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Samra Muhič

Negative regulation of the IFI16 and cGAS DNA sensing pathways

Negativní regulace IFI16 a cGAS DNA senzorických drah

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Samra Muhič

Abstract

Abstract

DNA sensors are molecules with the ability to sense DNA constituting an important tool of innate immunity. They are initiators of various signalling pathways, one of them being the production of interferons, which induce not only an anti-viral cell state but also protect cells of treats not related to pathogens. At least, fourteen DNA sensors have been described so far, among them – IFI16 and cGAS. Both sensors signal via an adaptor protein STING resulting in the production of type I IFN. All three of these molecules (cGAS, IFI16, STING) are strictly regulated either by host-cells in order to prevent immune over-activation or by viruses for the immune evasion. This work focuses on the mechanisms of negative regulation of the three molecules: post-translational modifications such as phosphorylation, ubiquitination, SUMOylation, acetylation and methylation; protein-protein interactions; degradation by the proteasomal system or by autophagy. Not surprisingly, viruses encode proteins able to down-regulate IFN responses for example, some proteins of herpes viruses interact with cGAS, IFI16 or STING preventing their activation or leading to their degradation. Other proteins of herpes viruses cause the degradation of the mRNA of the sensors or the adaptor. Dengue protease factor NS2B degrades cGAS or the viral polymerase of hepatitis B virus, E7 protein of human papilloma virus and E1A of adenoviruses bind STING and cause its inactivation.

Key words: cGAS, IFI16, STING, IFN, negative regulation, innate immunity, interferons, DNA sensors

Abstrakt

DNA senzory jsou molekuly schopné detekovat DNA a důležitým nástrojem vrozené imunity. Stojí na počátku různých signálních drah, kdy jednou z nich je produkce interferonů, které navozují jednak tzv. proti-virový stav a zároveň chrání buňky od nebezpečí netýkajících se patogenů. Nejméně čtrnáct DNA senzorů bylo doposud objeveno, mezi nimi – IFI16 a cGAS. Oba signalizují pomocí adaptorového proteinu STING, což vede k produkci interferonu typu I. Všechny tři tyto molekuly (cGAS, IFI16, STING) podléhají striktní regulaci buď ze strany vlastních buněk k zabránění přehnané imunitní reakce, nebo ze strany virů k útěku před imunitním systémem. Tato práce se zaměřuje na mechanismy negativné regulace těchto tří molekul, do nichž patří: post-translační modifikace jako např. fosforylace, ubiquitinace, SUMOylace, acetylace a metylace; protein-proteinové interakce; degradaci proteasomálním systémem nebo autofagií. Není překvapivé, že viry kódují proteiny schopné negativně regulovat interferonovou odpověď, např. některé proteiny herpes virů interagují s cGAS, IFI16 nebo STING a brání jejich aktivaci nebo způsobují jejich degradaci. Jiné proteiny herpes virů navozují degradaci mRNA senzorů či jejich adaptoru. Faktor

proteázy dengue viru NS2B degraduje cGAS stejně jako virová proteáza viru hepatitidy B, E7 protein lidského papillomaviru a E1A adenoviru váží STING, což vede k jeho inaktivaci.

Klíčová slova: cGAS, IFI16, STING, IFN, negativní regulace, vrozená imunita, interferony, DNA senzory

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List of abbreviations

AA	amino acid
AC	assembly complex
AdV	Adenovirus
AIM2	absent in melanoma 2
AP-1	activator protein-1
ATG	autophagy-related ubiquitin-like protein
ATP/ADP	adenosine-triphosphate/adenosine diphosphate
BLK	B-lymphoid tyrosine kinase
bp	base pair
CBD	cyclic dinucleotide binding domain
CDK1	cyclin dependent kinase 1
cGAMP	cyclic guanosine monophosphate-adenosine monophosphate
cGAS	cyclic guanosine monophosphate-adenosine monophosphate synthase
CTD	C-terminal domain
CTT	carboxy-terminal tail
DAI	DNA-dependent activator of interferon-regulatory factors
DDX3	DEAD-box protein 3
DDX41	DEAD-box helicase 41
DENV	Dengue virus
DHX36	DEAH-box protein 36
DHX9	DEAH-box protein 9
DNA-PK	DNA-dependent protein kinase
ds	double stranded
EBV	Epstein Barr virus
ER	endoplasmic reticulum
ERGIC	ER-Golgi intermediate compartment
FTO	fat mass and obesity-associated protein
G3BP1	GTPase activity protein (SH3 domain) binding protein 1

GTP/GDP	guanosine triphosphate/guanosine diphosphate
HBV	hepatitis B virus
HCMV	human cytomegalovirus
hnRNPA2B1	heterogeneous nuclear ribonucleoprotein A2B1
HPV	human papilloma virus
HSCs	hematopoietic stem cells
HSV-1	herpes simplex virus-1
IFI16	interferon-inducible protein 16
IFIX	interferon-inducible protein X
IFN	interferon
IL	interleukin
IRF3	interferon regulatory factor 3
IRF7	interferon regulatory factor 7
KDM5B/C	H3K4 Lys demethylase 5B/C
KSHV	Kaposi's sarcoma-associated herpesvirus
LANA	latency-associated nuclear antigen
LLPS	liquid-liquid phase separation
LRRFIP1	leucine-rich repeat flightless-interacting protein 1
Mab21	male abnormal 21
MAVS	mitochondrial antiviral signalling protein
MRE11-Rad50	meiotic recombination 11 homolog-Rad50
MyD88	myeloid differentiation primary response 88
NCOA3	nuclear receptor co-activator 3
NF- κ B	nuclear factor κ -light-chain-enhancer of activated B cells
NLRC3	NOD-like receptor family CARD domain containing 3
NLRX1	NLR family member X1
NLS	nuclear localization signal
Nrf2	nuclear factor erythroid 2-related factor 2
NT	nucleotidyl transferase
OAS	2'-5' oligo adenylate synthase

OASL	oligoadenylate synthetase-like
OB	oligonucleotides/oligosaccharides fold
p204	protein 204
PAD	pyrin association domain
PPM1A	protein phosphatase 1A
PPP6C	protein phosphatase 6
PRMT5	arginine methyltransferase 5
PTM	post-translational modification
PTPN1/2	protein tyrosine phosphatase non-receptor type 1/2
RECON	Reductase Controlling NF- κ B
RNA pol III	RNA polymerase III
SEN2	SUMO specific peptidase 2
SLE	systemic lupus erythematosus
SOX2	sex determining region Y-box 2
STIM1	stromal interaction molecule 1
STING	stimulator of interferon genes
TBK1	TANK-binding kinase 1
TLR9	toll-like receptor 9
TNF- α	tumour necrosis factor- α
TRIM	tripartite motif containing
TTLLs	tubulin tyrosine ligase-like glutamylases
ULK	UNC-51-like kinase
ZBP1	Z-DNA binding protein

1. Introduction

Intracellular DNA sensing by pattern recognition receptors has been discovered and established as a crucial innate immunity mechanism used by cells in defence against pathogens. These pathogens, either viruses or bacteria, contain DNA as their genomic nucleic acid and in some moments of their life and/or replication cycle expose it to these cellular sensors, which then activate various anti-pathogen signalling pathways leading to the production of certain defence molecules or degrading pathways – cytokines such as interferon (IFN), proinflammatory or inflammasome related cytokines or autophagy. These sensors can be localized in different intracellular compartments, exclusively cytosol, in both the cytosol and the nucleus, or in membranes. All DNA sensors require an adaptor protein upon their interaction with DNA to transmit the signal further to transcription factors, which are responsible for the production of the correct response molecules. However, the importance of DNA sensing does not end with foreign DNA sensing, these sensors play an important role in sensing self-DNA as well. In this context, the nuclear DNA can be mislocalized in the cytosol, e.g., in cells undergoing stress. Another similar case could be the sensing of leaked mitochondrial DNA in the cytosol or the sensing of damaged DNA in the nucleus. Lastly, DNA sensing has been also described in cancerous cells, where in some cases the DNA either is mislocalized or damaged serving as a substrate for the sensors. Thus, it can be concluded the absence of these mechanisms could result in serious pathologies. On the other hand, excessive signalling and overexpression of the anti-pathogen molecules can be equally dangerous and sometimes even more damaging than the initial infection. Precisely for this reason, cells have developed various complex mechanisms of negative regulation. However, not only cells need to inhibit these pathways. Pathogens, especially viruses, have evolved many intricate mechanisms to escape the sensors or suppress certain steps of the signalling cascades. Two sensors have been often reported as sensors of DNA of pathogens or self-DNA in various cells, cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) and interferon inducible protein 16 (IFI16). Both sensors signal via stimulator of interferon genes protein (STING) to induce production of type I IFN. In this thesis, I will focus on describing how these three key players of innate immunity, cGAS, IFI16 and STING, are negatively regulated in the context of the IFN response.

2. Aims

1. Describe the structure and properties of DNA sensors, cGAS and IFI16 and the STING adaptor protein, in the context of the innate immune responses.
2. Summarize the knowledge about the mechanisms of negative regulation of the cGAS, IFI16, and STING in the context of the immune responses leading to the production of IFN.

3. DNA immune sensing and its components

3.1 DNA sensors an overview

DNA sensors are molecules with the ability to bind to DNA and activate cell responses, both DNA of pathogens or self-DNA when mislocalized in the cytosol (Unterholzner et al., 2010, Chiu et al., 2009, Burdette et al., 2013). DNA sensing plays an important role in the activation of the innate immune system (Orzalli et al., 2015, Zhang et al., 2011, Sun et al., 2013, Takaoka et al., 2007, Ferguson et al., 2012, Unterholzner et al., 2010). Up to now, there are at least fourteen DNA sensors described – IFIX (interferon-inducible protein X), IFI16, AIM2 (interferon-inducible protein 2/absent in melanoma), ZBP1 (interferon-inducible protein – Z-DNA binding protein also referred to as DAI – DNA-dependent activator of IFN-regulatory factors), DNA-PK (DNA dependent protein kinase), Ku70 protein, RNA pol III (RNA polymerase III), TLR9 (toll-like receptor 9), LRRFIP1 (leucine-rich repeat flightless-interacting protein 1), MRE11-Rad 50 (meiotic recombination 11 homolog-Rad 50), DDX41 (DEAD-box helicase 41), DHX36 (DEAH-box protein 36), DHX 9 (DEAH-box protein 9) and cGAS. Activation of DNA sensors induces the production of IFN (or other cytokines, e.g., inflammasome-related cytokines). Production of IFN or inflammasome activation is related to the type of sensors activated. Notably there are three types of IFNs – type I, type II and type III. Type I includes α and β forms, type II consists of the γ form and the IFN III includes the λ form. This division is based on the type of the cell that produces the IFN and the functional characteristics of the protein (Huérffano et al., 2022)*. The pathways through which each sensor is signaling are being elucidated and will be briefly described as follows.

IFIX and IFI16 contain a nuclear localisation signal (NLS), these are one of the few sensors found in the nucleus and not exclusively in cytosol (Diner et al., 2015). It has been shown both IFIX and IFI16 interact with the endoplasmic reticulum (ER) localised adaptor protein – STING (Diner et al., 2015, Almine et al., 2017) which then results in the activation of the TANK-binding kinase 1 (TBK1), interferon regulatory factor 3 (IRF3) or/and nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) (Morrone et al., 2014, Stratmann et al., 2015). Both transcription factors, induce production of type I IFNs (Sato et al., 2000). TBK1 is IRF3's essential activator via phosphorylation (Fitzgerald et al., 2003). It was described that after binding of IFI16 to DNA in the nucleus IFI16 is translocated to cytosol. Acetylation of IFI16 was found to be essential for the protein translocation to cytosol (Howard et al., 2021). The activation of the PYHIN interferon-inducible proteins in some conditions leads to the formation of the inflammasome (Ansari et al., 2013, Bürckstümmer et al., 2009). From this family, IFI16 has been widely shown to play an important role in sensing viral genomes and other sources of intracellular DNA for the production of IFN in immune and non-immune cells (Diner et al., 2015)*.

Another dsDNA sensing protein DAI is mainly activated by Z and B forms of DNA (Takaoka et al., 2007). Takaoka et al., showed that immediately after activation DAI forms complexes or associates with both TBK1 and IRF3 (Takaoka et al., 2007). Moreover, studies during a cytomegalovirus infection have shown interesting cooperation between DAI and DEAD box protein 3 (DDX3) (DeFilippis et al., 2010). DDX3 is a helicase that binds to the IFN β promoter independently of IRF3. However, in these experiments it was suggested DDX3 plays a vital role in IRF3-dependent transcription. As well as IRF3, DDX3 is a target of TBK1 and is activated upon its phosphorylation. The kinase activity of TBK1 is triggered by DAI.

DNA-PK is a multifunctional protein first discovered as a DNA damage response protein, specifically DNA-PK recognizes double-stranded DNA (dsDNA) breaks and repairs them via the mechanism of non-homologous end joining recombination (Walker et al., 2001, Gell et al., 1999, Singleton et al., 1999, Mimori et al., 1986). Recently it has been discovered as a DNA sensor leading to type I IFN production as well as type III IFN (Ferguson et al., 2012, Burleigh et al., 2020, Zhang et al., 2011). DNA-PK consists of three proteins – Ku70, Ku80 and DNA-PK catalytic subunit (DNA-PK_{CS}), where Ku70 and Ku80 form a heterodimer (Nussenzweig et al., 1996, Gu et al., 1997). It was discovered Ku70 sensing leads to type III IFN production via STING (Sui et al., 2017) and DNA-PK sensing to type I IFN production in a STING-independent or dependent manner (Ferguson et al., 2012, Burleigh et al., 2020).

RNA pol III recognizes cytosolic dsDNA, mainly sequences rich in adenine and thymine, transcribes it into an RNA intermediate serving as a ligand for RIG-I which then leads to the production of type I IFN (Chiu et al., 2009) via an adaptor mitochondrial antiviral signalling protein (MAVS) (Kawai et al., 2005).

MRE11 was shown to interact with dsDNA in the cytoplasm, the STING adaptor protein was found to be required for the TBK1-IRF3 pathway activation. Interestingly, in this pathway the importance of RAD50, a protein that binds to MRE11 was suggested (Kondo et al., 2013).

