org/10.1038/nm1415>.
76. Mondini M, Vidali M, Airò P, De Andrea M, Riboldi P, Meroni PL, Gariglio M, Landolfo S. 2007. Role of the interferon-inducible gene *IFI16* in the etiopathogenesis of systemic autoimmune disorders. *Ann. N. Y. Acad. Sci.* 1110:47–56. <http://dx.doi.org/10.1196/annals.1423.006>.
77. Walker JR, Corpina RA, Goldberg J. 2001. Structure of the ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412:607–614. <http://dx.doi.org/10.1038/35088000>.
78. Dawson MJ, Trapani JA. 1995. The interferon inducible autoantigen, IFI16: localization to the nucleolus and identification of a DNA-binding domain. *Biochem. Biophys. Res. Commun.* 214:152–162. <http://dx.doi.org/10.1006/bbrc.1995.2269>.
79. Brian JF, Daniel SM, Nicholas EP, Hongwei R, Geoffrey LS. 2012. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *Elife* 1:00047. <http://dx.doi.org/10.7554/eLife.00047>.
80. Zhang X, Brann TW, Zhou M, Yang J, Oguariri RM, Lidie KB, Imamichi H, Huang D-W, Lempicki RA, Baseler MW, Veenstra TD, Young HA, Lane HC, Imamichi T. 2011. Cutting edge: Ku70 is a novel cytosolic DNA sensor that induces type III rather than type I IFN. *J. Immunol.* 186:4541–4545. <http://dx.doi.org/10.4049/jimmunol.1003389>.