Cytosolic DNA sensors regulating type I interferon induction

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Abstract

Type I interferon induction is a critical anti-pathogen response mediated by innate immune stimulation. While it has been appreciated for some time that the presence of pathogen DNA within a cell leads to a type I interferon response, it is only in the past few years that some of the key signalling proteins and DNA sensors that regulate this response have been uncovered. Here we review the nature of these DNA sensors, which includes a new family of pattern recognition receptors termed the 'AIM2-like receptors (ALRs), and consider the implications of their discovery for understanding emerging principles of innate immune DNA sensing. Furthermore, we discuss how their discovery provides a rationale as to why accumulation of self-DNA mediates IFN-dependent autoimmunity.

The type I interferon response to DNA

The innate immune system responds to the presence of pathogens or danger by recognizing pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) respectively, leading to altered gene expression, immune effector mechanisms and ultimately a return to homeostasis. PAMPs and DAMPs are detected by a number of classes of host pattern recognition receptors (PRRs) including the Toll-like receptors (TLRs), RIG-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin receptors (CLRs). The induction of both pro-inflammatory cytokines and type I interferons (IFNs) are essential components of the PRR response. As such, chronic or inappropriate activation of PRRs is linked to many inflammatory and autoimmune diseases, which provides a strong rationale to fully elucidate PAMP and DAMP sensing mechanisms by PRRs.

It is well known that microbial nucleic acids trigger the induction of type I IFNs such as IFN-α and IFN-β, and that this represents a key host defence strategy to limit the replication of invading micro-organisms. Innate immune responses to self-DNA have been linked to autoimmunity. However, in contrast to the wealth of knowledge about how pathogen RNA is detected and discriminated (from host RNA), the cellular mechanisms of innate DNA sensing are only just emerging.

The seminal discovery that mice lacking TLR9 could not elicit an immune response to CpG DNA [1] marked the first identification of an innate immune receptor for the detection of foreign DNA. TLR9 is primarily expressed in plasmacytoid dendritic cells (pDCs) and B cells, and is one of a subset of nucleic acid-specific TLRs that signal from within endosomes. TLR9-independent DNA sensing mechanisms also exist. This was demonstrated with reports in mice showing that uncontrolled IFN-β induction and ensuing lethality associated with DNAse II deficiency are not reversed in the absence of TLR9 or its downstream signalling adaptor MyD88 [2]. Also, DNA-associated systemic lupus erythematosus (SLE) auto-antigens trigger DC activation via TLR9-independent pathways [3,4]. Intracellular delivery of mammalian or bacterial dsDNA, using cationic liposomal transfection reagents, triggered macrophage and DC activation accompanied by potent TNF-α and IL-6 release independently of TLR9 [5,6]. This and other work hinted that innate DNA sensing was not confined to endosomes, but could also occur in the cytosol, and it is now appreciated that intracellular DNA is likely to constitute a major PAMP following infection with DNA viruses and intracellular bacteria such as *Listeria monocytogenes* and *Legionella pneumophila* [7].

In this review, we discuss recently identified cytosolic innate immune DNA sensors and how these sensors signal. Furthermore, we define a new family of PRRs involved in DNA sensing, termed the AIM2-like receptors (ALRs). The discovery of the ALRs together with other novel DNA sensors reveals that multiple DNA sensors exist, many of which operate in a cell-type specific manner, and that these DNA sensors are unlikely to discriminate between self and non-self DNA, thus providing a rationale for their likely roles in autoimmune responses to self-DNA.

Signalling components involved in the cytosolic DNA response

Although the specific receptor molecules involved in triggering TLR9-independent DNA sensing pathways were unknown until recently, studies examining the cellular responses induced by transfecting different types of DNA molecules into cells, or infecting cells with DNA viruses, uncovered a number of signalling proteins functioning downstream of DNA sensors that are required for cytosolic DNA responses (see Figure 1). Apart from the transcription factors NF-κB and IFN regulatory factor 3 (IRF3), strong evidence exists for a central role for TANK-binding kinase-1 (TBK1) and stimulator of IFN genes (STING) in DNA-dependent pathogen responses.