The DNA sensor TLR9 is present in the membrane of the endosome and recognises unmethylated CpG DNA (Hemmi et al., 2000) (CpG DNA are motifs in the sequence where cytosine is followed by guanine in the 5' -> 3' direction). In mammals, these sites are overall methylated, while in bacteria we find these sites to be hypomethylated. Recently, another feature of DNA was shown to be recognized by TLR9 and it is the presence of cytosine at the second position from the 5' end (5'-xCx DNA) (Ohto et al., 2018). Like other toll-like receptors, TLR9 also interacts with the adaptor protein myeloid differentiation primary response 88 (MyD88). Depending on the cell type downstream of MyD88, there is an activation of NF- κ B for the production of proinflammatory cytokines (e.g., interleukin-6 (IL-6) or interleukin-1 (IL-1)) or the activation of interferon regulatory factor 7 (IRF7) resulting in the production of type I IFN (Hemmi et al., 2000).

Cytosolic LRRFIP1 senses foreign DNA and viral dsRNA subsequently inducing type I IFN production via an adaptor protein β -catenin (Liu et al., 2015). β -catenin then binds to IRF3, which binds to the type I IFN promoter leading to the production of IFN (Yang et al., 2010).

RNA helicases are a group of cytosolic DNA sensors in plasmacytoid dendritic cells. These enzymes can be divided into two groups: DHX and DDX (Abdelhaleem et al., 2003). DHX9 and DHX36 are known to detect CpG DNA. However, only DHX36 is a type I IFN inducer via MyD88 and IRF7, while DHX9 leads to the production of IL-6 and tumour necrosis factor α (TNF- α) after the activation of MyD88 and NF- κ B (Kim et al., 2010). DDX41 binds to dsDNA and interacts with STING activating the TBK1-IRF3 pathway (Zhang et al., 2011).

Perhaps the most studied sensor is cGAS, which produces a cyclic guanosine monophosphate-adenosine monophosphate (cGAMP dinucleotide), specifically 2'-3'-cGAMP after binding to DNA. This nucleotide then binds to STING promoting the TBK1-IRF3 pathway (Ablasser et al., 2013) leading to type I IFN production (Ishikawa et al., 2008, Sun et al., 2013). This enzyme can be found in cytosol, nucleus and also in membranes on many cell surfaces serving an important role in distinguishing between exogenous and endogenous DNA. While both cytosolic and membrane cGAS are important DNA sensors, nuclear cGAS shows very low sensing activity (Barnett et al., 2019).

Table 1 summarises the localization of the DNA sensor proteins and the main signalling pathways used for each sensor for the production of IFNs and other cytokines.

Sensor	Localization	Signalling pathway	References
cGAS	Nucleus and cytosol	STING-TBK1-IRF3 for production of type I IFN	(Ablasser et al., 2013, Ishikawa et al., 2008)
IFI16	Nucleus and cytosol	1. STING-TBK1-IRF3/NF- κ B for production of type I IFN; 2. Inflammasome for activation of IL-1 β and IL-18	(Diner et al., 2015, Almine et al., 2017, Morrone et al., 2014, Stratmann et al., 2015, Gugliesi et al., 2005)
IFIX	Nucleus and cytosol	STING-TBK1-IRF3 for production of type I IFN	(Diner et al., 2015)
DAI	Cytosol	1. STING-TBK1-IRF3 for production of type I IFN 2. STING-TBK1-IRF3-DDX3 for production of type I IFN	(Takaoka et al., 2007) (DeFilippis et al., 2010)
RNA pol III	Nucleus and cytosol	RIG-I-MAVS-TBK1-IRF3 for type I IFN production	(Chiu et al., 2009, Kawai et al., 2005)
MRE11-RAD50	Nucleus	STING-TBK1-IRF3 for production of type I IFN	(Kondo et al., 2013)
AIM2	Cytosol	Inflammasome induction for activation of IL-1 β and IL-18	(Diner et al., 2015)
DNA-PK	Nucleus	1. STING-TBK1-IRF3 for type I IFN production 2. STING-independent pathway for production of type I IFN	(Ferguson et al., 2012, Burleigh et al., 2020)
Ku70	Cytosol	IRF1/7 for production of type III IFN	(Zhang et al., 2011)
LRRFIP1	Cytosol	β -catenin for production of type I IFN	(Liu et al., 2015, Yang et al., 2010)
TLR9	Endosomal membrane	1. MyD88-IRAK 1,2,4, and TRAF6, TRAF3 for production of type I IFN 2. Myd88-IRAK 1,2,4 and TRAF6, IRF7 for production of type I IFN	(Hemmi et al., 2000) (Kumagai et al., 2008)*
DDX41	Cytosol	STING-TBK1-IRF3 for production of type I IFN	(Zhang et al., 2011, Abdelhaleem et al., 2003)
DHX36	Cytosol	STING-TBK1-IRF3 for production of type I IFN	(Abdelhaleem et al., 2003, Kim et al., 2010)
DHX9	Cytosol	MyD88-IRF7 for production of proinflammatory cytokines IL-6 and TNF- α	(Abdelhaleem et al., 2003, Kim et al., 2010)

Table 1. Overview of the DNA sensors - their localization and the signaling pathways they induce.

3.2 DNA sensor cGAS: structure and mechanism of binding to DNA

Human cGAS contains 522 amino acid (AA) residues. Residues 1-160 AA form a positively charged N-terminus. This part of the protein is disordered and is important together with other protein domains for DNA binding (Gentili et al., 2019, Sun et al., 2013). Studies of the detail of the protein structure suggest

the N-terminus plays a role in the stability of the protein (Kranzusch et al., 2013). The C-terminus contains the catalytic nucleotidyl-transferase (NT) core domain. Overlapping with NT domain in residues 213-513 AA is located the Male abnormal 21 domain (Mab21) (Xie et al., 2019, Kranzusch et al., 2019). The domains of the cGAS protein are presented in figure 1.

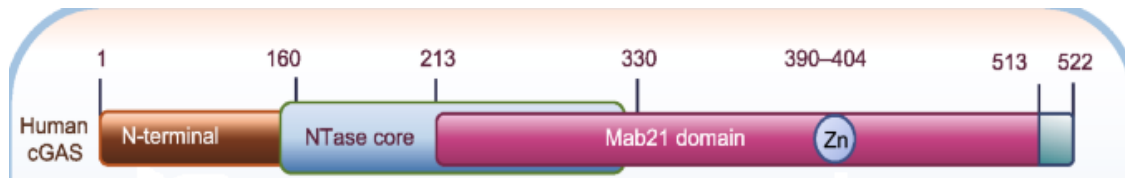


Figure 1. cGAS structure. A model of cGAS domains showing the N-terminal, catalytic core and the Mab21 domain including the zinc-ribbon domain. Modified from (Bai et al., 2022).

cGAS catalytic domain is homologous to that of the RNA sensor OAS (2'-5' oligo adenylate synthase). Crystal structures have shown a zinc-ribbon DNA-binding domain inside the Mab21 domain, which seems to play a crucial role in cGAS-STING signalling and the detection of exogenous DNA as well as in the deciding of the specificity of cGAS towards dsDNA by forming a loop in the protein, which enables the binding to dsDNA (Kranzusch et al., 2013).

cGAS requires a minimum DNA length of 20 base pairs (bp) (Andreeva et al., 2017). The DNA interaction is mediated through electrostatic interactions and hydrogen bonds (Li et al., 2013). Upon dsDNA binding cGAS forms a dimer which seems crucial for the stabilizing its enzymatic activity. Furthermore this dimerization changes the dsDNA's conformation into a high-affinity form enabling the binding of other cGAS dimers and ultimately forming a ladder-like structure of cGAS dimers (Andreeva et al., 2017). The enzymatic activity correlates with the accumulation of DNA. It was discovered cGAS-DNA complex condensates into droplet structures by liquid-liquid phase separation (LLPS), which serves as an optimal environment for the cGAMP synthesis and induces a conformational change in cGAS crucial for the cGAMP production (Kranzusch et al., 2019, Sun et al., 2013, Du et al., 2018). In an inactive state, cGAS forms a complex with GTPase activity protein (SH3 domain) binding protein 1 (G3BP1), a very low level of condensation occurs already in this complex. However, by the accumulation of DNA, G3BP1 is replaced by DNA promoting LLPS and producing cGAMP (Liu et al., 2019). Analogously this happens with dsRNA, but no conformational change takes place thus no cGAMP is produced (Du et al., 2018). Figure 2 summarises the formation of ladder-like structure and cGAS-DNA condensation into droplets.

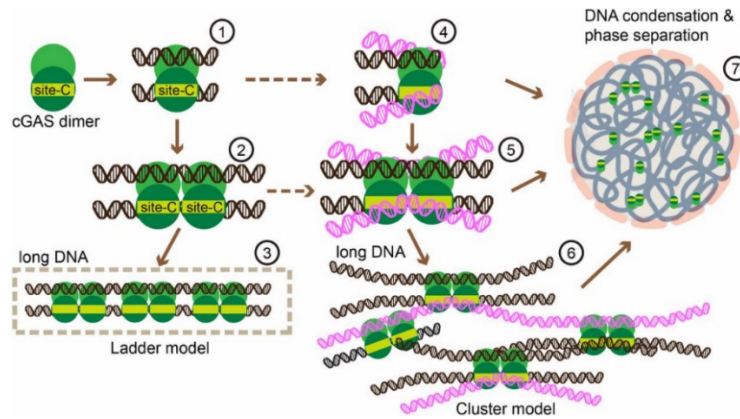


Figure 2. Model of cGAS-DNA complex condensation into droplets. 1) cGAS dimer binds dsDNA, 2) other cGAS dimers with dsDNA are recruited and 3) gradually form a ladder-like structure (ladder model), 4)/5) cGAS dimer or more cGAS dimers bind additional long DNA sequences, 6) through which they form a so-called cluster model, 7) DNA condensates and cGAS undergoes LLPS forming droplets. Modified from (Xie et al., 2019).

The product of this enzyme, cGAMP, contains mixed 2'-5' and 3'-5' phosphodiester bonds, unlike prokaryotic second messenger molecules with exclusively 3'-5' bonds (Kranzusch et al., 2015). The docking site in STING's C-terminal domain (CTD) for c-di-GMP or cGAMP was shown to be very small and thus unable to bind adenosine triphosphate/adenosine diphosphate (ATP/ADP) or guanosine triphosphate/guanosine diphosphate (GTP/GDP) (Ouyang et al., 2012).

In humans, cGAS (hcGAS) was shown to be subject to stricter regulation than in other mammals in a manner that hcGAS is more efficient in distinguishing between short and long exogenous dsDNA than in mice. This is determined by two amino acid residues, Lys187 and Leu195, which substitute these two in mouse's cGAS in fact promoted human-like regulation in mice (Zhou et al., 2018).

3.3 DNA sensor IFI16: structure and mechanism of binding to DNA

IFI16 is a part of the PYHIN family proteins and contains 785 amino acids. These proteins use their HIN domains to recognize and bind dsDNA. IFI16 contains two HIN domains, specifically HINa and HINb, and one PYRIN domain. The HIN domain binds DNA in a sequence-independent manner mediated through electrostatic interactions of positively charged HIN and negatively charged DNA sugar-phosphate backbone (Jin et al., 2012). Inside the HIN domains are two oligonucleotides/oligosaccharides (OB) folds that are held together by a long linker containing two alpha helices. Figure 3 summarises the IFI16 protein structure.

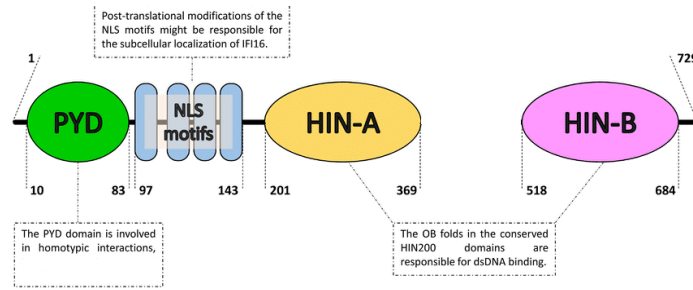


Figure 3. IFI16 structure. Model showing the PYD/PYRIN domain mediating protein-protein interactions, NLS motifs responsible for its nuclear localization and the two HIN domains HINa and HINb both crucial for binding DNA. Modified from (Caneparo et al., 2018).

It was reported that IFI16's affinity to DNA depends on its length (Unterholzner et al., 2010). Studies show there to be more factors regarding the affinity and the ability to recognize dsDNA. It seems IFI16 prefers cruciform and supercoiled DNA forms (Brázda et al., 2012) as well as quadruplex DNA structures (Hároníková et al., 2016).

Some studies showed the presence of both HINa and HINb in IFI16 or its mouse ortholog p204 to strengthen DNA binding (Unterholzner et al., 2010).

For IFI16 it was shown that longer DNA fragments induce a higher IFN response. However, 16bp fragments were reported to induce activation of IFI16 (Unterholzner et al., 2010). Later, it was discovered that 150bp long fragments are the most favourable for induction of IFN by this sensor. In fact, it was elucidated that ten IFI16 molecules are optimum for the activation of signalling (15bp/per one molecule of IFI16) (Morrone et al., 2014).

The HIN domains contain OB1 fold, OB2 fold and a linker in between. These are used to bind DNA, however, each in a slightly different manner. HINa binds DNA by using both OB1 and OB2 and binds to only one strand, thus is able to bind both ss (Brázda et al., 2012) and ds DNA by viewing DNA as two single strands enabling IFI16 to sense various nicks and damaged DNA either pathogen-derived or self-derived. HINb utilises the linker region and OB2 fold and binds specifically dsDNA (Ni et al., 2016). For p204, it was revealed the DNA is sensed by both of the HIN domains simultaneously by a HINab complex. Three of these HINab complexes have been shown to bind dsDNA and form a C-shaped structure around it. Upon this formation, HINa forms a dimer leading to the PYRIN domains' aggregation of other IFI16 molecules forming a filamentous structure, which then binds to STING and activates the type I IFN signalling pathway (Fan et al., 2021). The model of IFI16 binding to DNA and oligomerizing is summarised in figure 4.

