



Potentiality of DNA Sensors in Activating Immune System in Emerging Viral Infectious Diseases

16

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Abstract

Viruses are obligatory intracellular parasites and hijack the host cell machinery to make more identical copies of it and continue self-propagation. They attach and replicate in the susceptible and permissive hosts and host derived cell lines. They enter the cells either through direct attachment, receptor-mediated endocytosis, or phagocytosis. Hence, to thwart the invasion by viruses, hosts have developed immunity in ascending stages—innate, intrinsic and adaptive immunity. A robust intrinsic and innate immune response governs an effective adaptive immune response, should that be needed. Both enveloped as well as non-enveloped viruses are subject to distinct types of DNA sensors, subject to their site of replication. DNA sensors of viral PAMPs can be classified into three types, based on the location of their PAMPs in the host cellular compartment viz. cell surface, cytoplasmic and nuclear. The host cell membrane both, surface as well as intra cellular, is continuously monitored for the non-host, pathogenic components or PAMPs. Among the intracellular sensors of the viral genome, there are two types—essentially due to the two types of major viral genomes i.e. RNA and DNA sensors. The cytosolic DNA sensors include AIM2, IFI16, cGAS, RNA Pol III, DNA-PK, DDX9, DHX36, DDX41, DDX60, DAI, LRRFIP1, HMGB, ABCF1 and MRE11. PYHIN family of sensors include AIM2, IFI16, IFIX and MNDA. Another recently discovered family of sensor called stimulator of interferon (IFN) genes (STING), specifically houses on the endoplasmic reticulum (ER) and functions in association with its upstream sensor, cGAS. Some DNA sensors shuttle between the cytosol and nucleus pre- and post-extraneous DNA binding. These include IFI16, IFIX, RNA Pol III, etc. There is no exclusive nuclear DNA sensor. Many enzymes known to be present in the cells for their

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obvious primary functions also additionally function as DNA sensors. The DNase family of sensors include DNase II and TREX1, which are ubiquitously present in the cell for their housekeeping functions. The RNase family of sensor includes one member—RNA Pol III. Additionally, DNA-PK also functions to cater to viral DNA sensing. The endosomal DNA sensors include TLR7 and TLR9, which belong to the Toll-like receptor (TLR) family. The DExD/H-box helicase family include the putative DNA sensors recently discovered including DDX9, DHX36, DDX41 and DDX60. Several other sensors remain to be characterised or are less classified viz. DAI, LRRFIP1, HMGB, ABCF1, MRE11. In general, response to a viral RNA or DNA produces three types of responses, namely, production of antiviral cytokines including Types I and III IFNs, release of pro-inflammatory and inflammatory cytokines and chemotactic factors. This chapter discusses the structure, function and mechanism of action of the viral DNA sensors explored till date.

Keywords

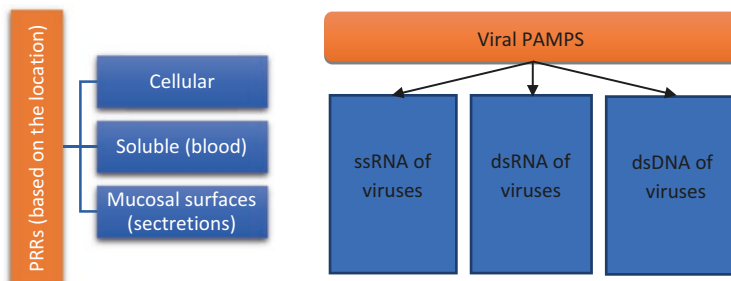
Viral DNA sensors · Immune responses · Immune activation

16.1 Introduction

Defence mechanisms employed by the innate branch of immunity include myriad types of cells and soluble molecules in tissues and circulatory system. Such pre-existing deployments constantly thwart the attacks on the organism by the pathogenic microorganisms ubiquitously present throughout the biosphere, thus averting the invasion, establishment and multiplication of such agents. In fact, if microbes do institute a niche, the innate immune responses equip early defence, before the involvement of adaptive branch of the immune system.

The peculiarities of the recognition by players of innate immune system have emerged to contest microbes which have outstanding common features. The innate immunity has evolved to perceive the molecular structures which are present on the surface or inside the microorganism in question, as well as the induced pathogenic microbial products. Such biomolecules which spur the innate immune system are often shared by classes of microbes are called pathogen-associated molecular patterns (PAMPS), or even more appropriately, microbial-associated molecular patterns (MAMPs). Such moieties include nucleic acids (single-stranded RNA/DNA), characteristic feature(s) on protein, carbohydrate and lipid products restricted to microorganisms, or even a combination of the biochemical elements. Such building blocks are often indispensable for the survival of microorganisms. Not only is it capable of recognising the foreign biomolecular structures, but also some, which are derived from self: in aberrant conditions like damaged or dying cells. These substances are known as death- or damage-associated molecular patterns (DAMPs).