TBK-1 and IRF3

In 2006, TBK1 was shown to be required for the synthetic DNA poly(dA:dT) to induce IFN-β production via activation of IRF3 [8]. TBK1 directly phosphorylates and thus activates IRF3, in most cells types examined to date. Similar to poly(dA:dT), a synthetic 45 bp non-CpG DNA oligomer (termed ISD) enhanced type I IFN expression in an IRF3-dependent manner [9]. Type I IFN responses to the DNA viruses

herpes simplex virus 1 (HSV-1) and murine gammaherpesvirus 68 (MHV-68), are also TBK1-dependent [10], as is the immune response to DNA-based vaccines [11]. IRF3 also conferred resistance to MHV-68 infection *in vivo* [10] providing evidence for a crucial role of a TBK1-IRF3 axis in the IFN response to DNA viruses. Transfected DNA triggers IRF3 and IFN-β promoter activation via a TBK1/IKKε dependent process but significant NF-κB activation still occurs in the absence of TBK1/IKKε [8].

STING

STING was identified as a transmembrane-spanning endoplasmic reticulum-resident protein essential for IFN-β induction by exogenous DNA ligands and also by *L. monocytogenes* and HSV-1 infection [12]. An immune function for STING was discovered independently by four groups and as such it is also known as MITA [13], MPYS [14] and ERIS [15] (while its gene name is *Tmem173*). A follow-up study in STING-deficient mice showed impaired IFN induction in response to bacterial, viral and mammalian DNA, as well as to a number of DNA viruses including HSV-1, human cytomegalovirus (HCMV), vaccinia virus (VACV) and baculovirus [16]. Further, survival of mice lacking STING was reduced following infection with HSV-1. STING functions 'upstream' of TBK1 in DNA sensing, and like TBK1, is also required for a normal immune response to DNA vaccines [16]. Thus, STING is critical for DNA-mediated signalling to enhance adaptive immune responses. While STING over-expression mediates potent IRF3 induction, the corresponding NF-κB activation is not very strong [12]. Thus, the question of whether intracellular DNA triggers a STING-independent pathway to NF-κB activation warrants further study.

The gram-negative bacterium *Francisella tularensis* has now also been shown to trigger IFN production in macrophages in a STING-dependent manner [17] and human immunodeficiency virus (HIV-1) recognition also appears to require STING [18]. A forward genetic screen revealed that a mutation in *Tmem173* (encoding STING) led to impaired recognition of cyclic-di-nucleotides (which are produced during *L. monocytogenes* infection), placing STING downstream not only of *L. monocytogenes* DNA recognition but also of *L. monocytogenes* cyclic-di-nucleotide ligands [19]. The identity of the upstream sensor for these type I IFN-inducing bacterial nucleotide ligands is currently a major focus. Finally, it has recently been shown that human SNPs in STING render cells defective in sensing DNA, emphasising the importance of STING to DNA responses in humans [20].

Novel intracellular DNA sensors for IFN induction

Neither TBK1 nor STING directly recognise DNA in the cytosol. Rather, upstream cytosolic DNA sensors have recently been described, which are proposed to have the capacity to bind directly to DNA, and engage signalling pathways converging on NF-κB and IRF activation, leading to type I IFN induction (Figure 1). These sensors display some selectivity in terms of cell type, the pathogens sensed, and the exact nature of the DNA ligand tested (Table 1). However, work to date suggests that it is unlikely that these diverse sensors display differential selectivity for different types of DNA. Rather they may all simply sense cytosolic dsDNA in different cell types.

DAI

The identification of DNA-dependent activator of IFN-regulatory factors (DAI; also known as Z-DNA binding protein or ZBP-1) in 2007 represented the first

identification of a TLR9-independent sensor for intracellular DNA [21]. DAI directly interacts with DNA and over-expression of DAI in murine L929 cells enhanced IFN-β induction triggered by transfected poly(dA:dT). Also, HCMV-induced IFN-β in human fibroblasts is dependent on DAI [22]. The role of DAI in DNA sensing pathways, however, may be restricted to certain cell types because inhibiting DAI expression has limited effects on poly(dA:dT)-induced IFN-β in MEFs [23]. Furthermore, in contrast to TBK-1 and STING-deficient mice, mice lacking DAI displayed normal immune responses to DNA virus infection and DNA vaccines [11]. The receptor interacting proteins, RIP1 and RIP3 facilitate NF-κB activation by DAI (Figure 1) [24,25]. Whether they regulate NF-κB activation by other DNA sensing pathways is an open question.