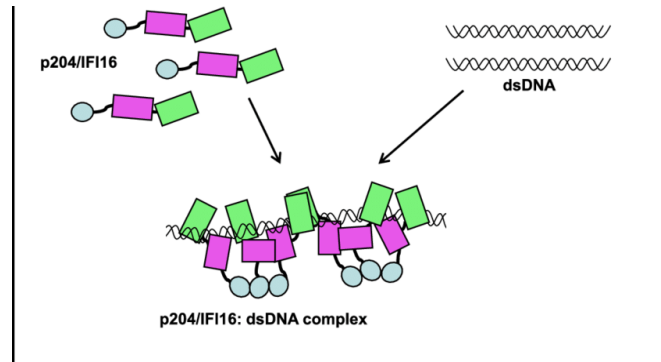


Figure 4. Model of IFI16 binding to DNA. HINa is presented in purple, HINb in green and the PYRIN domains in blue. The HIN domains of multiple IFI16 molecules bind to dsDNA, HINa dimerizes and PYRIN domains of different IFI16 molecules aggregate. Modified from (Fan et al., 2021).

3.4 STING adaptor protein: structure and mechanism of activation by cGAS and IFI16

STING is an important innate immunity molecule containing 379 amino acids. It resides in the ER membrane containing four transmembrane domains and a cytoplasmic CTD (Burdette et al., 2013). The CTD comprises of the dimerization domain (DD) and the carboxy-terminal tail (CTT). CTD forms a dimer via hydrophobic interactions in the cell resembling the letter V or a butterfly (Shu et al., 2012). This protein serves as an adaptor protein for many DNA sensors mentioned in chapter 2. The best-known mechanism is the one mediated by cGAMP after DNA sensing (Shang et al., 2012). Upon binding of the cGAMP to STING, the ligand binding cytoplasmic CTD dimer changes its conformation and rotates relatively to the transmembrane helices (Shang et al., 2019). This rotation creates a conformational change and a pocket for cGAMP sealed by a newly formed beta-sheet lid (Gao et al., 2013). The cyclic dinucleotide binding domain (CBD) is localised in the central area and is oriented towards the cytoplasm. It contains a connector element serving as a connecting point between two STING proteins (Liu et al., 2015). The structure of STING is summarised in figure 5.

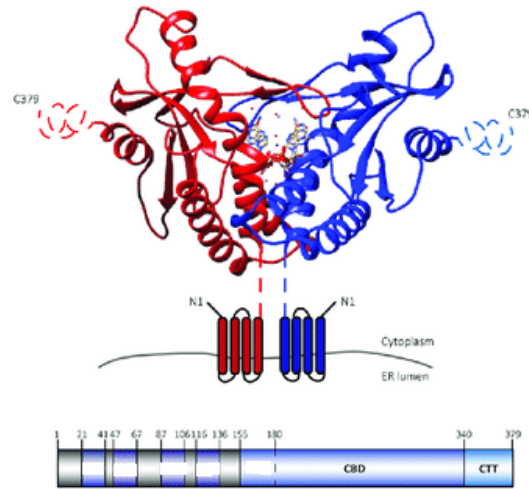


Figure 5. Model of STING structure. A STING dimer with C-termini in the cytosol and N-termini bound to the ER membrane is visible in the upper part. The lower part domain model shows the CBD domain and the CTT domain. Modified from (Landman et al., 2020).

STING also contains a TBK1 binding site crucial for the activation of IFN production. TBK1-STING interaction seems to be strengthened by the cGAMP-STING complex (Zhang et al., 2019). TBK1 as well as STING forms a dimer, and each subunit contains a kinase domain, scaffold and dimerization domain.

The binding of cGAMP to STING leads to its activation (Sun et al., 2013). The activation comprises two important steps. Firstly, the STING dimers oligomerize into linear tetramers. This oligomerization is possible by cGAMP binding to STING's CBD which then rotates by 180° and enables STING to oligomerize (Zhang et al., 2019). At the same time, the second step of activation takes place – the translocation of STING from the ER into Golgi via the ER-Golgi intermediate compartment (ERGIC). This process seems to be promoted by cGAMP (Gui et al., 2019). After translocation, TBK1 is recruited to STING's TBK1-binding motif on its C-terminus. TBK1 also forms a dimer meaning one TBK1 dimer interacts with two TBK1-binding motifs. For its activation TBK1 needs to be phosphorylated by autophosphorylation. This is possible exclusively when STING is in its oligomer form. Once activated, TBK1 phosphorylates STING, which is now fully activated and able to bind IRF3. IRF3 is immediately phosphorylated upon its recruitment (Zhang et al., 2019). Phosphorylated IRF3 bound to STING recruits an unphosphorylated IRF3. These two IRF3s form a dimer preceding the phosphorylation of the newly recruited IRF3. This dimer then detaches itself and translocates into the nucleus (Liu et al., 2015).

The activation of STING by cGAS is summarised in figure 6.

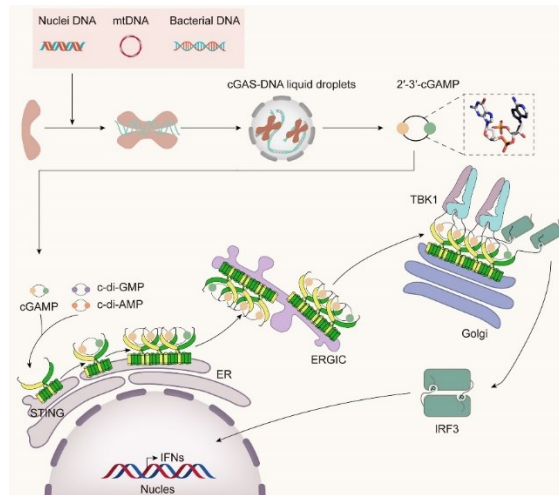


Figure 6. Model of activation of STING by cGAS. cGAS senses DNA forms liquid droplets and starts producing cGAMP. cGAMP binds to STING in the ER and STING then translocates into Golgi via the ERGIC compartment. In Golgi, it associates with and is phosphorylated by TBK1, which then leads to the phosphorylation of IRF3, its translocation into the nucleus and the production of type I IFN. Source (Liu et al., 2022).

Multiple studies showed that IFI16 associates with STING upon DNA recognition. After the activation of STING by DNA sensing mechanisms, the ER-residual protein translocates to a perinuclear compartment – the so-called non-ER microsome compartment (Ishikawa et al., 2009). Other studies managed to explain this phenomenon in a more detailed manner (Parker et al., 2015., Stetson et al., 2006, Reinert et al., 2016). After applying brefeldin A, a molecule blocking the ER-Golgi trafficking, STING was not found in the perinuclear vesicles, leading to the conclusion that it is transported via the Golgi apparatus. Furthermore, in these same vesicles, which contained STING, both IFI16 and TBK1 were present.

It was established in the previous chapter, that IFI16 forms filamentous structures upon DNA sensing (Fan et al., 2021). These filamentous IFI16 molecules then relocate from the nucleus to the ER and interact with STING, which enables and is followed by the recruitment of TBK1 and IRF3. Afterward, STING and IRF3 are phosphorylated, resulting in the canonical STING-TBK1-IRF3 induction of type I IFN (Morrone et al., 2014, Stratmann et al., 2015, Lum et al., 2019).

The mechanism of STING activation by IFI16 is summarised in figure 7.

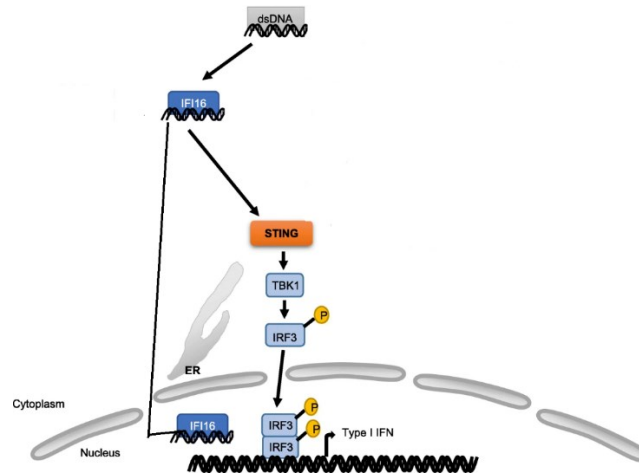


Figure 7. Model of STING activation by IFI16. IFI16 binds dsDNA either in the cytosol or in the nucleus. Cytosolic IFI16 then directly interacts with STING; nuclear IFI16 first translocates from the nucleus and then interacts with STING. Afterwards, TBK1 and IRF3 are recruited, IRF3 is phosphorylated and translocates to the nucleus, binds to DNA and type I IFNs are transcribed. Modified from (Li et al., 2021).

It is worth mentioning that cGAS and IFI16 pathways have been implied in multiple studies to be intertwined. The first such study conducted its research on human foreskin fibroblasts infected by the herpes simplex virus 1 (HSV-1). The results implied both cGAS and IFI16 were required for HSV-1 sensing. They determined that it is IFI16 that senses the virus itself and is recruited to the site of the infection, while cGAS produces small amounts of cGAMP and directly interacts with IFI16. In cGAS depleted and HSV-1 infected cells, the half-life of IFI16 was reduced twofold and upon restoration of cGAS, returned to normal (Orzalli et al., 2015). Another study done on cancerous cells and keratinocytes hinted at an interplay of cGAS and IFI16 pathways. In cancer-derived cells, where many aspects and components of the DNA sensing apparatus are absent, two findings were observed – 1) cGAS activates STING in IFI16 depleted cells, 2) IFI16 activates STING in cGAS depleted cells. However, when these two experiments were repeated on human keratinocytes, the authors realised neither was sufficient for the activation of STING without the other. They found that cGAMP interacts with IFI16 and stabilises it in order to activate STING (Almine et al., 2017). Logically, it seems there is a big difference and a higher level of complexity of the regulation of these pathways in the physiological conditions and healthy cells than in the cancerous cells and laboratory conditions. Lastly, a study on human macrophages revealed that IFI16 promotes the enzymatic activity of cGAS and its subsequent activation of STING. Interestingly, the authors postulated that IFI16 is a co-factor of cGAS in macrophages, ensuring an adequate response in these first-line innate immunity cells (Jönsson et al., 2017). It is apparent that cGAS and IFI16 are connected but many questions remain to be answered.

4. Negative regulation of the interferon responses induced by cGAS and IFI16

4.1 Negative regulation of cGAS

There are several mechanisms, which negatively regulate DNA sensor cGAS in the context of IFN production, among them – the blockage of cGAS DNA binding site, the blockage of cGAS catalytic site, cGAS degradation or the low production of cGAMP. Specific details of these mechanisms – activated by cells or viruses, will be discussed below.

In recent studies, the mechanisms by which cGAS is negatively regulated during mitosis were described. During cell division, it is crucial for cGAS not to be activated by genomic DNA. Firstly, during interphase, nuclear cGAS is suppressed via an interaction with the nucleosome, specifically with the histone H2A-H2B. The mechanism of this inhibition was described as competitive inhibition, meaning cGAS was revealed to bind these nucleosomes with higher affinity than dsDNA (Zierhut et al., 2019). Another study provided more details on the interaction of the nucleosome and cGAS. The authors experimented with two types of nucleosomes – one with a 40bp long linker DNA and one with no linker DNA. The absence of linker DNA was proven to negatively regulate cGAS. Furthermore, cryo-electron microscopy of the complex cGAS:H2A-H2B revealed cGAS to have very small to no contact with nucleosomal DNA (Michalski et al., 2020). Furthermore, the DNA binding protein barrier-to-autointegration factor 1 (BAF) competes with cGAS for DNA binding. Down-regulation of BAF results in activation of IFN response in cells (Guey et al., 2020).

After the interphase ends and the nuclear envelope breaks down, a significant amount of cytosolic cGAS is exposed to nuclear DNA, thus, the chromatin-mediated inhibition is insufficient. During this phase, the N-terminus of cGAS is phosphorylated by mitotic kinases, including the aurora kinase B. In detail, seven serine sites and two threonine sites were identified as the phosphorylated AA residues. Apart from Ser305, in the catalytic domain, all the remaining sites are situated in the N-terminus and are hyperphosphorylated. The authors postulated hyperphosphorylation of these amino acids, which are then significantly negatively charged, disable the interaction with negatively charged DNA (Li et al., 2021).

In agreement, it was shown that Akt kinase, also known as protein kinase B or case cyclin-dependent kinase 1 (CDK1), phosphorylate hcGAS in position Ser305, this post-translational modification (PTM) occurs in close proximity to cGAS catalytic site and therefore leads to low or no production of cGAMP (Seo et al., 2015, Zhong et al., 2020).

cGAS was found to translocate from the cytosol into the nucleus after treating cells with DNA damage-inducing agents. The translocation was shown to be dependent on dephosphorylation at Tyr215 of cGAS.

In depth, a kinase phosphorylating cGAS at this position, the B-lymphoid tyrosine kinase (BLK) was identified as a negative regulator of cGAS by preventing its translocation to the nucleus (Liu et al., 2018).

DNA-PK, a sensor described in chapter 2, induces type I IFN response via STING independent pathway in cells lacking STING. However, in cells where STING is present, DNA-PK was discovered to serve as a homeostatic regulator of the immune response mediated through cGAS by phosphorylating it at positions Thr68 and Ser213. Thr68 is located in the N-terminus and its modification leads to the impairment of cGAS oligomerization, while Ser213 is located near the catalytic domain and the cGAMP production is inhibited (Sun et al., 2020).

Interestingly, the protein phosphatase 6 (PPP6C) dephosphorylates cGAS at Ser435 and decreases its affinity towards GTP (one of the substrates of cGAS together with ATP) suppressing its enzymatic activity. PPP6C was found to be in close proximity to cGAS in un-infected cells (Li et al., 2020).

Another PTM shown to modulate cGAS activity is ubiquitination. This modification leads to proteolytic and non-proteolytic outcomes. Adding ubiquitin is provided by three enzymes – E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase) (Wilkinson et al., 2000, Mukhopadhyay et al., 2007). As for cGAS, this modification works both as a positive and a negative regulatory mechanism. The result depends on the type of ubiquitin ligase and the target site for ubiquitination. In the first case, ubiquitin is added with Lys28-linked bonds by E3 ubiquitin ligase tripartite motif containing 14 (TRIM14) – this stabilises cGAS and enhances the production of type I IFN (Wang et al., 2017). On the other hand, Lys48-linked ubiquitination leads to cGAS degradation and hinders the production of type I IFN (Chen et al., 2016).