The cell-associated recognition counterpart that assists in perceiving the molecular patterns are called pattern recognition receptors (PRRs). PRRs constitute various types of cell receptors, present in distinct premises of the cell (surface as well as intracellular), as soluble factors in the circulation and other body secretions.



Cell-associated molecules are mostly restricted to the cells of the immune system like phagocytes—Macrophages and Neutrophils; antigen presenting cells (APCs)—Dendritic cells; cells that form the obstacle between the internal milieu and the external environment—epithelial cells; as well as other cells like mast cells and tissue resident cells.

Pathogens like viruses, bacteria, fungi and protozoa can establish themselves within any compartment of the cell viz. cytosol, nucleus, endosomes or on the surface of the cell, tissue, etc. The innate immune system efficiently tackles the microbial invasion in all these compartments by activating the signal transduction pathways downstream of the recognition molecules which ultimately promote the pro-inflammatory and anti-microbe activity.

Broadly though, the pattern recognition receptors can be either cell-associated or soluble.

The cell-associated receptors include:

1. Toll-like receptors (TLRs)
2. NOD-like receptors (NLRs)
3. RIG-I-like receptors (RLRs)
4. Cytosolic DNA sensors (CDSs)
5. C-type lectin-like receptors (CLRs)
6. Scavenger receptors
7. *N*-formyl met-leu-phe receptors

The soluble receptors include:

1. Pentraxins
2. Collectins
3. Ficolins
4. Complement

The scope of discussion on each of the above PRRs is wide. However, since this chapter specifically deals with the DNA-sensing molecules, further discussion would be restricted to the viral DNA sensors.

16.2 Sources of Cytosolic DNA

There are several ways by which DNA can be present in the cell cytoplasm.

These routes include (but are not limited to):

1. Intracellular pathogen infection
2. Impaired ability of clearing exogenous DNA innately metabolised in the endo-lysosomal compartment
3. An asymmetric management of endogenous DNA products and turnover.

Modes of entry of pathogen DNA in the cytoplasm are depicted below.

The following table summarises the currently known DNA sensors in brief—proposed DNA sensor families and the examples:

PRR	Cell types	Site of DNA sensing	Response	References
<i>Toll-like receptor (TLR) family</i>				
TLR9	pDCs	Endosomes	Type I IFN	(Hemmi et al. 2000; Latz et al. 2004, 2007)
<i>PYHIN family</i>				
AIM2	Macrophages, DCs	Cytoplasm	IL-1 β , IL-18	(Hornung et al. 2009; Fernandes-Alnemri et al. 2009; Burckstummer et al. 2009; Roberts et al. 2009)
IFI16	Macrophages, endothelial cells	Cytoplasm, nucleus	IFN- β , CXCL10, IL-6, IL-1 β	(Unterholzner et al. 2010; Horan et al. 2013; Kerur et al. 2011)
IFIX	Macrophages	Nucleus	IL-1 β , IL-18	(Diner et al. 2015a)
MNDA	Less explored			
<i>STING activator family</i>				
cGAS	L929, THP-1, HEK293	Cytoplasm	IFN- β	(Sun et al. 2013)
<i>DNase family</i>				
DNase II	Ubiquitous	Lysosomes	DNA degradation	(Okabe et al. 2005)
TREX1	Ubiquitous	Cytoplasm-ER associated	Degradation of DNA elements derived from endogenous retroviruses	(Stetson et al. 2008; Yang et al. 2007)
<i>RNase family</i>				

PRR	Cell types	Site of DNA sensing	Response	References
RNA Pol III	EBV ⁺ B cell, macrophage, cell line	Cytoplasm, nucleus	IFN- β	(Ablasser et al. 2009; Chiu et al. 2009)
<i>Protein kinase (PK) family</i>				
DNA-PK	293T, MEFs	Cytoplasm	IFN- λ 1, IFN- β , IL-6	(Zhang et al. 2011a; Ferguson et al. 2012)
<i>DExD/H-box helicase family</i>				
DDX9	pDCs	Cytoplasm	TNF- α	(Kim et al. 2010)
DHX36	pDCs	Cytoplasm	IFN- α	(Kim et al. 2010)
DDX41	DCs	Cytoplasm	IFN- α , β	(Zhang et al. 2011b)
DDX60	HeLa cells	Cytoplasm	IFN- β , CXCL10	(Miyashita et al. 2011)
<i>Others/less characterised family</i>				
DAI	Fibroblasts	Cytoplasm	IFN- β , necrosis	(Takaoka et al. 2007; Upton et al. 2012)
LRRFIP1	Less studied	Cytoplasm	Type I IFN induction	(Sabbah et al. 2009; Yanai et al. 2009; Yang et al. 2010)
HMGB		Cytoplasm		
ABCF1		Cytoplasm	Less known	
MRE11	MEFs, DCs	Cytoplasm	IFN- β , CXCL10, IL-6	(Kondo et al. 2013)