RNA polymerase III

Some of the redundancy in DAI-mediated sensing of poly(dA:dT) can be attributed to the indirect sensing of this AT-rich DNA by RNA polymerase III. In 2009, RNA polymerase III was shown to transcribe AT-rich dsDNA into 5'triphosphate-containing dsRNA which acts as a ligand for the RNA sensor retinoic acid inducible gene I (RIG-I) [26,27]. Since MAVS is a downstream adaptor molecule for RIG-I signalling, this explains observations that MAVS-dependent signalling is sometimes required for DNA sensing (Figure 1) [8,28]. The RNA polymerase III-dependent pathway has been implicated in sensing Epstein Barr virus (EBV)-encoded EBER RNA as well as HSV-1, adenovirus and *L. pneumophila* DNA. However, other studies have shown that RNA polymerase III is dispensible for IFN-β induction by HSV-1 [29,30] and *L. pneumophila* [31]. RNA has been proposed as the primary IFN-inducing ligand in *L. pneumophila* infection of BMDMs and both RIG-I and Mda5

(an RLR related to RIG-I) have been implicated in this response [31]. Given the importance of pDCs in responding to DNA, it may be that DNA contributes more significantly to the *L. pneumophila*-induced IFN response in these cells. RNA polymerase III appears to operate in a number of cell types, and the response to poly(dA:dT) in human cell lines is at least partially attributable to this pathway [26,27,29]. However, the physiological relevance of the RNA polymerase III pathway in the context of a whole animal model of pathogen infection will be difficult to assess, because RNA polymerase III is essential for normal cell function.

LRRFIP1

In 2010, using a synthetic siRNA library screen in mouse primary peritoneal macrophages, the leucine-rich repeat (LRR)-containing protein, LRRFIP1, was implicated in the induction of IFN-β in *L. monocytogenes* infection, and also in response to transfected DNA [32]. LRRFIP1 was found to regulate a very specific β-catenin-dependent pathway whereby rather than mediating NF-κB or IRF3 activation, LRRFIP1 triggered phosphorylation and subsequent nuclear translocation of β−catenin, which then interacted with IRF3 (Figure 1). This facilitated p300 recruitment and histone acetylation at the *ifnb1* promoter locus. LRRFIP1 was proposed to directly recognise DNA because it immunoprecipitated with synthetic dsDNA. However, physiologically relevant direct DNA binding remains to be demonstrated. Furthermore, LRRFIP1 regulated a highly specific pathway to IFN-β induction utilising β-catenin in response to both DNA and RNA ligands while having no effect on *L. monocytogenes*- or DNA-mediated TNF-α induction.

DHX9 and DHX36

Very recently, in pDCs a TLR9-independent, but MyD88-dependent CpG DNA sensing mechanism led to the discovery of a role for the DEAD/H-box helicases, DHX9 and DHX36, in CpG-dependent gene induction [33]. DHX9 and DHX36 were initially identified as CpG-interacting proteins by mass spectrometry. siRNA knock-down experiments revealed that DHX36 was specifically required for the IFN-α response to HSV-1 infection in pDCs while DHX9 expression was primarily required for HSV-1-mediated TNF-α induction. Both DHX36 and DHX9 interact with the TIR domain of MyD88. Thus, it was proposed that two TLR9-independent, MyD88-dependent DNA sensing pathways exist in pDCs whereby DHX36 governs a pathway specific for IRF7 activation and IFN-α induction while DHX9 triggers nuclear translocation of the p50 NF-κB subunit and subsequent upregulation of NF-κB-dependent genes such as TNF-α and IL-6.

Ku70

Another protein very recently implicated in DNA sensing in a specific context is Ku70. HEK293 cells are refractory to DNA-induced IFN-β induction, except for RNA polymerase III sensing of AT-rich DNA [26,27,29]. However, plasmid DNA induces the type III interferon, IFN-λ1, as opposed to type I IFN-α or IFN-β in HEK293 cells [34]. Ku70, a protein involved in DNA repair, was identified as a dsDNA-binding protein by mass spectrometry, and using siRNA and cells from $Ku70^{-/-}$ mice subsequently implicated as the sensor responsible for triggering this pathway. DNA-induced IFN-λ1 induction correlated with binding of IRF1 and IRF7, but not NF-κB or IRF3, to the *IL29* (IFN-λ1) promoter, suggesting that Ku70 might engage a signalling pathway regulating IRF1 and IRF7 activation. As Ku70-mediated IFN-λ1 induction required DNA of greater than 500 bp, the authors suggest that Ku70

proteins may need to bind to multiple sites along a dsDNA molecule in order to stimulate signal transduction to IRFs [34].