Similar to ubiquitination, SUMOylation also either stabilises or destabilises (inhibits) cGAS. In a study, TRIM38 was described as an E3 SUMO ligase adding SUMO to cGAS and SENP2 as a SUMO-specific protease. The SUMOylation happens at two sites on cGAS – Lys231 and Lys479. During an early phase of viral infection or in uninfected cells, it is crucial for cGAS to be active. This is enabled by SUMOylation in these specific sites, which disables Lys48-linked ubiquitination of cGAS and its degradation. On the other hand, in the late stages of an infection, SENP2 degrades SUMO chains from cGAS, releasing the sites for Lys48-linked ubiquitination and cGAS is degraded (Hu et al., 2016). Another study showed SUMOylation of cGAS in different sites – Lys335, Lys372, and Lys382. These sites lie in close proximity of DNA binding sites disabling cGAS's ability to bind DNA (Cui et al., 2017).

The next PTM negatively regulating cGAS is glutamylation. Generally, during glutamylation two types of enzymes play an important role – tubulin tyrosine ligase-like glutamylases (TTLs), which mediate glutamylation and enzymes of the cytosolic carboxypeptidase family, which work antagonistically to

TTLLs (Rogowski et al., 2010). Two glutamylations have been described as negatively regulating cGAS – 1) polyglutamylation at Glu272 by TTLL6 and 2) monoglutamylation at Glu302 by TTLL4. The first modification interferes with DNA binding while the second one with the enzymatic activity, both leading to the decrease of cGAMP and type I IFN production (Xia et al., 2016).

In a study dealing with interferonopathies (e.g., lupus) the role of acetylation of cGAS has been brought to light. The authors conducted experiments with various medicines that could negatively regulate cGAS – one of them being aspirin. Aspirin proved to acetylate cGAS at the Lys414 residue, which was identified as the key to the suppression of cGAS by decreasing its DNA binding properties. A genetically engineered system producing acetylated cGAS was constructed, and indeed, its function was dramatically reduced. Apart from Lys414, two other acetylation sites were described, Lys384 and Lys394, including Lys414. These are conserved residues among different species and their mutation was shown to lead to a significant reduction of IRF3 phosphorylation (Dai et al., 2019).

Recently, palmitoylation has been described as a mechanism of negative regulation as well. This study showed the role of a palmitoyl transferase ZDHHC18, which palmitoylates cGAS at the Cys474 site inhibiting its enzymatic ability. This modification mainly hinders the catalytic part of cGAS. However, in the same study, there were results pointing to a decreased DNA binding ability as well. It seems likely this is caused by a mechanistic interference to the DNA binding site (Shi et al., 2022).

The final PTM and also recently discovered, is methylation. A study elucidated the role of arginine methyltransferase 5 (PRMT5) in the negative regulation of cGAS. PRMT5 modifies Arg124 resulting in dimethylation of this site and blockage of the ability to bind DNA. Intriguingly, Ma et al. experimented with orally administered inhibitors of PRMT5 in mice during HSV-1 infection and these mice showed increased resistance to this virus (Ma et al., 2021).

There are two pathways found to influence the activity of cGAS, autophagy and inflammasome. Autophagy is a mechanism by which cell components are detained into organelles called autophagosomes and degraded (Takeshige et al., 1992, Baba et al., 1994, Tsukada et al., 1993). It was shown that beclin-1, an autophagy protein, interacts with the catalytic domain of cGAS and inhibits the production of cGAMP (Liang et al., 2014).

Interestingly, cGAS was shown to target micronuclei (containing nuclear DNA leaked to cytosol) to autophagosomes. It was revealed cGAS interacts with autophagy receptors from the autophagy-related ubiquitin-like protein family (ATG8) – LC3A, LC3B and LC3A. These proteins are known to bind the targets of autophagy and bind them to the membrane of the autophagosome (Xie et al., 2008). Upon this

interaction, cGAS is degraded in the presence of another autophagy-related protein ATG7 (Zhao et al., 2021).

Inflammasome, as well as autophagy (in some cases), serves as an important defence mechanism of the innate immune system (Kanneganti et al., 2006, Gross et al., 2009, Allen et al., 2009, Thomas et al., 2009). A component of the inflammasome, caspase-1, was found to interact with cGAS and cut it at sites D140/157 (Hornung et al., 2009). This was proven true solely during DNA virus infections (HSV-1 and vaccinia virus) and not RNA virus infections (Sendai virus) (Wang et al., 2017).

The ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP1), an enzyme generating diphosphate and responsible for breaking down ATP or GTP to produce pyrophosphate, was found to be a negative regulator of cGAS by hydrolysing the product of the enzyme – cGAMP and subsequently disabling the activation of STING-mediated type I IFN response. ENPP1 is a part of cells' signalling of the insulin hormone (Paola et al., 2011). Li et al. managed to construct chemically modified cGAMP resistant to hydrolysis by ENPP1, which could serve as a potential cure in some pathologies with dysfunctional cGAS regulation (Li et al., 2014). Another enzyme antagonizing cGAS is the interferon-inducible human oligoadenylate synthetase-like (OASL) (Ghosh et al., 2019). Ghosh et al. discovered that OASL acts in an opposite way during infections by DNA viruses, repressing the enzymatic activity of cGAS (Ghosh et al., 2019).

Wu et al. discovered two hypoxia-responsive microRNAs, identified as the molecules used by hypoxic tumours to evade the immune system. Specifically, microRNA25 and microRNA93 were the RNAs responsible for suppressing cGAS and its immune signalling. In detail, the high levels of expression of both microRNAs were related to low levels of cGAS in the cancer cells, moreover, authors found that the cGAS promotor region has a binding site for the activator protein-1 (AP-1), transcription factor and that the miRNAs target the AP-1 co-activator nuclear receptor co-activator 3 (NCOA3) (Wu et al., 2017).

In addition, cGAS was found to influence the activation of hematopoietic stem cells (HSCs) by promoting the exit of their dormant state to the active state using type I IFN signalling (Wilson et al., 2008, Essers et al., 2009). However, overstimulation of type I IFN signalling can lead to the exhaustion of the HSCs (Sato et al., 2009). Thus, a mechanism of negative regulation of cGAS in HSCs was described as mediated through a circular RNA called cia-cGAS. Circular RNA are non-coding RNAs, which form a covalently closed continuous loop; they are usually the products of RNA splicing (Zhang et al., 2016, Jeck et al., 2013). Cia-cGAS RNA binds to cGAS with strong affinity and inactivates its enzymatic (Xia et al., 2018).

The first virus I will discuss will be a dsDNA virus - Kaposi's sarcoma-associated herpesvirus (KSHV). KSHV, as well as other herpesviruses, has the ability to induce latency in host cells (Lu et al., 2012). An important protein expressed during this latent cycle is a latency-associated nuclear antigen (LANA). LANA

has two isoforms – a) containing the N-terminus, and b) not containing the N-terminus. The first one translocates into the nucleus while the second stays in the cytosol. Zhang et al. found that LANA cytosolic isoform binds to cGAS and inhibits the cGAS-STING pathway (Zhang et al., 2016). KSHV has developed more mechanisms to evade the immune system, another being its tegument protein ORF52. This study showed ORF52's ability to bind both cGAS and dsDNA independently of one another. This seems to be a mechanism for inhibiting DNA sensing of cGAS by reducing its catalytic activity and, on the other hand disabling its ability to bind DNA. In addition, homologues of ORF52 in Epstein Barr virus (EBV), Ross River virus and Murine Gammaherpesvirus 68 also block cGAS (Wu et al., 2015).

HSV-1 was proven to inhibit cGAS. The first study examined the virion host shutoff protein UL41, a protein that acts as an endoribonuclease. The results were as follows – UL41 degraded the mRNA of cGAS with its endoribonuclease activity in the nucleus and consequently decreased the actual levels of cGAS (Su et al., 2017). A different study focused on a tegument protein VP22, which bound to cGAS and impeded its catalytic activity (Huang et al., 2018).

Dengue virus (DENV) is a virus with single-stranded positively oriented RNA and without any DNA production during its life cycle. However, it was discovered that cGAS senses mitochondrial DNA, which is released into the cytoplasm during DENV infection (West et al., 2015). DENV has developed a way to degrade cGAS by using its protease factor NS2B (Aguirre et al., 2017). Aguirre et al. postulated this degradation happens in an autophagy-dependent pathway.

There are opposing opinions regarding the Hepatitis B virus (HBV) and its immune system evasion. One study argued that since the expression of cGAS in hepatocytes is generally lower, HBV is able to evade detection passively solely because of this (Lauterbach-Rivière et al., 2020). Another study obtained results suggesting HBV does, in fact, actively negatively influence cGAS by suppressing its expression and cGAS-related genes (Verrier et al., 2018). However, more studies need to be conducted concerning this subject.

Negative regulation of cGAS is summarised in table 2.

Mechanism	Details of the mechanism	Result	Reference
Competitive inhibition (by interaction with cellular proteins)	1. H2A-H2B 2. BAF	Does not bind DNA	(Zierhut et al., 2019), (Michalski et al., 2020)
PTMs	Phosphorylation by: 1. mitotic kinases 2. Akt kinase 3. CDK1 4. BLK 5. DNA-PK	1. No binding DNA 2.,3. No production of cGAMP 4. No translocation to the nucleus 5. No oligomerization	(Li et al., 2021), (Seo et al., 2015), (Zhong et al., 2020), (Liu et al., 2018), (Sun et al., 2020)
	Dephosphorylation by PPP6C	Reduced affinity towards GTP	(Li et al., 2020)
	Ubiquitination by E3 ubiquitin ligases	Proteasomal degradation	(Chen et al., 2016)
	SUMOylation by SENP2	Proteasomal degradation	(Cui et al., 2017)
	Glutamylation by: 1. TTL6 2. TTL4	1. No binding to DNA 2. Reduced enzymatic activity	(Xia et al., 2016)
	Acetylation by aspirin	Does not bind DNA	(Dai et al., 2019)
	Palmitoylation by ZDHHC18	No cGAMP produced	(Shi et al., 2022)
	Methylation by PRMT5	Does not bind DNA	(Ma et al., 2021)
Autophagy related	Protein interactions with: 1. Beclin-1 2. ATG8 family	1. Inhibition of the enzymatic activity of cGAS 2. cGAS degradation	(Liang et al., 2014), (Zhao et al., 2021)
Inflammasome pathway related	Enzymatically by caspase-1	Sequestration of cGAS	(Wang et al., 2017)
Cellular enzymes dependent	1. ENPP1 2. OASL	1. Degraded cGAS 2. Inhibited enzymatic activity	(Li et al., 2014), (Ghosh et al., 2019)
Non-coding RNAs related	By: 1. microRNA25 2. microRNA93	Suppression of cGAS expression	(Wu et al., 2017)
	By cia-cGAS	Reduced enzymatic activity	(Xia et al., 2018)
Virus induced	By KSHV viral proteins: 1. LANA 2. ORF52	No cGAMP produced	(Wu et al., 2015)
	By HSV-1 viral proteins: 1. UL41 2. VP22	1. Degradation of cGAS mRNA 2. No cGAMP produced	(Su et al., 2017), (Huang et al., 2018)
	By DENV viral protein NS2B	Degradation of cGAS	(Aguirre et al., 2017)
	By HBV suppression of the expression of cGAS	cGAS is not transcribed	(Verrier et al., 2018)

Table 2. Summary of mechanisms, which negatively regulate cGAS.

4.2 Negative regulation of IFI16

In comparison to cGAS, IFI16 has been less studied. Nevertheless, studies of IFN responses to Herpesvirus have brought some insights into how viral proteins or viral infection leads to the downregulation of the

IFI16 IFN pathways. In addition, some cellular proteins have also been shown to regulate the activation of this sensor, for example, the presence of nucleosomes has been discovered to prevent IFI16 clustering (Stratmann et al., 2015).

In 2012 a study was conducted on IFI16 during HSV-1 infection in human foreskin fibroblasts by Orzalli et al. This study suggested a general model starting with IFI16 detecting viral DNA in the nucleus followed by signalling to STING in the ER and lastly activating TBK1-IRF3. Simultaneously HSV-1 produces ICP0 in the nucleus, which acts as an E3 ubiquitin ligase, which selects IFI16 as its target for ubiquitination, ultimately leading to the degradation of IFI16 in the proteasome (Orzalli et al., 2012). Results supporting this theory were based on the fact that IFI16 was not degraded under these conditions – during the inhibition of the proteasome or during infection by ICP0 defective mutants. A year later, another study was conducted disputing these findings completely, claiming ICP0 is not required for IFI16 degradation at all. The following results made them come to this conclusion – ICP0 expressed without an infection did not induce the degradation of IFI16; on the other hand, degradation happened during an ongoing infection where ICP0 was not present. Upon this, they postulated the degradation is more likely dependent on some cellular factors activated during HSV-1 infection (Cuchet-Lourenço et al., 2013). In 2016 another study performed by Orzalli et al. used both tumour-derived and normal cells and found that not only ICP0 but also UL41 played a role in the negative regulation of IFI16. As for UL41, results pointed to the downregulation of the expression of IFI16 by degrading IFI16 mRNA in tumour-derived cells. Authors showed ICP0 is sufficient to promote the degradation of IFI16 in normal cells but does not induce degradation in tumour cell lines (Orzalli et al., 2016).

Human cytomegalovirus (HCMV), a DNA virus, has been found to negatively regulate IFI16 via the tegument protein pUL83. HCMV DNA is sensed in the nucleus and activates the canonical STING-TBK1-IRF3 type I IFN inducing pathway. As mentioned in previous chapters, the oligomerization of IFI16 is crucial for its DNA binding activity, which is exactly what is blocked by pUL83. Specifically pUL83 contains an evolutionary conserved N-terminal pyrin association domain (PAD), which is able to bind the pyrin domain of IFI16 and preclude its oligomerization (Li et al., 2013). Another study explaining the interplay of IFI16 and HCMV revealed an intriguing evasion mechanism, where IFI16 is entrapped within new mature virions. They found that IFI16 is bound to viral DNA during the early phases of the infection in the nucleus and in the late stages, mislocalized into the cytoplasmic so-called virus assembly complex (AC) with viral structural proteins. The mechanism was described as follows, IFI16 binds to HCMV protein kinase pUL97, which results in its phosphorylation, the phosphorylated IFI16 is translocated into the cytoplasm and is transported into the ACs by the endosomal-sorting complex required for transport, ultimately confining IFI16 into the newly mustered virions. The incorporation of IFI16 into mature virions

was demonstrated by electron microscopy. It is intriguing whether there is another role for IFI16 molecules, which are packaged into the virions apart from evasion of the innate immunity (Dell'Oste et al., 2014).