16.3 TLR Family

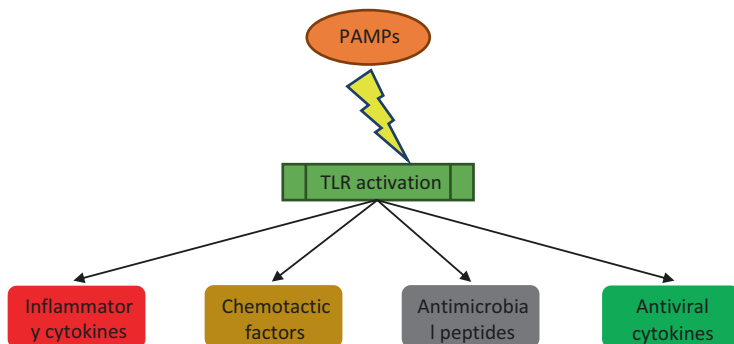
16.3.1 Structure

TLRs belong to the type I integral membrane glycoproteins embedded in the surface membrane or in the endosomal membrane. They possess an extracellular (or intra-endosomal) region—characteristic leucine-rich repeats (LRRs) which are surrounded by cysteine-rich motifs which essentially bind ligand(s), a transmembrane region, and an intracellular (or cytosolic) region—known as the Toll/IL-1 receptor (TIR) involved as a part of the cytoplasmic tail. There are about 18–26 copies of LRRs which vary in different TLRs. Each LRR of TLR protein is composed of around 20–25 amino acids and multiple such LRRs make up a typical question mark hook shaped protein scaffold which adapts for ligand binding. Both, the concave as well as convex surfaces, are involved in ligand binding. The N-terminus of a TLR is at its LRR end, whereas the C-terminus is at its TIR end.

16.3.2 Function

There are nine different functional TLRs in humans (TLR1-9). The function of TLR-10 is unknown. TLRs always act as dimers. Some of them form heterodimers like TLR-1 and TLR-2, whereas others form homodimers like TLR-3, TLR-4,

TLR-5, TLR-5, TLR-6, TLR-7, TLR-8, TLR-9 and TLR-10. TLR-1, 2, 4, 5 and 6 are expressed on the surface of the membrane, whereas TLR-3, 7, 8 and 9 are expressed inside the endosomal membrane. TLR-4 utilises accessory proteins MD2, LPS-binding protein (LBP) and CD14.



Amongst all, TLR-9 recognises unmethylated CpG dinucleotides. In the human genome, the DNA methyltransferases heavily methylate cytosine residues. However, in the genome of bacteria and many viruses, CpG dinucleotides remain unmethylated and thus serve as PAMP. Delivery of TLR-9 (and TLR-3, 7, 8) from the endoplasmic reticulum to the endosome relies on UNC-93B, a protein composed of 12 transmembrane domains. Deficiency of UNC-93B1 due to rare human mutations increases the susceptibility to herpes simplex encephalitis.

16.3.3 Mechanism of Action

Ligand binding to the TLR brings about dimerization of the respective TLRs involved. This brings about the cytoplasmic tails to come in proximity. TIR domain containing adaptor proteins are now recruited, which bind the TIR domains in the cytoplasmic tails. Further, this brings about recruitment and activation of distinct protein kinases, which activate the transcription factors. Nuclear factor κ -B (NF- κ B), activation protein-1 (AP-1), interferon response factor 3 (IRF3) and IRF7 are the major transcription factors which are activated by TLR signalling pathways. NF- κ B and AP-1 are responsible for the expression of genes encoding inflammatory response molecules, including (but not limited to) inflammatory cytokines, endothelial adhesion molecules and chemokines. IRF3 and IRF7 stimulate the production type I interferons (IFNs), which are central to antiviral innate immune responses. Different TLRs use different combination of adaptors and signalling intermediates and thus mediate unique downstream effects. TLR9 uses the MyD88-dependent, TRIF-independent pathway and activates both NF- κ B and IRFs. Hence, they induce both inflammatory and antiviral responses.