IFI16 and p204

An IFN-β-inducing 70bp dsDNA from VACV was used to affinity purify DNA-binding proteins from cytosolic extracts of human monocytes, leading to the identification of IFI16 [29]. IFI16 was found to recruit STING to activate a TBK1-IRF3-dependent pathway to IFN-β induction, while siRNA showed that both DNA and HSV-1-induced IFN-β was IFI16-dependent. Interestingly, IFI16 is part of a larger protein family termed the pyrin and HIN domain (PYHIN) family, of which there are four human and 13 murine members (Figure 2). The closest murine structural counterpart to IFI16, p204, is the only murine PYHIN family member that, like IFI16, contains two HIN200 DNA binding domains and a pyrin domain (Figure 2). Inhibition of p204 expression in murine cells showed that p204 was essential for IRF3 and NF-κB activation, and type I IFN and pro-inflammatory gene induction driven by HSV-1 infection or by dsDNA oligonucleotides. A prerequisite for a bona fide DNA sensor is a demonstration of direct binding to immune-stimulatory DNA and as such, IFI16 not only colocalised with DNA in intact cells, but purified IFI16 protein also directly bound DNA *in vitro* [29].

Another member of the PYHIN family, AIM2, is a cytosolic DNA receptor that forms an inflammasome with ASC to trigger caspase 1 activation [17,35-37]. Very recently, IFI16 has also been proposed to mediate inflammasome activation, in response to Kaposi's sarcoma-associated virus (KSHV) infection of endothelial cells [38]. In this case IFI16 was proposed to sense viral DNA in the nucleus, raising the possibility that

IFI16 may have alternative DNA sensing functions in the nucleus and the cytosol. Recent studies with AIM2, IFI16 and p204 suggest the existence of a brand new family of PRRs, previously unappreciated, and termed 'AIM2-like receptors' (ALRs). Table 2 compares the characteristics of the DNA sensing ALRs with the RNA sensing RLRs. It will be of great interest to assess the role of the other PYHIN family members (Figure 2) in innate immune responses to DNA, which may be easier to address in the human system, given that two out of the four known human PYHIN proteins have already been assigned innate DNA sensing roles. As PYHIN proteins have other defined roles in cellular processes [39-41], they may be analogous to the NOD proteins whereby some family members function as PRRs (the NLRs) and some do not [42].

Negative regulation of intracellular DNA sensing pathways

The inappropriate induction of type I IFNs leads to the on-set of such auto-immune disorders as SLE and Aicardi-Goutieres syndrome (AGS). The detrimental effects of dysregulated type I IFN production is underscored by the fatal encephalopathy induced by overproduction of IFN-α in AGS [43]. Further, *irf3* or *ifnαβR* deletion can reverse the lethal phenotype of autoimmune disorders in mice [44-46]. Accordingly, inappropriately generated self nucleic acid must be cleared efficiently in order to prevent autoimmune responses. The existence of the cytosolic DNA sensing pathways mediating IFN induction described above provide a rationale for this, since they do not seem to recognise any particular structural motif of DNA that would discriminate between self and non-self. It may be that cytosolic DNA sensing pathways evolved to sense 'danger' (such as free dsDNA in the cytosol) more so than 'stranger' (the presence of a pathogen), whereas cytosolic RNA sensing by the RLRs does seem

capable of detecting structural moieties specific to pathogen RNA, such as 5'-triphosphates (Table 2).

A role for host DNases in the elimination of mislocalised host DNA to prevent autoimmunity is emerging. Mice deficient in DNase I, the most abundant nuclease found in serum, develop SLE-like autoimmune disorders [38] while mutation of the DNase I gene has been associated with SLE in humans [47,48]. DNase II plays a major role in the clearance of DNA from apoptotic cells within macrophage phagosomes. In mice lacking DNase II, accumulating DNA from phagocytosed apoptotic cells drives excessive IFN-β and TNF production inducing embryonic lethality [49]. Further, in DNase II-deficient mice, DNA released from erythroid precursor cells during erythrocyte development is not properly digested and so accumulates in macrophage lysosomes within foci of erythropoiesis known as blood islands [50]. This triggers constitutive IFN-β production in the liver along with an ensuing upregulation of IFN-inducible genes. These embryos suffer severe anaemia and die *in utero*. However, DNase II-IFN-α/βR double deficient mice developed normally and appeared healthy despite the abnormal accumulation of DNA in blood-island macrophages confirming the contribution of uncontrolled DNA-induced IFN to the lethality observed.