An interesting non-viral mechanism of negative regulation was described to be mediated by STING in order to protect the cells from an excessive production of type I IFN. STING recruits ER ubiquitin ligase TRIM21, and then IFI16 is bound to STING via its PYRIN domain. TRIM21 enhances Lys48-linked polyubiquitination of IFI16 at the N-terminus of the protein (lysines 3, 4 and 6), leading to its degradation via proteasome (Li et al., 2019).

Similar to cGAS, methylation by PRMT5 leads to the inhibition of IFI16 as well. The study was performed on IFI16 and its mouse homolog. The methylation of both of these proteins led to the attenuation of TBK1-IRF3 activation and the subsequent decrease in IFN and chemokine production induced by dsDNA (Kim et al., 2020).

Lastly, heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) was revealed to enhance the type I IFN response. This protein interacts with mRNAs of cGAS, IFI16 and STING and mediates the creation of N⁶-methyladenosines as well as the subsequent nucleocytoplasmic trafficking of these RNAs. The catalyzation of this reaction is entirely dependent on the methylation of hnRNPA2B1 itself, specifically at Arg226. A demethylase fat mass and obesity-associated protein (FTO) was found by mass spectrometry to interact with hnRNPA2B1 in the nucleus and keep it in unmethylated state – unable to bind RNA. However, during HSV-1 infection, FTO was found unbound from hnRNPA2B1 enabling its methylation and the methylation of cGAS, IFI16 and STING mRNAs, translocating them into the cytoplasm leading to type I IFN production (Wang et al., 2019). The negative regulation of IFI16 is summarised in table 3.

Mechanism	Details of the mechanism	Result	Reference
Virus-induced	HSV-1: 1. ICP0 2. UL41	1. Proteasomal degradation 2. Degradation of IFI16 mRNA	(Orzalli et al., 2016)
	HCMV: 1. pUL83 2. pUL97	1. Inhibition of oligomerization 2. Entrapment into virions	(Li et al., 2013), (Dell'Oste et al., 2014)
PTMs	Recruitment of TRIM21 via STING	Induce ubiquitination and consequently proteasomal degradation	(Li et al., 2019)
	PRMT5 induced IFI16 methylation	Leads to down-regulation of IFN response	(Kim et al., 2020)
mRNA related	Suppression of methylation of hnRNPA2B1	Detainment of cGAS, IFI16 and STING mRNAs in the nucleus	(Wang et al., 2019)

Table 3. Summary of mechanisms, which negatively regulate IFI16.

4.3 Negative regulation of STING

Regulation of STING is similarly well understood and studied as that of cGAS.

There are several mechanisms that negatively regulate the DNA sensor adaptor STING, among them, blockage of binding sites required for interaction with downstream proteins, STING degradation, proteins competing for the binding of the messenger dinucleotides or inhibition of STING subcellular trafficking. Specific details of these mechanisms activated by cells or by viruses will be discussed below.

The PTMs of STING bear a few similarities with the PTMs of cGAS. STING also undergoes phosphorylation, which modulates its activity. It is known STING requires phosphorylation at Ser358 for the subsequent interaction with TBK1 (Zhong et al., 2008). TBK1 then phosphorylates STING at a different site, Ser366. This PTM is necessary for the recruitment of IRF3 (Liu et al., 2015). Recently, a protein phosphatase 1A (PPM1A) was shown to dephosphorylate both STING and TBK1, disabling the initial recruitment of TBK1 and subsequently of IRF3 (Li et al., 2015).

Intriguingly, phosphorylation at Ser366, apart from activating STING in one particular case actually leads to its inhibition. This reaction is catalysed by the UNC-51-like kinase (ULK1/2). ULK1/2 is typically attached to its repressor adenosine-monophosphate-activated protein kinase. Incredibly, the detachment of ULK1/2 is caused by cGAMP, creating negative feedback against overstimulation by the immune system. It has been postulated that phosphorylation at the same site leads to dramatically different outcomes depending on the phase of the immune response or the phase of the infection (Konno et al., 2013).

Another phosphorylation that enables the activation of STING was identified during the late phases of HSV-1 infection, this phosphorylation at the Tyr245 site by an SRC Tyr kinase (Hu et al., 2019) was found to be negatively regulated by the protein tyrosine phosphatase non-receptor type 1/2 (PTPN1/2). Interestingly, the loss of this PTM leads to 20S proteasomal degradation in a ubiquitin non-dependent pathway. Xia et al. proved the function of PTPN1/2 by measuring the half-life of STING phosphorylated and dephosphorylated at Tyr245, the results were 9h and 3h respectively (Xia et al., 2019).

Similar to cGAS, Akt kinase serves as a negative regulatory mechanism of STING, however, in a slightly different manner. Before the activity of Akt kinase, a different kinase begins the process – receptor Tyr kinase HER2, this kinase interacts with STING directly and enables Akt kinase to phosphorylate but unlike with cGAS, it is TBK1 that is phosphorylated, making it impossible for STING and TBK1 to interact (Wu et al., 2019).

Ubiquitination is another PTM regulating STING. Analogously to cGAS, a Lys48-linked polyubiquitination leads to the proteasomal degradation of STING. This is mediated by an E3 ubiquitin ligase RNF5 in the later phases of infection by the Sendai virus (Zhong et al., 2009). On the other hand, Lys27/33-linked and K63-linked ubiquitinations stabilise STING, thus, removing these modifications lead to its inhibition. Two deubiquitinating enzymes were identified in two studies – USP13 and USP49, which

precisely remove these two types of polyubiquitination destabilising STING and hindering its activation (Sun et al., 2017, Ye et al., 2019).

SUMOylation at Lys337 by a SUMO ligase TRIM38 was described as an activating and stabilising PTM of STING and the same deSUMOylase as with cGAS, SENP2, was identified to reverse this modification. This ultimately leads to lysosomal degradation of STING (Hu et al., 2016).

In a study focusing on a type of interferonopathy systemic lupus erythematosus (SLE), a negative regulatory role of endogenous nitro-fatty acids was revealed. These fatty acids catalyse a covalent modification of STING by nitro-alkylation; this prevents STING from being palmitoylated, which is a stabilising PTM in this case. The same group then tried administering nitro-fatty acids to SLE-derived fibroblasts and obtained positive results in the decrease of type I IFN signalling (Hansen et al., 2018).

During bacterial infection, the cyclic dinucleotides, c-di-GMP, c-di-AMP and 3'3'-cGAMP are produced. They act as second messengers that regulate a variety of cellular processes such as cellular growth, homeostasis among others. These dinucleotides are different from the mammalian-derived 2'3'-cGAMP, which is recognized by STING. However, c-di-AMP has been involved in STING-dependent type I IFN responses during bacterial infection. It was demonstrated during the infection of macrophages by intracellular bacteria the c-di-AMPs are recognized by the oxidoreductase Reductase Controlling NF- κ B (RECON). RECON affinity for c-di-AMP is higher than the affinity of STING to c-di AMP, thus, binding of the c-di-AMP to RECON prevents activation of the type I IFN signalling pathway but leads to the activation of NF- κ B (McFarland et al., 2017).

A protein stromal interaction molecule 1 (STIM1), a calcium sensor, interacts with STING and traps it in the ER inhibiting its translocation necessary for type I IFN signalling (Srikanth et al., 2019). STIM1 is not the only molecule hindering STING's proper trafficking, NOD-like receptor family CARD domain containing 3 (NLRC3) was elucidated to prevent STING from leaving the ER (Zhang et al., 2014).

The NLR family member X1 (NLRX1) was shown to prevent STING activation in the context of infection by human immunodeficiency virus 1. Upon entry into cells, the HIV viral RNA genome is converted into viral DNA. This intermediate DNA genome can be recognized by cGAS via STING (Gao et al., 2013). NLRX1 was observed to hinder STING from binding to TBK1 and led to the depletion of type I IFN production. Furthermore, when STING was able to bind TBK1 in these infected cells, type I IFN production and antiviral state was restored (Guo et al., 2016).

There are two mechanisms regulating STING at the genetic and epigenetic levels. The first influences the mRNA of STING. A transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), regulates

STING by destabilising its mRNA, which is then degraded and cannot be translated into a functional protein (Olagnier et al., 2018). The second regulation uses H3K4 Lys demethylases KDM5B and KDM5C, which demethylate histone 3 and repress the transcription of the STING gene (Wu et al., 2018).

Recently, a novel property of STING was elucidated. Yu et al. identified spherical condensed structures in the ER and discovered STING formed these via the LLPS mechanism. Either infection with DNA pathogens induced this formation or administration of cGAMP, which led to the blockade of STING by being unable to translocate from the ER and also to constraining of TBK1 in its interaction with STING. The formation of these condensates was postulated by Yu et al. to be a homeostatic negative regulation mechanism against the overactivation of the innate immunity system (Yu et al., 2021).

One of the more common cancers in humans – head and neck squamous cell carcinoma, also caused by a viral infection of human papillomavirus (HPV) as well as other cancers, spreads by evading the immune system and in this case, by negatively regulating STING. In this pathology, STING is inhibited by the sex-determining region Y-box 2 (SOX2) protein, which promotes its degradation via the autophagy pathway (Tan et al., 2018). SOX2 functions as a transcriptional regulator in different stages of mammalian development, thus, its function as a regulator of the STING pathway is novel.

KSHV protein vIRF1 inhibits phosphorylation of STING at Ser366 by TBK1 (Ma et al., 2015). After depleting infected cells from vIRF1, the reactivation of KSHV was abolished, thus, IFN response was launched. In addition, cells which expressed vIRF1 but were not infected by KSHV showed reduced type I IFN response upon infection with other DNA pathogens. Another KSHV protein antagonizing STING was discovered, the protein ORF64 (Sun et al., 2015). This protein acts as a deubiquitinating enzyme, removing the stabilising Lys63-linked ubiquitination and hindering further signalling by STING.

The next virus reducing IFN production is the HCMV. A viral tegument protein UL82 inhibits STING via two different mechanisms, entirely abolishing its activation and proper function (Fu et al., 2017). Firstly, UL82 represses the translocation of STING from the ER necessary for further signalling. The other mechanism of UL82 seems to be the impairment of STING's ability to interact with both TBK1 and IRF3 properly. HCMV protein UL42 also hinders the proper translocation of STING from the ER (Fu et al., 2019). According to Fu et al. this happens by the degradation of translocone-associated protein β , which helps with the translocation of STING in standard conditions. Lastly, an HCMV glycoprotein US9 suppresses STING oligomerization and its recruitment of TBK1 by the mechanism of competitive inhibition (Choi et al., 2018).

In HSV-1 infection, two proteins were identified to suppress type I IFN signalling via inhibition of STING. UL46 interacts directly with both STING and TBK1 by its N-terminus binding the first and C-terminus

binding the latter inhibiting interaction of the two proteins (Deschamps et al., 2017). E3 ubiquitin ligase TRIM30 α was shown to be induced by HSV-1 infection (Wang et al., 2015). This ligase promotes the Lys48-linked polyubiquitination of STING, ultimately leading to proteasomal degradation. Knockdown of TRIM30 α in mice resulted in heightened resistance to HSV-1 and different DNA pathogens. EBV induces a different ubiquitin ligase TRIM29, which also triggers the K48-linked polyubiquitination of STING, degrading it in the proteasome (Xing et al., 2017).

HBV encodes a viral polymerase (Pol) containing several domains. One of these is a reverse transcriptase domain, which was found to bind STING (Liu et al., 2015). This interaction suppresses the stabilising Lys63-linked polyubiquitination disabling further proper activation and STING-mediated signalling.

HPV and adenoviruses (AdV) encode two STING hindering proteins E7 and E1A, respectively. Both contain an LXCXE motif, which they use to bind and inhibit STING directly. After depleting the cells of E1A and/or E7, STING-mediated signalling was restored activation (Shaikh et al., 2019, Lau et al., 2015).

The mechanisms, which negatively regulate STING are summarised in table 4.

Mechanism	Details of the mechanism	Result	Reference
PTMs	Dephosphorylation by: 1. PPM1A 2. PTPN1/2	1.No interaction with TBK1 2. Destabilisation of STING	(Li et al., 2015), (Xia et al., 2019)
	Phosphorylation by: 1. Ulk1/2 2. Akt, HER2	1. No activation 2. No TBK1 recruitment	(Wu et al., 2019), (Konno et al., 2013)
	Ubiquitination and deubiquitylation by: 1. RNF5 2. USP13, USP49	1. Proteasomal degradation 2. Destabilisation	(Zhong et al., 2009), (Sun et al., 2017, Ye et al., 2019)
	deSUMOylation by SENP2	Lysosomal degradation	(Hu et al., 2016)
	Nitro-fatty acids- Nitro-alkylation	No palmitoylation	(Hansen et al., 2018)
Competitive/ inhibition (by interaction with cellular proteins)	Enzymatically by RECON	No substrate binding	(McFarland et al., 2017)
	By protein NLRX1	No interaction with TBK1	(Guo et al., 2016)
Blocking of subcellular trafficking	1. STIM1 2. NLRC3	No translocation from ER	(Srikanth et al., 2019), (Zhang et al., 2014)
Genetic and epigenetic related	1. Nrf2 2. KDM5B, KDM5C	1. Destabilisation of STING mRNA 2.Repression of transcription	(Olagnier et al., 2018), (Wu et al., 2018)
Condensation	LLPS	STING trapped in ER	(Yu et al., 2021)
Autophagy related	Head and neck cancer by protein SOX2	Degradation via autophagy	(Tan et al., 2018)
Virus related	KSHV: 1. vIRF1 2. ORF64	1. No TBK1 recruitment 2.Destabilising deubiquitylation	(Ma et al., 2015), (Sun et al., 2015)
	HCMV: 1. UL82 2. UL42 3. US9	1. No translocation from ER and no TBK1 recruitment 2. No translocation 3. No oligomerization	(Fu et al., 2017), (Choi et al., 2018)
	HSV-1: 1. By protein UL46 2. Induction of TRIM30α	1. No TBK1 recruitment 2. Proteasomal degradation	(Deschamps et al., 2017), (Wang et al., 2015)
	EBV : induction of TRIM29	Proteasomal degradation	(Xing et al., 2017)
	HBV: viral polymerase	No stabilising ubiquitination	(Liu et al., 2015)
	HPV : E7	No activation	(Shaikh et al., 2019)
	AdV : E1A	No activation	(Lau et al., 2015)

Table 4, Summary of mechanisms negatively regulating STING.