Two protein domains of MyD88 allow it to function as an adaptor protein: a TIR domain at its carboxy terminus that associates with the TIR domains of the TLR

cytoplasmic tails and a death domain at its amino terminus, which associates with the death domains present in other intracellular signalling proteins. It is worthwhile to note that both the domains of MyD88 are essential for signalling. The MyD88 death domain recruits and activates IL-1 receptor associated kinase (IRAK4) and IRAK1, which are both serine-threonine protein kinases. The complex involving IRAK, TIR of cytoplasmic tails and MyD88 executes two functions—executing the enzymes that produce a signalling scaffold, employing the scaffold to recruit other molecules which are then phosphorylated by the IRAKs. The formation of signalling scaffold is a multi-step process: The IRAK complex brings in the enzyme tumour necrosis factor receptor-associated factor 6 (TRAF6), which functions as an E3 ubiquitin ligase in association with TRIKA1 (composed of UBC13, which is an E2 ubiquitin ligase with a cofactor for Uve1A). TRAF6 and UBC13 together have the function of polyubiquitination. This polyubiquitin has linkages between lysine63 of previous subunit and C terminus of the next, leading to K63 linkages. The same process can be initiated on vivid proteins, including TRAF6 or the multi-ubiquitin chains can exist independently as free linear chains which can be extended to form polyubiquitin chains, which can bind to other signalling proteins. Further, the scaffold brings in signalling complex that is made up of TAB1, TAB2-ubiquitin binding adaptor molecules, and TAK1—a serine-threonine kinase. IRAK complex phosphorylates TAK1, which propagates signalling by activation of certain MAPKs like c-Jun terminal kinase (JNK) and MAPK14 (p38 MAPK). This brings about downstream activation of AP-1 family of transcription factors which transcribe cytokine genes.

TAK1 additionally phosphorylates and activates the I κ B kinase (IKK) complex. IKK α , IKK β and IKK γ constitute the IKK complex (the IKK γ is also known as NF- κ B essential modifier or NEMO). NEMO binds to polyubiquitin chains, which leads to IKK complex close to TAK1. TAK1 then activates IKK β by its phosphorylation. IKK β then phosphorylates inhibitor of κ B (I κ B), which is a cytoplasmic protein that natively binds to the transcription factor NF- κ B. I κ B is made up of two subunits—p50 and p65. The binding of I κ B prevents the movement of NF- κ B to the nucleus from cytoplasm. Post phosphorylation of IKK, the I κ B is released from the functional subunit of NF- κ B, which leads to translocation of NF- κ B to nucleus, where it leads to transcription of genes for pro-inflammatory cytokines like TNF- α , IL-1 β and IL-6. It is noteworthy that the effect of TLR activation varies depending on the cell type in which it occurs.

The nucleic acid sensing TLRs, including TLR9 activate IRF family of proteins. Natively present in the cytoplasm, they only get activated upon phosphorylation of serine and threonine residues in their C terminal. Upon activation, they move to the nucleus to act as transcription factors. Among all the IRFs, IRF3 and IRF7 are particularly important for TLR signalling and expression of antiviral type I IFNs. For TLR9 signalling in plasmacytoid dendritic cells, MyD88 exclusively is used as an adaptor protein. The TIR domain of MyD88 employs IRAK1/IRAK4 complex as described earlier. However, the IRAK complex carries out a different function beyond recruiting TRAFs which generates a signalling scaffold. In these cells, IRAK1 can also interact with IRF7, which is highly expressed by plasmacytoid

Table 16.1 Containing location of different TLRs, their ligand (Janeway)