Mutations in human cytosolic 3′ – 5′ exonuclease Trex1 (DNase III) are associated with both SLE and AGS. Trex1 deficiency in mice manifests in the development of lethal autoimmunity due to excessive type I IFN and auto-antibody production [44]. Cytosolic ssDNA derived from endogenous retroelements were shown to accumulate in cells lacking Trex1 leading to aberrant type I IFN induction. Whether a specific cytosolic sensor that triggers type I IFN induction in response to these ssDNA Trex1

substrates exists remains to be elucidated. Such a ssDNA sensor may be central to HIV-1 innate immunity as HIV was found to subvert Trex1 activity to rapidly degrade ssDNA intermediates generated during reverse transcription of this retroviral genome [18]. Given the link between nuclease deficiency and autoimmunity, it is of interest to determine which, if any, of the new DNA sensors described above are responsible for responding to endogenous DNA in contexts where nucleases are deficient.

Due to the expression of high basal levels of IRF7, pDCs are the most potent IFN-α–producing cells (reviewed in [51]). The production of type I IFN by pDCs is a key component of the pathogenesis of many autoimmune diseases. Polymorphisms in genes of the type I IFN signalling system, including *IRF5* and *STAT4*, have been identified as susceptibility factors in many autoimmune diseases, including Sjogren's syndrome (SS) and SLE [52-55]. Serum levels of type I IFN are increased [56] while blood pDC populations are reduced and infiltrating pDCs are enhanced in the salivary glands in SS patients [57]. Similarly, in SLE patients, circulating pDCs are significantly lower [58] due to migration of these cells to skin [59,60] and renal tissue [61]. Nucleoproteins and immunoglobulins complexed to self DNA have been shown to continually activate pDCs to produce type I IFN in SLE [62,63]. This leads to myeloid DC differentiation and continual activation of autoreactive T cells [64]. Thus, any intracellular DNA sensor implicated in sensing host DNA to trigger an autoimmune response will likely be active in pDCs. Recently, dsDNA-activated human DCs were shown to be more potent than LPS- or inflammatory cytokine-activated DCs at triggering an adaptive immune response and IFI16 was found to contribute to this response [65].

Viral evasion of innate DNA sensing mechanisms

Most PRRs identified to date are subject to targeting by viral evasion mechanisms [66], and this is also becoming apparent for some of the novel cytosolic DNA sensors (Table 2). Prior to the discovery of DNA sensors, it was already appreciated that multiple viral mechanisms to antagonize TBK1 signalling existed (reviewed in [66]), however it has also now been shown that some viruses also target the novel DNA sensors more directly. For example, murine CMV encodes a RHIM-containing protein, M45, which blocks DAI signalling to NF-κB [25]. The HCMV tegument protein, pUL83, can associate with the human PYHIN proteins IFI16 and IFIX (Figure 2), and a mutant virus with impaired pUL83 expression was more susceptible to type I IFN treatment [67]. Thus, IFI16 and/or IFIX targeting by HCMV may represent a strategy to evade innate sensing of its dsDNA genome. The poxvirus-encoded M13L protein contains an N-terminal pyrin domain and interacts with ASC to antagonise caspase 1 activation and subsequent IL1β processing [68]. Disruption of the M13L gene in myxoma virus gave rise to an attenuated virus displaying reduced disease progression in rabbits due to a potent inflammatory response to infection. Thus, M13L-expressing poxviruses can potentially antagonise inflammasome activation triggered by AIM2 upon sensing viral DNA. If this viral immunomodulatory protein targets the pyrin domain of the other PYHIN family members, for example IFI16, this may represent a very clever viral strategy evolved by poxviruses to simultaneously suppress two arms of the innate machinery for sensing viral DNA.