5. Conclusions

The role of DNA sensors in pathologies has been extensively studied in the past years. More and more sensors are being identified, which either induce similar pathways e.g., cGAS, IFI16, DNA-PK, etc. via STING or entirely different responses e.g., RNA pol III, Ku70, LRRFIP1, etc. However, all of these share one similarity – they are indispensable for the cells to defend themselves against pathogens and other treats.

Fully understanding the cell type, where they are produced, under which conditions they are induced and how they are regulated are very relevant questions. This thesis reviews the structure and properties of the molecules cGAS, IFI16 and STING and the mechanisms that result in the negative regulation of these proteins in the context of the IFN response.

cGAS is a sensor that has been extensively studied concerning its activation, signalling and regulation. It was previously thought to be situated exclusively in the cytosol; however, recent studies have proven its localization in the nucleus, where it remains in an inactive state regarding immune signalling. The reason it is not active in the nucleus is not completely understood yet, but clearly, it coincides with the need to protect the genomic DNA from self-sensing and over-activation of the type I IFN response. Negative regulations of cGAS are multifarious, however, most of the time hinder its ability to bind or sense DNA either by protein binding or PTMs. Viruses use similar mechanisms. HSV-1 and KSHV have been described in this thesis to have two proteins of negative regulation each. Moreover, mechanism such as non-coding RNAs has been described to down-regulate the cGAS pathway, in detail, micro RNAs against cGAS co-activator or circular RNAs that bind cGAS active site. Interestingly, despite the many mechanisms uncovered for cGAS negative regulation, the researchers presume there to be more. This points to the fact there is still much more to be discovered even in the already known negative regulators of cGAS.

IFI16 lacks the extent of studies and information available compared to cGAS. Similarly to cGAS, it is also localized in both the cytosol and the nucleus, but on the contrary, IFI16 could be active in specific conditions in the nucleus as during sensing of DNA of herpes viruses. In fact, IFI16 does not sense self-DNA in the nucleus, at least in part due to the presence of the nucleosomes. Other mechanisms, which prevent the sensing of DNA in the nucleus have been suggested. Apart from the methylation by PRMT5 and ubiquitination by TRIM21, no other negative regulation PTMs have been described in not infected cells. However, HSV-1 promotes ubiquitination and the degradation of IFI16. Being a part of the PYHIN family enables a mechanism of negative regulation used by HCMV with the evolutionarily conserved PAD domain of pUL83. Intriguingly, this mechanism is very likely to be similar in other viruses, which encode proteins with the same domain; however, whether they do remains under speculation. The questions regarding the negative regulation of IFI16 are numerous and require further studies. Nevertheless, a novel mechanism such as blocking the methylation of hnRNPA2B1 via the FTO protein, which prevents the migration of the mRNAs of IFI16, cGAS and STING to the cytosol, has shed new light into the mechanisms of negative regulation on transcriptional level.

STING is the adaptor protein of many DNA sensors – cGAS, IFI16, IFIX, DAI, MRE11-RAD50, DNA-PK, DDX41 and DHX36. Thus, its role in immune signalling is utterly essential. Without STING, many cells would lose their ability to defend themselves against pathogens and indeed, the lack of STING causes

serious pathologies. On the other hand, the lack of negative regulation of STING can have dire consequences on an organism, also resulting in serious illnesses e.g., interferonopathies. Many negative regulation PTMs have been described, which resemble those of cGAS and usually hinder the oligomerization or interactions with TBK1 or IRF3. HCMV and certain cell proteins – STIM1 and NLRC3 have developed a strategy targeting the necessary subcellular trafficking of STING and abolishing its proper signalling. Fascinatingly, STING subjects to certain mechanisms so far scarce in the negative regulation of IFI16 and cGAS. Firstly, the epigenetic regulation by histone demethylases KDM5B and KDM5C, which prevent the transcription of STING. Secondly, the LLPS mechanism, when STING forms condensed droplets in the ER, which hinder its translocation and subsequent interaction with TBK1 and IRF3. It is very interesting how LLPS poses as a necessary step for cGAS enzymatic function but abolishes the activation of the STING pathway when in detail; the LLPS of STING can be induced by the administration of cGAMP, which would not be produced without LLPS. Understanding the negative regulation of STING could help answer questions about various viruses and sensors, thus, I strongly feel the mechanisms regarding STING are of tremendous importance in further research.

6. References

The symbol “*” marks a secondary citation.

- Abdelhaleem, Mohamed, Lois Maltais, a Hester Wain. 2003. „The Human DDX and DHX Gene Families of Putative RNA Helicases". *Genomics* 81 (6): 618–22. [https://doi.org/10.1016/S0888-7543\(03\)00049-1](https://doi.org/10.1016/S0888-7543(03)00049-1).
- Ablasser, Andrea, Marion Goldeck, Taner Cavlar, Tobias Deimling, Gregor Witte, Ingo Röhl, Karl-Peter Hopfner, Janos Ludwig, a Veit Hornung. 2013. „cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING". *Nature* 498 (7454): 380–84. <https://doi.org/10.1038/nature12306>.
- Aguirre, Sebastian, Priya Luthra, Maria T. Sanchez-Aparicio, Ana M. Maestre, Jenish Patel, Francise Lamothe, Anthony C. Fredericks, et al. 2017. „Dengue Virus NS2B Protein Targets CGAS for Degradation and Prevents Mitochondrial DNA Sensing during Infection". *Nature Microbiology* 2 (březen): 17037. <https://doi.org/10.1038/nmicrobiol.2017.37>.
- Allen, Irving C., Margaret A. Scull, Chris B. Moore, Eda K. Holl, Erin McElvania-TeKippe, Debra J. Taxman, Elizabeth H. Guthrie, Raymond J. Pickles, a Jenny P.-Y. Ting. 2009. „The NLRP3 Inflammasome Mediates in Vivo Innate Immunity to Influenza A Virus through Recognition of Viral RNA". *Immunity* 30 (4): 556–65. <https://doi.org/10.1016/j.immuni.2009.02.005>.
- Almine, Jessica F., Craig A. J. O'Hare, Gillian Dunphy, Ismar R. Haga, Rangeetha J. Naik, Abdelmadjid Atrih, Dymrna J. Connolly, et al. 2017. „IFI16 and CGAS Cooperate in the Activation of STING during DNA Sensing in Human Keratinocytes". *Nature Communications* 8 (1): 14392. <https://doi.org/10.1038/ncomms14392>.

- Andreeva, Liudmila, Björn Hiller, Dirk Kostrewa, Charlotte Lässig, Carina C. de Oliveira Mann, David Jan Drexler, Andreas Mäyser, et al. 2017. „CGAS Senses Long and HMGB/TFAM-Bound U-Turn DNA by Forming Protein–DNA Ladders". *Nature* 549 (7672): 394–98. <https://doi.org/10.1038/nature23890>.
- Ansari, Mairaj Ahmed, Vivek Vikram Singh, Sujoy Dutta, Mohanan Valiya Veetil, Dipanjan Dutta, Leela Chikoti, Jie Lu, David Everly, a Bala Chandran. 2013. „Constitutive Interferon-Inducible Protein 16-Inflammasome Activation during Epstein-Barr Virus Latency I, II, and III in B and Epithelial Cells". *Journal of Virology* 87 (15): 8606–23. <https://doi.org/10.1128/JVI.00805-13>.
- Baba, M., K. Takeshige, N. Baba, a Y. Ohsumi. 1994. „Ultrastructural Analysis of the Autophagic Process in Yeast: Detection of Autophagosomes and Their Characterization". *The Journal of Cell Biology* 124 (6): 903–13. <https://doi.org/10.1083/jcb.124.6.903>.
- Bai, Juli, a Feng Liu. 2022. „Nuclear CGAS: Sequestration and Beyond". *Protein & Cell* 13 (2): 90–101. <https://doi.org/10.1007/s13238-021-00869-0>.
- Barnett, Katherine C., Julia M. Coronas-Serna, Wen Zhou, Michael J. Ernandes, Anh Cao, Philip J. Kranzusch, a Jonathan C. Kagan. 2019. „Phosphoinositide Interactions Position CGAS at the Plasma Membrane to Ensure Efficient Distinction between Self- and Viral DNA". *Cell* 176 (6): 1432-1446.e11. <https://doi.org/10.1016/j.cell.2019.01.049>.
- Brázda, Václav, Jan Coufal, Jack C. C. Liao, a Cheryl H. Arrowsmith. 2012. „Preferential Binding of IFI16 Protein to Cruciform Structure and Superhelical DNA". *Biochemical and Biophysical Research Communications* 422 (4): 716–20. <https://doi.org/10.1016/j.bbrc.2012.05.065>.
- Bürckstümmer, Tilmann, Christoph Baumann, Stephan Blüml, Evelyn Dixit, Gerhard Dürnberger, Hannah Jahn, Melanie Planyavsky, et al. 2009. „An Orthogonal Proteomic-Genomic Screen Identifies AIM2 as a Cytoplasmic DNA Sensor for the Inflammasome". *Nature Immunology* 10 (3): 266–72. <https://doi.org/10.1038/ni.1702>.
- Burdette, Dara L., a Russell E. Vance. 2013. „STING and the Innate Immune Response to Nucleic Acids in the Cytosol". *Nature Immunology* 14 (1): 19–26. <https://doi.org/10.1038/ni.2491>.
- Burleigh, Katelyn, Joanna H. Maltbaek, Stephanie Cambier, Richard Green, Michael Gale, Richard C. James, a Daniel B. Stetson. 2020. „Human DNA-PK Activates a STING-Independent DNA Sensing Pathway". *Science Immunology* 5 (43): eaba4219. <https://doi.org/10.1126/sciimmunol.aba4219>.
- Caneparo, Valeria, Santo Landolfo, Marisa Gariglio, a Marco De Andrea. 2018. „The Absent in Melanoma 2-Like Receptor IFN-Inducible Protein 16 as an Inflammasome Regulator in Systemic Lupus Erythematosus: The Dark Side of Sensing Microbes". *Frontiers in Immunology* 9 (květen): 1180. <https://doi.org/10.3389/fimmu.2018.01180>.
- Cuchet-Lourenço, Delphine, Gail Anderson, Elizabeth Sloan, Anne Orr, a Roger D. Everett. 2013. „The Viral Ubiquitin Ligase ICP0 Is neither Sufficient nor Necessary for Degradation of the Cellular DNA Sensor IFI16 during Herpes Simplex Virus 1 Infection". *Journal of Virology* 87 (24): 13422–32. <https://doi.org/10.1128/JVI.02474-13>.
- Cui, Ye, Huansha Yu, Xin Zheng, Rui Peng, Qiang Wang, Yi Zhou, Rui Wang, et al. 2017. „SEN7 Potentiates CGAS Activation by Relieving SUMO-Mediated Inhibition of Cytosolic DNA Sensing". *PLoS Pathogens* 13 (1): e1006156. <https://doi.org/10.1371/journal.ppat.1006156>.
- Dai, Jiang, Yi-Jiao Huang, Xinhua He, Ming Zhao, Xinzhen Wang, Zhao-Shan Liu, Wen Xue, et al. 2019. „Acetylation Blocks cGAS Activity and Inhibits Self-DNA-Induced Autoimmunity". *Cell* 176 (6): 1447-1460.e14. <https://doi.org/10.1016/j.cell.2019.01.016>.

- DeFilippis, Victor R., David Alvarado, Tina Sali, Stefan Rothenburg, a Klaus Früh. 2010. „Human Cytomegalovirus Induces the Interferon Response via the DNA Sensor ZBP1". *Journal of Virology* 84 (1): 585–98. <https://doi.org/10.1128/JVI.01748-09>.
- Dell'Oste, Valentina, Deborah Gatti, Francesca Gugliesi, Marco De Andrea, Mandar Bawadekar, Irene Lo Cigno, Matteo Biolatti, et al. 2014. „Innate Nuclear Sensor IFI16 Translocates into the Cytoplasm during the Early Stage of In Vitro Human Cytomegalovirus Infection and Is Entrapped in the Egressing Virions during the Late Stage". *Journal of Virology* 88 (12): 6970–82. <https://doi.org/10.1128/JVI.00384-14>.
- Deschamps, Thibaut, a Maria Kalamvoki. 2017. „Evasion of the STING DNA-Sensing Pathway by VP11/12 of Herpes Simplex Virus 1". *Journal of Virology* 91 (16): e00535-17. <https://doi.org/10.1128/JVI.00535-17>.
- Diner, Benjamin A., Tuo Li, Todd M. Greco, Marni S. Crow, John A. Fuesler, Jennifer Wang, a Ileana M. Cristea. 2015. „The Functional Interactome of PYHIN Immune Regulators Reveals IFIX Is a Sensor of Viral DNA". *Molecular Systems Biology* 11 (1): 787. <https://doi.org/10.15252/msb.20145808>.
- Diner, Benjamin A., Krystal K. Lum, a Ileana M. Cristea. 2015. „The Emerging Role of Nuclear Viral DNA Sensors *". *Journal of Biological Chemistry* 290 (44): 26412–21. <https://doi.org/10.1074/jbc.R115.652289>.*
- Du, Mingjian, a Zhijian J. Chen. 2018. „DNA-induced liquid phase condensation of cGAS activates innate immune signaling". *Science* 361 (6403): 704–9. <https://doi.org/10.1126/science.aat1022>.
- Essers, Marieke A. G., Sandra Offner, William E. Blanco-Bose, Zoe Waibler, Ulrich Kalinke, Michel A. Duchosal, a Andreas Trumpp. 2009. „IFN α Activates Dormant Haematopoietic Stem Cells in Vivo". *Nature* 458 (7240): 904–8. <https://doi.org/10.1038/nature07815>.
- Fan, Xiaojiao, Jiansheng Jiang, Dan Zhao, Feng Chen, Huan Ma, Patrick Smith, Leonie Unterholzner, Tsan Sam Xiao, a Tengchuan Jin. 2021. „Structural mechanism of DNA recognition by the p204 HIN domain". *Nucleic Acids Research* 49 (5): 2959–72. <https://doi.org/10.1093/nar/gkab076>.
- Ferguson, Brian J., Daniel S. Mansur, Nicholas E. Peters, Hongwei Ren, a Geoffrey L. Smith. 2012. „DNA-PK Is a DNA Sensor for IRF-3-Dependent Innate Immunity". *ELife* 1 (prosinec): e00047. <https://doi.org/10.7554/eLife.00047>.
- Fitzgerald, Katherine A., Sarah M. McWhirter, Kerrie L. Faia, Daniel C. Rowe, Eicke Latz, Douglas T. Golenbock, Anthony J. Coyle, Sha-Mei Liao, a Tom Maniatis. 2003. „IKKepsilon and TBK1 Are Essential Components of the IRF3 Signaling Pathway". *Nature Immunology* 4 (5): 491–96. <https://doi.org/10.1038/ni921>.
- Fu, Yu-Zhi, Yi Guo, Hong-Mei Zou, Shan Su, Su-Yun Wang, Qing Yang, Min-Hua Luo, a Yan-Yi Wang. 2019. „Human cytomegalovirus protein UL42 antagonizes cGAS/MITA-mediated innate antiviral response". *PLOS Pathogens* 15 (květen): e1007691. <https://doi.org/10.1371/journal.ppat.1007691>.
- Fu, Yu-Zhi, Shan Su, Yi-Qun Gao, Pei-Pei Wang, Zhe-Fu Huang, Ming-Ming Hu, Wei-Wei Luo, et al. 2017. „Human Cytomegalovirus Tegument Protein UL82 Inhibits STING-Mediated Signaling to Evade Antiviral Immunity". *Cell Host & Microbe* 21 (2): 231–43. <https://doi.org/10.1016/j.chom.2017.01.001>.
- Gao, Daxing, Jiayi Wu, You-Tong Wu, Fenghe Du, Chukwuemika Aroh, Nan Yan, Lijun Sun, a Zhijian J. Chen. 2013. „Cyclic GMP-AMP Synthase Is an Innate Immune Sensor of HIV and Other Retroviruses". *Science (New York, N.Y.)* 341 (6148): 903–6. <https://doi.org/10.1126/science.1240933>.