Toll-like receptor	Functional association	Cellular or subcellular location	Ligand	Adaptor proteins
TLR1	TLR1:TLR2 heterodimer	Monocytes, dendritic cells, mast cells, eosinophils, basophils	Lipomannans (mycobacteria), Lipoproteins (diacyl lipopeptides; triacyl lipopeptides); Lipoteichoic acids (gram-positive bacteria); cell-wall β -glucans (bacteria and fungi)	MyD88/MAL
TLR2	TLR2-TLR6 heterodimer			
TLR3	TLR3-TLR3 homodimer	Macrophages, dendritic cells, intestinal epithelium	Double-stranded RNA (viruses), poly I:C	TRIF
TLR4	TLR4-TLR4 homodimer	Macrophages, dendritic cells, mast cells, eosinophils	LPS (gram-negative bacteria)	MyD88/MAL; TRIF/TRAM
TLR5	TLR5-TLR5 homodimer	Intestinal epithelium, macrophages, dendritic cells	Flagellin (bacteria)	MyD88
TLR7	TLR7-TLR7 homodimer	Plasmacytoid dendritic cells, macrophages, eosinophils, B cells	Single-stranded RNA (viruses)	MyD88
TLR8	TLR8-TLR8 homodimer	Macrophages, neutrophils	Single-stranded RNA (viruses)	MyD88
TLR9	TLR9-TLR9 homodimer	Plasmacytoid dendritic cells, eosinophils, B cells, basophils	DNA with unmethylated CpG (bacteria and herpesviruses)	MyD88
TLR10	TLR3-TLR3 homodimer	Plasmacytoid dendritic cells, eosinophils, B cells, basophils	Unknown	Unknown

dendritic cells. This enables IRAK1 to phosphorylate IRF7 which leads to induction of type I IFNs (Table 16.1).

16.4 PYHIN Family

There are 4 PYHIN proteins in humans, and 13 in mice. Two human proteins in this class, namely AIM2 and IFN- γ inducible (IFI16) have been predicted based on the studies so far, to be necessary for different DNA-modulated immune responses and perhaps, also function as DNA sensors (Hornung et al. 2009; Roberts et al. 2009;

Unterholzner et al. 2010). PYHIN family of proteins can also activate the formation of inflammasome.

Inflammasome Inflammasomes is a complex structure which consists of many proteins which form in the cytosol upon stimulation with cytosolic PAMPs and DAMPs, the outcome of which is production of active forms of IL-1 β and IL-18. IL-1 β and IL-18 are actually produced as inactive precursors and their activation is dependent on their proteolytic cleavage by the enzyme caspase-1. These cytokines are then released from the cell, which then promote inflammatory responses. Inflammasomes are made up of oligomers of a sensor, caspase-1, and an adaptor which links the interaction between the rest of the two components. The oligomeric complexes only form upon stimulation with DNA detected by sensors.

16.4.1 Structure of PYHIN

The PYHIN family proteins contain an N-terminal pyrin (PY) domain and an H inversion (HIN) domain

16.4.1.1 AIM2

Absent in melanoma (AIM2) is one such example of a PYHIN family of proteins. The HIN region of AIM2 recognises dsDNA genome and triggers caspase 1 activation. This occurs through the simultaneous interaction of pyrin domain with ASC. Primarily AIM2 is involved in the inflammasome formation (Schattgen and Fitzgerald 2011). Microbial DNA is one such PAMP. It is usually located in the cytosol and is key to responses in vitro to vaccinia virus (Janeway). However, it ubiquitously oligomerises upon stimulation. The downstream aggregates so formed recruit and activate a protease-caspase-1 that leads to the maturation of pro-inflammatory cytokines IL-1 β and IL-18 which ultimately culminates into a programmed cell death, termed 'pyroptosis' (Bergsbaken et al. 2009; Miao et al. 2011).

16.4.1.2 IFI16

Interferon-inducible protein 16 (IFI16) was recently characterised as the first viral DNA sensor to function within the nucleus. It is a member of PYHIN protein family (Diner et al. 2015a; Schattgen and Fitzgerald 2011). IFI16 has two HIN domains. At its C terminus, it has two HIN200 domains which bind to DNA. At its N-terminal, is a pyrin (PY) domain which mediates homotypic interactions within the molecules as well as cooperative assembly of small subunits of IFI16 (Li et al. 2013; Morrone et al. 2014). This binding is sequence-independent manner (Jin et al. 2012). It primarily functions in the nucleus, where it is located. It recognises viral dsDNA.

The function of IFI16 depends on the type of cell in which it functions. In the immune cells, IFI16 binds to cytosolic viral DNA, engaging sting and induces IFN production (Unterholzner et al. 2010; Horan et al. 2013; Jakobsen et al. 2013). However, in non-immune cells, IFI16 majorly functions in the nucleus by localising