DNA sensing: future perspectives and outstanding questions

Understanding of innate intracellular DNA sensing pathways has advanced rapidly in recent years. As described above, a number of candidate sensors of intracellular DNA have now been described. However, key questions remain to be addressed. Whether all of the current described DNA sensors are actually true receptors requires clarification. For example, specific regulation of IFN-β by LRRFIP1 independently of any role in TNF-α induction coupled with no role in IRF3 or p65 activation points to a more downstream role for LRRFIP1 in nucleic acid mediated IFN-β induction. Consistent with this, infection with both VSV (an RNA virus) and *L. monocytogenes,* as well as synthetic dsRNA and dsDNA analogues appear to activate this pathway [32], suggesting LRRFIP1 may regulate multiple, and not just DNA sensing, PRR pathways to type I IFN transcriptional activation. Furthermore, DHX9 is required for DNA-mediated NF-κB activation only, while DHX36 mediates IRF7 activation in response to DNA but is not required for NF-κB induction [33]. How these molecules act as separate sensors upstream of MyD88 to differentially activate specific transcription factors requires further examination.

The apparent redundancy of some of the DNA sensors raises the question of which ones are physiologically relevant in sensing viral and bacterial infection in primary cells? Are specific sets of DNA sensors engaged by particular pathogens? Cell-type specificity may explain the existence of so many different pathways (Table 1).

A major question still outstanding is whether these newly described DNA sensing mechanisms function in the nucleus or whether they only detect foreign DNA in the cytosol? It would seem more rational to restrict immune surveillance for DNA to the cytosol, where its presence could alert the cell to ‘danger’ either in the form of a

pathogen DNA, or mis-localised or accumulating host DNA. However, the proposal that IFI16 can sense viral DNA in the nucleus [38], tied in with the fact that it, and other DNA sensors such as DHX9 and RNA polymerase III are expressed abundantly in the nucleus, may suggest that the nucleus does not enjoy immune privilege when it comes to DNA. If so, then the issue of what prevents the aberrant activation of a deleterious IFN-β response to host chromosomal DNA will need to be resolved. As DNA sensors such as IFI16 and Ku70 are also implicated in DNA damage responses, they may simply signal IFN-β induction upon encountering any type of naked DNA not complexed with protein in chromatin.

These questions pose some major challenges and addressing them will advance our understanding of how the innate immune system detects and responds not only to invading pathogens but also to self nucleic acid leading to inflammation and autoimmunity.

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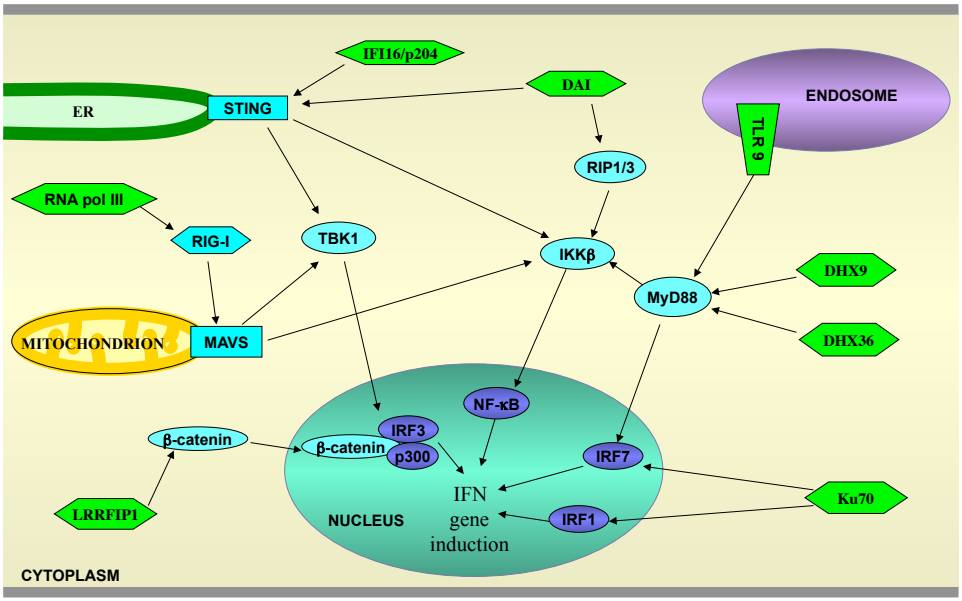

Figure 1. Cytosolic sensors of DNA mediating interferon induction.