- Gao, Pu, Manuel Ascano, Thomas Zillinger, Weiyi Wang, Peihong Dai, Artem A. Serganov, Barbara L. Gaffney, et al. 2013. „Structure-Function Analysis of STING Activation by c[G(2',5')pA(3',5')p] and Targeting by Antiviral DMXAA". *Cell* 154 (4): 748–62. <https://doi.org/10.1016/j.cell.2013.07.023>.
- Gell, D, a S P Jackson. 1999. „Mapping of protein-protein interactions within the DNA-dependent protein kinase complex." *Nucleic Acids Research* 27 (17): 3494–3502.
- Gentili, Matteo, Xavier Lahaye, Francesca Nadalin, Guilherme P. F. Nader, Emilia Puig Lombardi, Solène Herve, Nilushi S. De Silva, et al. 2019. „The N-Terminal Domain of CGAS Determines Preferential Association with Centromeric DNA and Innate Immune Activation in the Nucleus". *Cell Reports* 26 (9): 2377-2393.e13. <https://doi.org/10.1016/j.celrep.2019.01.105>.
- Ghosh, Arundhati, Lulu Shao, Padmavathi Sampath, Baoyu Zhao, Nidhi V. Patel, Jianzhong Zhu, Bharat Behl, et al. 2019. „Oligoadenylate-Synthetase-Family Protein OASL Inhibits Activity of the DNA Sensor CGAS during DNA Virus Infection to Limit Interferon Production". *Immunity* 50 (1): 51-63.e5. <https://doi.org/10.1016/j.immuni.2018.12.013>.
- Gross, Olaf, Hendrik Poeck, Michael Bscheider, Catherine Dostert, Nicole Hanneschläger, Stefan Endres, Gunther Hartmann, et al. 2009. „Syk Kinase Signalling Couples to the Nlrp3 Inflammasome for Anti-Fungal Host Defence". *Nature* 459 (7245): 433–36. <https://doi.org/10.1038/nature07965>.
- Gu, Y., S. Jin, Y. Gao, D. T. Weaver, a F. W. Alt. 1997. „Ku70-Deficient Embryonic Stem Cells Have Increased Ionizing Radiosensitivity, Defective DNA End-Binding Activity, and Inability to Support V(D)J Recombination". *Proceedings of the National Academy of Sciences of the United States of America* 94 (15): 8076–81. <https://doi.org/10.1073/pnas.94.15.8076>.
- Guey, Baptiste, Marilena Wischnewski, Alexiane Decout, Kristina Makasheva, Murat Kaynak, Mahmut S. Sakar, Beat Fierz, a Andrea Ablasser. 2020. „BAF Restricts CGAS on Nuclear DNA to Prevent Innate Immune Activation". *Science (New York, N.Y.)* 369 (6505): 823–28. <https://doi.org/10.1126/science.aaw6421>.
- Gugliesi, Francesca, Michele Mondini, Raffaella Ravera, Andrea Robotti, Marco de Andrea, Giorgio Gribaudo, Marisa Gariglio, a Santo Landolfo. 2005. „Up-Regulation of the Interferon-Inducible IFI16 Gene by Oxidative Stress Triggers P53 Transcriptional Activity in Endothelial Cells". *Journal of Leukocyte Biology* 77 (5): 820–29. <https://doi.org/10.1189/jlb.0904507>.
- Gui, Xiang, Hui Yang, Tuo Li, Xiaojun Tan, Peiqing Shi, Minghao Li, Fenghe Du, a Zhijian J. Chen. 2019. „Autophagy Induction via STING Trafficking Is a Primordial Function of the CGAS Pathway". *Nature* 567 (7747): 262–66. <https://doi.org/10.1038/s41586-019-1006-9>.
- Guo, Haitao, Renate König, Meng Deng, Maximilian Riess, Jinyao Mo, Lu Zhang, Alex Petrucelli, et al. 2016. „NLRX1 Sequesters STING to Negatively Regulate the Interferon Response, Thereby Facilitating the Replication of HIV-1 and DNA Viruses". *Cell Host & Microbe* 19 (4): 515–28. <https://doi.org/10.1016/j.chom.2016.03.001>.
- Hansen, Anne Louise, Gregory J. Buchan, Michael Rühl, Kojiro Mukai, Sonia R. Salvatore, Emari Ogawa, Sidsel D. Andersen, et al. 2018. „Nitro-fatty acids are formed in response to virus infection and are potent inhibitors of STING palmitoylation and signaling". *Proceedings of the National Academy of Sciences* 115 (33): E7768–75. <https://doi.org/10.1073/pnas.1806239115>.
- Hároníková, Lucia, Jan Coufal, Iva Kejnovská, Eva B. Jagelská, Miroslav Fojta, Petra Dvořáková, Petr Muller, Borivoj Vojtesek, a Václav Brázda. 2016. „IFI16 Preferentially Binds to DNA with Quadruplex Structure and Enhances DNA Quadruplex Formation". *PLoS ONE* 11 (6): e0157156. <https://doi.org/10.1371/journal.pone.0157156>.

- Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, et al. 2000. „A Toll-like Receptor Recognizes Bacterial DNA". *Nature* 408 (6813): 740–45. <https://doi.org/10.1038/35047123>.
- Hornung, Veit, Andrea Ablasser, Marie Charrel-Dennis, Franz Bauernfeind, Gabor Horvath, Daniel R. Caffrey, Eicke Latz, a Katherine A. Fitzgerald. 2009. „AIM2 Recognizes Cytosolic DsDNA and Forms a Caspase-1-Activating Inflammasome with ASC". *Nature* 458 (7237): 514–18. <https://doi.org/10.1038/nature07725>.
- Howard, Timothy R., Marni S. Crow, Todd M. Greco, Krystal K. Lum, Tuo Li, a Ileana M. Cristea. b.r. „The DNA Sensor IFIX Drives Proteome Alterations To Mobilize Nuclear and Cytoplasmic Antiviral Responses, with Its Acetylation Acting as a Localization Toggle". *mSystems* 6 (3): e00397-21. <https://doi.org/10.1128/mSystems.00397-21>.
- Hu, Ming-Ming, Wen-Rui He, Peng Gao, Qing Yang, Ke He, Li-Bo Cao, Shu Li, Yu-Qi Feng, a Hong-Bing Shu. 2019. „Virus-Induced Accumulation of Intracellular Bile Acids Activates the TGR5- β -Arrestin-SRC Axis to Enable Innate Antiviral Immunity". *Cell Research* 29 (3): 193–205. <https://doi.org/10.1038/s41422-018-0136-1>.
- Hu, Ming-Ming, Qing Yang, Xue-Qin Xie, Chen-Yang Liao, Heng Lin, Tian-Tian Liu, Lei Yin, a Hong-Bing Shu. 2016. „Sumoylation Promotes the Stability of the DNA Sensor CGAS and the Adaptor STING to Regulate the Kinetics of Response to DNA Virus". *Immunity* 45 (3): 555–69. <https://doi.org/10.1016/j.immuni.2016.08.014>.
- Huang, Jian, Hongjuan You, Chenhe Su, Yangxin Li, Shunhua Chen, a Chunfu Zheng. 2018. „Herpes Simplex Virus 1 Tegument Protein VP22 Abrogates cGAS/STING-Mediated Antiviral Innate Immunity". *Journal of Virology* 92 (15): e00841-18. <https://doi.org/10.1128/JVI.00841-18>.
- Huérffano, Sandra, Vojtech Šroller, Kateřina Bruštková, Lenka Horníková, a Jitka Forstová. 2022. „The Interplay between Viruses and Host DNA Sensors". *Viruses* 14 (4): 666. <https://doi.org/10.3390/v14040666>.*
- Chen, Meixin, Qingcai Meng, Yunfei Qin, Puping Liang, Peng Tan, Lian He, Yubin Zhou, et al. 2016. „TRIM14 Inhibits CGAS Degradation Mediated by Selective Autophagy Receptor P62 to Promote Innate Immune Responses". *Molecular Cell* 64 (1): 105–19. <https://doi.org/10.1016/j.molcel.2016.08.025>.
- Chiu, Yu-Hsin, John B. MacMillan, a Zhijian J. Chen. 2009. „RNA Polymerase III Detects Cytosolic DNA and Induces Type-I Interferons Through the RIG-I Pathway". *Cell* 138 (3): 576–91. <https://doi.org/10.1016/j.cell.2009.06.015>.
- Choi, Hyun jin, Areum Park, Sujin Kang, Eunhye Lee, Taeyun A. Lee, Eun A. Ra, Jiseon Lee, Sungwook Lee, a Boyoun Park. 2018. „Human Cytomegalovirus-Encoded US9 Targets MAVS and STING Signaling to Evade Type I Interferon Immune Responses". *Nature Communications* 9 (1): 125. <https://doi.org/10.1038/s41467-017-02624-8>.
- Ishikawa, Hiroki, a Glen N. Barber. 2008. „STING Is an Endoplasmic Reticulum Adaptor That Facilitates Innate Immune Signalling". *Nature* 455 (7213): 674–78. <https://doi.org/10.1038/nature07317>.
- Ishikawa, Hiroki, Zhe Ma, a Glen N. Barber. 2009. „STING Regulates Intracellular DNA-Mediated, Type I Interferon-Dependent Innate Immunity". *Nature* 461 (7265): 788. <https://doi.org/10.1038/nature08476>.
- Jeck, William R., Jessica A. Sorrentino, Kai Wang, Michael K. Slevin, Christin E. Burd, Jinze Liu, William F. Marzluff, a Norman E. Sharpless. 2013. „Circular RNAs Are Abundant, Conserved, and Associated with ALU Repeats". *RNA* 19 (2): 141–57. <https://doi.org/10.1261/rna.035667.112>.

- Jin, Tengchuan, Andrew Perry, Jiansheng Jiang, Patrick Smith, James A. Curry, Leonie Unterholzner, Zhaozhao Jiang, et al. 2012. „Structures of The HIN Domain:DNA Complexes Reveal Ligand Binding and Activation Mechanisms of The AIM2 Inflammasome and IFI16 Receptor". *Immunity* 36 (4): 561–71. <https://doi.org/10.1016/j.immuni.2012.02.014>.
- Jønsson, K. L., A. Laustsen, C. Krapp, K. A. Skipper, K. Thavachelvam, D. Hotter, J. H. Egedal, et al. 2017. „IFI16 Is Required for DNA Sensing in Human Macrophages by Promoting Production and Function of CGAMP". *Nature Communications* 8 (1): 14391. <https://doi.org/10.1038/ncomms14391>.
- Kanneganti, Thirumala-Devi, Mathilde Body-Malapel, Amal Amer, Jong-Hwan Park, Joel Whitfield, Luigi Franchi, Zenobia F. Taraporewala, et al. 2006. „Critical Role for Cryopyrin/Nalp3 in Activation of Caspase-1 in Response to Viral Infection and Double-Stranded RNA". *The Journal of Biological Chemistry* 281 (48): 36560–68. <https://doi.org/10.1074/jbc.M607594200>.
- Kawai, Taro, Ken Takahashi, Shintaro Sato, Cevayir Coban, Himanshu Kumar, Hiroki Kato, Ken J. Ishii, Osamu Takeuchi, a Shizuo Akira. 2005. „IPS-1, an Adaptor Triggering RIG-I- and Mda5-Mediated Type I Interferon Induction". *Nature Immunology* 6 (10): 981–88. <https://doi.org/10.1038/ni1243>.
- Kim, Hyungsoo, Heejung Kim, Yongmei Feng, Yan Li, Hironari Tamiya, Stefania Tocci, a Ze'ev A. Ronai. 2020. „PRMT5 control of cGAS/STING and NLRC5 pathways defines melanoma response to antitumor immunity". *Science Translational Medicine* 12 (551): eaaz5683. <https://doi.org/10.1126/scitranslmed.aaz5683>.
- Kim, Taeil, Shwetha Pazhoor, Musheng Bao, Zhiqiang Zhang, Shino Hanabuchi, Valeria Facchinetti, Laura Bover, et al. 2010. „Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells". *Proceedings of the National Academy of Sciences of the United States of America* 107 (34): 15181–86. <https://doi.org/10.1073/pnas.1006539107>.
- Kondo, Takeshi, Junya Kobayashi, Tatsuya Saitoh, Kenta Maruyama, Ken J. Ishii, Glen N. Barber, Kenshi Komatsu, Shizuo Akira, a Taro Kawai. 2013. „DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking". *Proceedings of the National Academy of Sciences of the United States of America* 110 (8): 2969–74. <https://doi.org/10.1073/pnas.1222694110>.
- Konno, Hiroyasu, Keiko Konno, a Glen N. Barber. 2013. „Cyclic Di Nucleotides Trigger ULK1 (ATG1) Phosphorylation of STING to Prevent Sustained Innate Immune Signaling". *Cell* 155 (3): 10.1016/j.cell.2013.09.049. <https://doi.org/10.1016/j.cell.2013.09.049>.
- Kranzusch, Philip J. 2019. „Xie". *Current Opinion in Structural Biology, Catalysis and Regulation • Protein Nucleic Interactions*, 59 (prosin): 178–87. <https://doi.org/10.1016/j.sbi.2019.08.003>.
- Kranzusch, Philip J., Amy Si-Ying Lee, James M. Berger, a Jennifer A. Doudna. 2013. „Structure of Human CGAS Reveals a Conserved Family of Second-Messenger Enzymes in Innate Immunity". *Cell Reports* 3 (5): 1362–68. <https://doi.org/10.1016/j.celrep.2013.05.008>.
- Kranzusch, Philip J., Stephen C. Wilson, Amy S.Y. Lee, James M. Berger, Jennifer A. Doudna, a Russell E. Vance. 2015. „Ancient Origin of cGAS-STING Reveals Mechanism of Universal 2',3' cGAMP Signaling". *Molecular cell* 59 (6): 891–903. <https://doi.org/10.1016/j.molcel.2015.07.022>.
- Kumagai, Yutaro, Osamu Takeuchi, a Shizuo Akira. 2008. „TLR9 as a Key Receptor for the Recognition of DNA". *Advanced Drug Delivery Reviews, Toll-like Receptor and Pattern Sensing for Evoking Immune Response*, 60 (7): 795–804. <https://doi.org/10.1016/j.addr.2007.12.004>.*