there (Diner et al. 2015a; Li et al. 2013, 2012; Orzalli et al. 2012). A plausible explanation for this is that there exists a multi-partite nuclear localisation signal on IFI16 and is necessary for its transit between nucleus and cytoplasm (Li et al. 2012). Especially in case of herpesvirus infections, both IFI16 and STING are required for inducing the expression of IFN and IFN-stimulated genes (Orzalli et al. 2012). The differences in IFI16 DNA-sensing are possibly due to the cell type-dependent process (Diner et al. 2015b). Prompt responses are required in case of immune cells, and thus, DNA-sensing components localised in the cytoplasm makes more sense, whereas, in non-immune cells, the IFI16 might play some housekeeping functions but additionally may respond to the successful viral infections in the nucleus. Additionally, IFI16 also mounts inflammatory and apoptotic responses to foreign DNA through inflammasome—a multiprotein assembly (Kerur et al. 2011; Johnson et al. 2013; Ansari et al. 2013; Singh et al. 2013; Monroe et al. 2014). In reality, the evidence that IFI16 elicits both, type I IFN response as well as inflammation is contradictory, since type I IFNs are known to exhibit anti-inflammatory effect (Theofilopoulos et al. 2005; Billiau 2006; Guarda et al. 2011). Recently, IFI16 was shown to play a direct role in inhibiting the formation of both, AIM2 and NLR family inflammasomes (Veeranki et al. 2011). Hence, it is quite possible that the role of IFI16 in inflammasome responses is cell type dependent and other yet unknown factors do play a role too. Recent evidences suggest that IFI16 and cGAS may function in harmony to execute immune signalling to nuclear foreign DNA (Orzalli et al. 2015). This also suggests that IFI16 is a dominant nuclear DNA sensor, whereas cGAS has auxiliary functions like stabilising the IFI16 to enable or prolong signal efficiency.

16.4.1.3 IFIX

Some of the PYHIN family members, namely, IFIX and MNDA also majorly localise in the nucleus. Yet, their functions were not known in the early phase and were suspected to have some immunological function. IFIX associates with antiviral factors and its expression is inversely associated with the capacity of herpesvirus replication (Diner et al. 2015a) as it binds to the DNA of the virus and remains localised in the nucleus. It binds DNA substrates in a sequence-dependent fashion and leads to type I IFN response (Diner et al. 2015a). IFIX expression, like IFI16, is dependent on the type of cell and tissue, thus making it likely that its function varies from one cell type to other (Ding et al. 2004; Haque et al. 2014).

16.5 STING Activator Family

16.5.1 STING (*a.k.a.* MITA, ERIS and TMEM173)

STING is an ER localised transmembrane adaptor protein that is anchored by an amino-terminal tetraspan transmembrane domain. Its carboxy-terminal domain extends into the cytoplasm and interacts to form an inactive form of STING—the STING homodimer. Type I IFN response is crucial for successful defence against

viral pathogens. Stimulator of IFN genes (STING) is one such pathway vital to the mechanism of dsDNA-induced stimulation of type I IFN responses. If a viral dsDNA happens to exist in the cytosol, post entry and uncoating, it activates the enzyme cGAS (cyclic GMP-AMP synthase) that generates cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), a signalling molecule. cGAS contains a protein motif as a part of the nucleotidyltransferase (NTase) family of enzymes, including adenylate cyclase and distinct DNA polymerases (Janeway). cGAS has an affinity for DNA and readily attaches to the cytosolic DNA. This interaction stimulates the cGAS enzymatic activity that leads to generation of cGAMP from GTP and ATP in the cytoplasm. cGAMP binds to both the subunits of STING dimer and activates STING signalling.

It activates TANK binding kinase 1 (TBK1) through interaction with cGAMP. TBK1 phosphorylates and activates a transcription factor IRF3 which induces expression of type I IFN genes. Besides these, the STING is also known to respond to other cytosolic DNA sensors like DAI and IFI16. Additionally, STING induces autophagy. In the innate immune system, autophagy is a potential mechanism of delivering cytosolic microbes to lysosome where they are acted upon by the proteolytic enzymes.

It is quite interesting to know that STING, MAVS and TRIF, all have similar amino acid sequence motif at their carboxy termini

16.6 DNase Family

16.6.1 DNase II

Cells possess DNases, which degrade the unwanted DNA in the compartment it is not expected to be present. DNase II is restricted to lysosomes which digests pathogenic and by-products of dead cells which enter this compartment (Okabe et al. 2005). Originally, this mechanism has evolved for the degradation of the cells undergoing programmed cell death, i.e. apoptosis, which are usually taken up by macrophages. In cells which lack DNase II activity, DNA may stimulate aberrant responses by entering cytoplasm, consequently stimulating the cytosolic DNA sensors (Okabe et al. 2005).

16.6.2 TREX1

Yet another cellular DNase is three primer repair endonuclease 1 (TREX1) that is present in association with the endoplasmic reticulum (ER) in the cytoplasm. Under housekeeping conditions, basal amount of DNA tends to accumulate in the cytosol. TREX1 regularly degrades such DNA in the cytosol.