Intracellular dsDNA produced during infection with pathogens such as dsDNA viruses and bacteria or from phagocytosed dead cells is a potent inducer of immune responses resulting in the production of proinflammatory cytokines and IFNs. A variety of intracellular sensors recognizing dsDNA (green) have been described. **TLR9** recognizes CpG DNA in endosomes and triggers MyD88-dependent activation of IRF7 and NF-κB via IKKβ. Binding of dsDNA by **DAI** results in STING- and TBK1-dependent IRF3 activation and induction of type I IFNs. It also activates NF-κB via RIP1 and RIP3. Human **IFI16** and murine **p204** induce STING-dependent activation of IRF3 and NF-κB transcription factors. **RNA polymerase III** converts AT-rich DNA into an endogenous 5' triphosphate-containing RNA ligand, which then activates RIG-I to trigger MAVS- and TBK1-dependent activation of transcription

factors. **Ku70** activates IRF1 and IRF7 to induce IFNλ1. Cytosolic recognition of CpG-A DNA by **DHX36** induces MyD88-dependent IRF7 activation and induction of IFN-α, whereas **DHX9** binds CpG-B DNA triggering MyD88-dependent activation of NF-κB and production of proinflammatory cytokines. **LRRFIP1** activation by DNA stimulates β-catenin phosphorylation at Ser552 promoting a relocalisation of β-catenin to the nucleus where it interacts with IRF3. This enhances the recruitment of the histone acetyltransferase, p300, to the IFN-β enhanceosome resulting in transcriptional activation. Induction of IFNs in response to intracellular dsDNA is a key step in early immune responses to various pathogens and danger signals, and is also involved in shaping the later adaptive immune response.

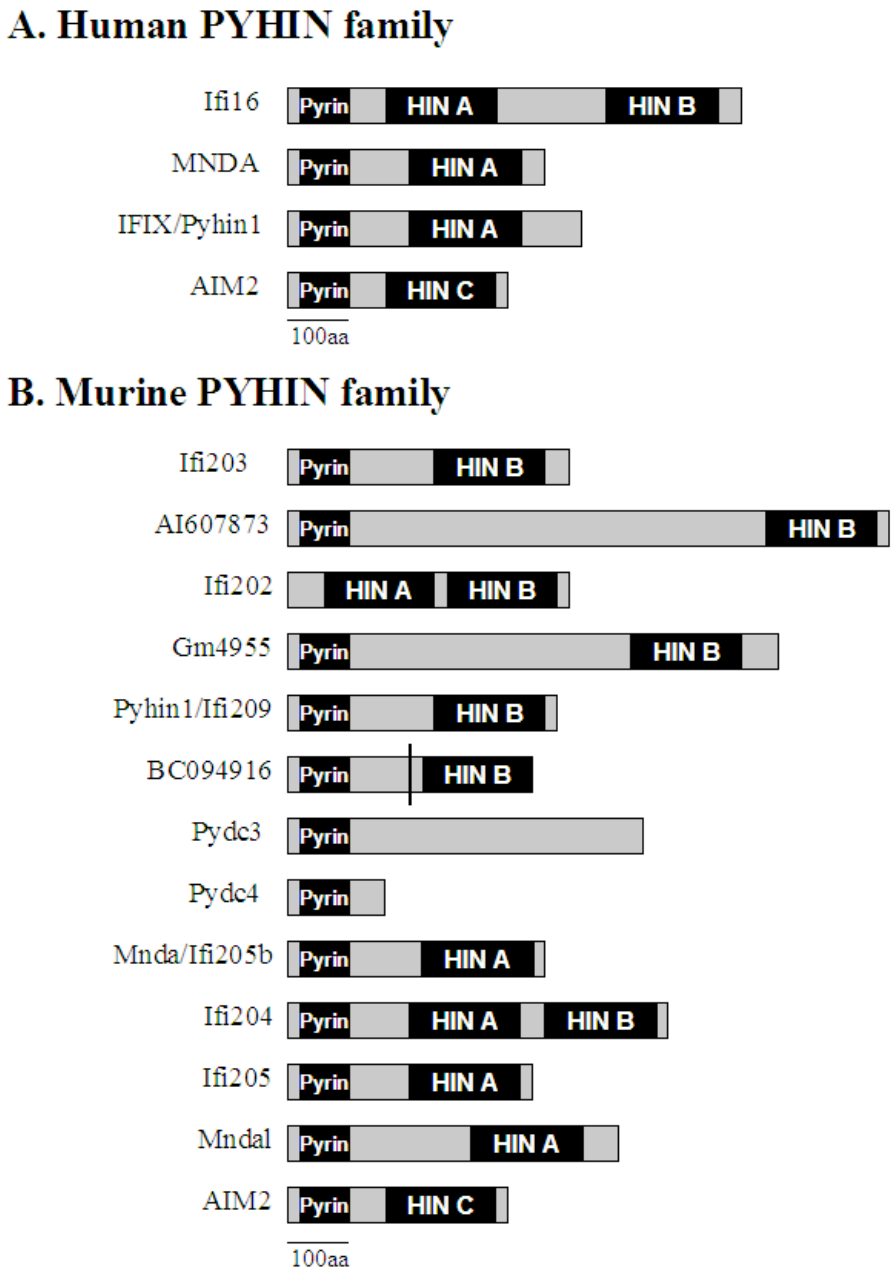

Figure 2. The human and murine PYHIN protein families.

PYHIN proteins consist of a pyrin domain and at least one HIN200 domain (black boxes) that can be divided into 3 subtypes (HIN A, HIN B and HIN C) based on their conserved amino acid content [69]. Both human and murine PYHIN genes are localized

at chromosome 1q21-23. The human PYHIN family (**A**) consists of 4 members and of these only IFI16 contains two HIN200 domains (HIN A and HIN B) while MNDA and IFIX/Pyhin1 only contain one HIN A domain. AIM2 is phylogenetically distinct from the other family members with both its pyrin and HIN domain (HIN C) having the lowest sequence similarity to the pyrin and HIN domains of other PYHIN proteins. The murine family (**B**) is much more diverse, predicted to consist of at least 13 members. As with human IFI16, IFI204 (p204) is the only murine PYHIN protein consisting of a pyrin and two HIN200 domains (HIN A and HIN B). Some conflicting data has been reported for BC094916. Thus it may be truncated (marked by↑↓) to give rise to a final gene product lacking a HIN B domain. IFI202 lacks a pyrin domain, but has two HIN200 domains (HIN A and HIN B) and was shown to be inhibitory. As with human AIM2, murine AIM2 is phylogenetically distinct from the other family members.

Table 1. Characteristics of cytosolic DNA sensors triggering type I IFN induction.

DNA sensor	Pathogens sensed	Ligands tested	Cell type	Signalling molecules involved	Ref
DAI	HCMV, HSV-1, *S. pneum*.	AT-rich B-DNA, Viral, bacterial, mammalian gDNA 500bp>>100bp>75bp	L929	TBK1, IRF3, RIP1, RIP3, NF-κB	21,70
RNA pol III	*L. pneum.*?, Adenovirus, EBV, HSV-1?	AT-rich B- DNA, EBERs	HEK293, HeLa, MEF, mBMDM, RAW264.7, human monocytes, L929, Mutu III	RIG-I, MAVS, TBK1, DDX3, IRF3	26,27
LRRFIP1	*L. mono.,* VSV	dsDNA, dsRNA AT-rich B-DNA, GC-rich Z-DNA	murine 1° PM[a], RAW264.7	β-catenin, p300, IRF3	32
DHX9	HSV	CpG-A	human pDCs	MyD88, NF-κB (p50)	33
DHX36	HSV	CpG-B	human pDCs	MyD88, IRF7	33
IFI16 /p204	HSV-1	dsDNA Sequence-indep. 70bp>>50bp	THP1, RAW264.7, MEF, HeLa, BMDM	STING, TBK1, IRF3, NF-κB	29
Ku70	HSV-2G	dsDNA, ssDNA, sequence-indep. 500bp>>50bp	HEK293, human MDM[b], murine spleen cells	IRF1, IRF7 IFNλ1	34

[a]PM, peritoneal macrophages; [b]MDM, monocyte-derived macrophages

Table 2. Comparison of innate sensing of DNA by ALRs compared to RNA by RLRs.

	ALRs	**RLRs**
Ligand	Self or non-self dsDNA	Viral RNA
IFN induction	IFI16, p204	RIG-I, Mda5
Inflammasome activation	AIM2, IFI16	RIG-I
Negative regulation	p202	LGP2
Viral evasion	M13L, pUL83	NS1, K7
Pathogen sensed	Virus and bacteria	Virus and bacteria
Cellular compartment	Cytosol (and nucleus?)	Cytosol