16.7 RNase Family

Another putative cytosolic DNA sensor discovered is RNA Polymerase III (PolIII). It uses AT-rich and herpesvirus DNA as a template to produce 5'triphosphate RNAs. RNAs so produced get detected by the cytosolic RNA sensors like RIG-I (Ablasser et al. 2009; Chiu et al. 2009). However, the broad mechanism being the Pol III as a potential DNA sensor remains to be completely defined.

16.8 Protein Kinase (PK) Family

Some proteins which are usually involved in DNA damage repair also serve as DNA sensors. DNA-PK (DNA-dependent protein kinase which is composed of Ku70, Ku80 and DNA-PKc).

16.9 DExD/H-Box Helicase Family

Both, RNA, as well as DNA helicases constitute the DExD/H-box (DDX) protein family which has its characteristic DExD/H-box domain. DDXs are multi-functional and control the gene induction at multiple points that includes signal transduction pathways, gene promoters, mRNA splicing, and translation regulation. Several DNA sensors have been identified in the DDX family (Kim et al. 2010; Zhang et al. 2011b; Yoneyama et al. 2004; Schroder et al. 2008).

16.9.1 DDX9 and DHX36

DHX9 and DHX36 are the other two DExD/H-box helicases which bind to CpG DNA and interact with MyD88 (Kim et al. 2010). The inflammatory response and type I interferon response depend partly on DHX9 and DHX36, respectively.

16.9.2 DDX41

DEAD box polypeptide 1 (DDX41) is closely related to RIG-I. It appears to signal through the STING. It has been reported that DDX41 interacts with dsDNA, both, in vitro and in vivo. In some cells, it has also been shown to be crucial to the DNA-dependent activation of type I IFN production involving STING and TBK1 (Zhang et al. 2011b). In cells where the IFI16 expression is less or restricted, the DDX41 acts as the primary DNA sensor of cytoplasmic DNA which further induced the IFN induction and IFI16 expression, the latter of which, amplifies the innate immune responses (Zhang et al. 2011b). The pattern of DNA sensor expression across different types of cells is vital to define the type of sensor which mediates the DNA primed innate immune responses. Also, recently, DDX41 has also been shown to

directly bind the cyclic di-nucleotides (CDNs) and thus, indirectly, the IFN response induced was DDX41 dependent (Parvatiyar et al. 2012)

16.9.3 DDX60

It is a novice antiviral factor among the pre-existing list of DExD/H box helicases and functions in association with RIG-I, MDA5 and LGP2 to induce the type I IFN response (Miyashita et al. 2011).

16.10 Other/Less Characterised Family

There are many unknown and known but less characterised candidate DNA sensors. They might play some vital role in other cellular processes, but their role as a DNA sensor is yet to be confirmed. Very less is known regarding their mechanism of recognition and signalling, or their in vivo function.

16.10.1 DAI

Another protein is DAI (*a.k.a.* ZBP1) (Takaoka et al. 2007). The peculiarity of this protein is that the response of DAI as a DNA sensor is decided by the cell type (Unterholzner et al. 2010; Upton et al. 2012, 2010; DeFilippis et al. 2010; Ishii et al. 2008). It interacts with the dsDNA and drives type I IFN response (DeFilippis et al. 2010).

16.10.2 LRRFIPI

It senses the DNA present in the cytoplasm and phosphorylates β -catenin. B-catenin translocates to the nucleus to induce IFN- β production.

16.10.3 HMGB

Three subtypes of HMGB, viz. HMGB1, HMGB2 and HMGB3 are known to respond to cytosolic DNA. Primarily, ABCF1 binds to the cytosolic DNA. This complex then binds to HMGB2 and IFI16 to stimulate further innate immune response (Yanai et al. 2009; Yang et al. 2010; Lee et al. 2013; Goubau et al. 2013)

16.10.4 ABCF1

They function in harmony with the HMGB group of sensors.

16.10.5 MRE11

Meiotic recombination 11 homolog a (MRE11A) has the capability to sense dsDNA in the cytosol and activates STING pathway.

16.11 Sensing of Viral Components by DNA Sensors

There are numerous mechanisms through which the DNA sensors in the host cells can detect the viral genomic components and trigger a cascade of downstream reactions. Some of them have been discussed below:

16.11.1 Herpesviridae

Till date, the role of DNA sensors has been best studied extensively using herpesviruses, HSV, in particular (Paludan et al. 2011). Quite evidently, currently known-well, established, as well as prospective DNA sensors have been shown to counteract herpesvirus infections. The very primary DNA sensor was TLR9, followed by the contemporary DNA sensors. To state a few, the HSV-1 infection in human origin primary MDMs (monocyte-derived macrophages) induces the production of pro-inflammatory cytokines like IL-1 β , which imitates the response post HSV-1 infection along IFI16 axis (Horan et al. 2013). However, this response is altered when the cell type differs. HSV-1 infection of primary murine dendritic cells (DCs) invokes a type I IFN response with the protein DDX41 involved (Zhang et al. 2011b). IRF3 is known to drive the type I IFN response as well. DAI drives IRF3 activation as well. However, contrastingly, DAI (or ZBP1) is shown to play a central role in inducing necroptosis, post infection with MCMV (Upton et al. 2012).

As discussed earlier, STING is central to all the DNA induced antiviral responses. This coincides with the study which demonstrated that STING deficiency in mice causes increased susceptibility to HSV-1 infection (Ishikawa et al. 2009). While it is not known if the cell-specific response applies to all the cell-lines, the above documented work irrefutably states that the response to herpesvirus infections in primary cells is cell type specific.

16.11.2 Retroviridae

HIV is the most widely studied retrovirus for known reasons on its complex pathogenesis, drug resistance and co-evolution with the host. As is known, HIV replication proceeds through and RNA–DNA double-stranded intermediate in the process of its RNA being converted to a dsDNA form. Hence, both, RNA as well as DNA sensors, play a crucial role in the detection of retroviral genome upon target cell entry (Solis et al. 2011; Berg et al. 2012; Doitsh et al. 2010; Yan et al. 2010). A recent study, intended to understand the type I IFN response against HIV proved

that ssDNA, rather than dsDNA form of HIV, is more potent at inducing the type I IFN response. TREX1 is a 3'/5'-exonuclease that degrades the unintegrated cytosolic cDNA, making it unavailable for sensing to the DNA sensors. Eliminating the expression of TREX1 leads to upregulation in type I IFN response and was more so in case of ssRNA intermediate of HIV, rather than dsDNA form. Undoubtedly, thus, cell does employ the intracellular sensors of DNA that culminates in type I IFN response. Although the definite sensors involved remain to be defined to date, they sure function through a STING-TBK1-IRF3 axis.

Other virus families and the respective DNA sensors have been summarised in Table 16.2

16.11.3 Mechanisms Employed By Viruses to Evade DNA Sensors

Viral genomes are subject to recognition by DNA sensors only if they happen to expose their characteristic features, leading to the consequent recognition as non-self genomic entity by the host DNA sensing mechanisms. However, myriad viruses evade and subvert the host immune responses so as to render these mechanisms of detecting the viral components ineffective.

One such strategy is to simply obscure their genome and replication intermediates involving potential ligand to the DNA sensors:

Viruses belonging to the *Herpesviridae* family inhibit DNA sensors like DAI, DHX9 and IFI16 responses.

1. HSV-1 encodes a protein ICP0, an E3 ubiquitin ligase, engages proteasomal degradation of IFI16 and also prevents its nuclear relocalisation. These further limits the activation of IRF3 (Orzalli et al. 2012).
2. HCMV possesses pUL83/pp65, a matrix protein. It inhibits ISG induction. In addition to this, it is known to interact with IFI16 (Cristea et al. 2010).

Table 16.2 Human nuclear replicating DNA viruses and implicated DNA sensors

Virus	Implicated DNA sensors
Adenovirus C (types 1, 2, 5 and 6)	cGAS, TLR9
Hepatitis B virus	AIM2, cGAS
Cytomegalovirus	AIM2, DAI/ZBP1, IFI16, TLR7, TLR9
Epstein–Barr virus	RNA Pol III, IFI16, TLR9
Herpes simplex virus type I	cGAS, DAI/ZBP1, DDX41, DDX60, DHX9, DHX36, DNA-PK, RNA Pol III, IFI16, TLR9, IFIX
Herpes simplex virus type II	DNA-PK, TLR9
Herpesvirus 8 (Kaposi sarcoma-associated virus)	IFI16
Varicella zoster virus	NLRP3, TLR9
Papillomavirus (>170 types)	TLR9

3. Proteases of viral origin like the NS2B3 protease, coronavirus papain-like proteases subvert STING in a direct manner (Aguirre et al. 2012; Sun et al. 2012; Yu et al. 2012).

Acknowledgements Authors are grateful to NIV Pune and NMIMS Pune for the support extended.

Conflict of Interest The authors declare that they have no competing interests.

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