- Landman, Sanne, Maaïke Rensing, a Annemarthé Veen. 2020. „Balancing STING in antimicrobial defense and autoinflammation". *Cytokine & Growth Factor Reviews* 55 (červen). <https://doi.org/10.1016/j.cytogfr.2020.06.004>.
- Lau, Laura, Elizabeth E. Gray, Rebecca L. Brunette, a Daniel B. Stetson. 2015. „DNA tumor virus oncogenes antagonize the cGAS-STING DNA-sensing pathway". *Science* 350 (6260): 568–71. <https://doi.org/10.1126/science.aab3291>.
- Lauterbach-Rivière, Lise, Mäiwenn Bergez, Saskia Mönch, Bingqian Qu, Maximilian Riess, Florian W. R. Vondran, Juliane Liese, Veit Hornung, Stephan Urban, a Renate König. 2020. „Hepatitis B Virus DNA Is a Substrate for the CGAS/STING Pathway but Is Not Sensed in Infected Hepatocytes". *Viruses* 12 (6): 592. <https://doi.org/10.3390/v12060592>.
- Li, Dapei, Rongsheng Wu, Wen Guo, Lifan Xie, Zigang Qiao, Shengchuan Chen, Jingfei Zhu, et al. 2019. „STING-Mediated IFI16 Degradation Negatively Controls Type I Interferon Production". *Cell Reports* 29 (5): 1249–1260.e4. <https://doi.org/10.1016/j.celrep.2019.09.069>.
- Li, Lingyin, Qian Yin, Pia Kuss, Zoltan Maliga, José L. Millán, Hao Wu, a Timothy J. Mitchison. 2014. „Hydrolysis of 2'3'-CGAMP by ENPP1 and Design of Nonhydrolyzable Analogs". *Nature Chemical Biology* 10 (12): 1043–48. <https://doi.org/10.1038/nchembio.1661>.
- Li, Mi, a Hong-Bing Shu. 2020. „Dephosphorylation of CGAS by PPP6C Impairs Its Substrate Binding Activity and Innate Antiviral Response". *Protein & Cell* 11 (8): 584–99. <https://doi.org/10.1007/s13238-020-00729-3>.
- Li, Siji, Lili Cao, Zeming Zhang, Ming Kuang, Luoying Chen, Yingchi Zhao, Yujie Luo, Zhinan Yin, a Fuping You. 2021. „Cytosolic and Nuclear Recognition of Virus and Viral Evasion". *Molecular Biomedicine* 2 (1): 30. <https://doi.org/10.1186/s43556-021-00046-z>.
- Li, Tuo, TuoZhi Huang, Mingjian Du, Xiang Chen, Fenghe Du, Junyao Ren, a Zhijian J. Chen. 2021. „Phosphorylation and Chromatin Tethering Prevent cGAS activation During Mitosis". *Science (New York, N.Y.)* 371 (6535): eabc5386. <https://doi.org/10.1126/science.abc5386>.
- Li, Tuo, Jin Chen, a Ileana M. Cristea. 2013. „Human Cytomegalovirus Tegument Protein PUL83 Inhibits IFI16-Mediated DNA Sensing for Immune Evasion". *Cell Host & Microbe* 14 (5): 591–99. <https://doi.org/10.1016/j.chom.2013.10.007>.
- Li, Xiao-Dong, Jiayi Wu, Daxing Gao, Hua Wang, Lijun Sun, a Zhijian J. Chen. 2013. „Pivotal Roles of cGAS-cGAMP Signaling in Antiviral Defense and Immune Adjuvant Effects". *Science* 341 (6152): 1390–94. <https://doi.org/10.1126/science.1244040>.
- Li, Zexing, Ge Liu, Liwei Sun, Yan Teng, Xuejiang Guo, Jianhang Jia, Jiahao Sha, Xiao Yang, Dahua Chen, a Qinmiao Sun. 2015. „PPM1A Regulates Antiviral Signaling by Antagonizing TBK1-Mediated STING Phosphorylation and Aggregation". *PLOS Pathogens* 11 (3): e1004783. <https://doi.org/10.1371/journal.ppat.1004783>.
- Liang, Qiming, Gil Ju Seo, Youn Jung Choi, Mi-Jeong Kwak, Jianning Ge, Mary A. Rodgers, Mude Shi, et al. 2014. „Crosstalk between the CGAS DNA Sensor and Beclin-1 Autophagy Protein Shapes Innate Antimicrobial Immune Responses". *Cell Host & Microbe* 15 (2): 228–38. <https://doi.org/10.1016/j.chom.2014.01.009>.
- Liu, Haipeng, Haiping Zhang, Xiangyang Wu, Dapeng Ma, Juehui Wu, Lin Wang, Yan Jiang, et al. 2018. „Nuclear CGAS Suppresses DNA Repair and Promotes Tumorigenesis". *Nature* 563 (7729): 131–36. <https://doi.org/10.1038/s41586-018-0629-6>.

- Liu, Nanxin, Xiaoxiao Pang, Hua Zhang, a Ping Ji. 2022. „The cGAS-STING Pathway in Bacterial Infection and Bacterial Immunity". *Frontiers in Immunology* 12. <https://www.frontiersin.org/article/10.3389/fimmu.2021.814709>.
- Liu, Siqi, Xin Cai, Jiayi Wu, Qian Cong, Xiang Chen, Tuo Li, Fenghe Du, et al. 2015. „Phosphorylation of Innate Immune Adaptor Proteins MAVS, STING, and TRIF Induces IRF3 Activation". *Science* 347 (6227): aaa2630. <https://doi.org/10.1126/science.aaa2630>.
- Liu, Yinghui, Jianhua Li, Jieliang Chen, Yaming Li, Weixia Wang, Xiaoting Du, Wuhui Song, Wen Zhang, Li Lin, a Zhenghong Yuan. 2015. „Hepatitis B Virus Polymerase Disrupts K63-Linked Ubiquitination of STING To Block Innate Cytosolic DNA-Sensing Pathways". *Journal of Virology* 89 (4): 2287–2300. <https://doi.org/10.1128/JVI.02760-14>.
- Liu, Yuan, Ziyang Zou, Bing Zhu, Zonghai Hu, Ping Zeng, a Lijuan Wu. 2015. „LRRFIP1 Inhibits Hepatitis C Virus Replication by Inducing Type I Interferon in Hepatocytes". *Hepatitis Monthly* 15 (5): e28473. [https://doi.org/10.5812/hepatmon.15\(5\)2015.28473](https://doi.org/10.5812/hepatmon.15(5)2015.28473).
- Liu, Zhao-Shan, Hong Cai, Wen Xue, Miao Wang, Tian Xia, Wan-Jin Li, Jia-Qing Xing, et al. 2019. „G3BP1 Promotes DNA Binding and Activation of CGAS". *Nature Immunology* 20 (1): 18–28. <https://doi.org/10.1038/s41590-018-0262-4>.
- Lu, Jie, Subhash C. Verma, Qiliang Cai, Abhik Saha, Richard Kuo Dzeng, a Erle S. Robertson. 2012. „The RBP-Jk Binding Sites within the RTA Promoter Regulate KSHV Latent Infection and Cell Proliferation". *PLoS Pathogens* 8 (1): e1002479. <https://doi.org/10.1371/journal.ppat.1002479>.
- Lum, Krystal K., Timothy R. Howard, Catherina Pan, a Ileana M. Cristea. b.r. „Charge-Mediated Pyrin Oligomerization Nucleates Antiviral IFI16 Sensing of Herpesvirus DNA". *mBio* 10 (4): e01428-19. <https://doi.org/10.1128/mBio.01428-19>.
- Ma, Dapeng, Min Yang, Qiushi Wang, Caiyu Sun, Hongbiao Shi, Weiqiang Jing, Yuxuan Bi, et al. 2021. „Arginine methyltransferase PRMT5 negatively regulates cGAS-mediated antiviral immune response". *Science Advances* 7 (13): eabc1834. <https://doi.org/10.1126/sciadv.abc1834>.
- Ma, Zhe, Sarah R. Jacobs, John A. West, Charles Stopford, Zhigang Zhang, Zoe Davis, Glen N. Barber, Britt A. Glaunsinger, Dirk P. Dittmer, a Blossom Damania. 2015. „Modulation of the CGAS-STING DNA Sensing Pathway by Gammaherpesviruses". *Proceedings of the National Academy of Sciences of the United States of America* 112 (31): E4306-4315. <https://doi.org/10.1073/pnas.1503831112>.
- McFarland, Adelle P., Shukun Luo, Fariha Ahmed-Qadri, Meghan Zuck, Elizabeth F. Thayer, Young Ah Goo, Kevin Hybiske, Liang Tong, a Joshua J. Woodward. 2017. „Sensing of Bacterial Cyclic Dinucleotides by the Oxidoreductase RECON Promotes NF-KB Activation and Shapes a Proinflammatory Antibacterial State". *Immunity* 46 (3): 433–45. <https://doi.org/10.1016/j.immuni.2017.02.014>.
- Michalski, Sebastian, Carina C. de Oliveira Mann, Che A. Stafford, Gregor Witte, Joseph Bartho, Katja Lammens, Veit Hornung, a Karl-Peter Hopfner. 2020. „Structural Basis for Sequestration and Autoinhibition of CGAS by Chromatin". *Nature* 587 (7835): 678–82. <https://doi.org/10.1038/s41586-020-2748-0>.
- Mimori, T., a J. A. Hardin. 1986. „Mechanism of Interaction between Ku Protein and DNA." *Journal of Biological Chemistry* 261 (22): 10375–79. [https://doi.org/10.1016/S0021-9258\(18\)67534-9](https://doi.org/10.1016/S0021-9258(18)67534-9).
- Morrone, Seamus R., Tao Wang, Leeza M. Constantoulakis, Richard M. Hooy, Michael J. Delannoy, a Jungsan Sohn. 2014. „Cooperative Assembly of IFI16 Filaments on DsDNA Provides Insights into Host Defense Strategy". *Proceedings of the National Academy of Sciences* 111 (1). <https://doi.org/10.1073/pnas.1313577111>.

- Mukhopadhyay, Debdyuti, a Howard Riezman. 2007. „Proteasome-Independent Functions of Ubiquitin in Endocytosis and Signaling". *Science* 315 (5809): 201–5. <https://doi.org/10.1126/science.1127085>.
- Ni, Xiangmin, Heng Ru, Feng Ma, Lixia Zhao, Neil Shaw, Yingang Feng, Wei Ding, et al. 2016. „New insights into the structural basis of DNA recognition by HINa and HINb domains of IFI16". *Journal of Molecular Cell Biology* 8 (1): 51–61. <https://doi.org/10.1093/jmcb/mjv053>.
- Nussenzweig, A., C. Chen, V. da Costa Soares, M. Sanchez, K. Sokol, M. C. Nussenzweig, a G. C. Li. 1996. „Requirement for Ku80 in Growth and Immunoglobulin V(D)J Recombination". *Nature* 382 (6591): 551–55. <https://doi.org/10.1038/382551a0>.
- Ohto, Umeharu, Hanako Ishida, Takuma Shibata, Ryota Sato, Kensuke Miyake, a Toshiyuki Shimizu. 2018. „Toll-like Receptor 9 Contains Two DNA Binding Sites That Function Cooperatively to Promote Receptor Dimerization and Activation". *Immunity* 48 (4): 649-658.e4. <https://doi.org/10.1016/j.immuni.2018.03.013>.
- Olagnier, David, Aske M. Brandtoft, Camilla Gunderstofte, Nikolaj L. Villadsen, Christian Krapp, Anne L. Thielke, Anders Laustsen, et al. 2018. „Nrf2 Negatively Regulates STING Indicating a Link between Antiviral Sensing and Metabolic Reprogramming". *Nature Communications* 9 (1): 3506. <https://doi.org/10.1038/s41467-018-05861-7>.
- Orzalli, Megan H., Nicole M. Broekema, Benjamin A. Diner, Dustin C. Hancks, Nels C. Elde, Ileana M. Cristea, a David M. Knipe. 2015. „CGAS-Mediated Stabilization of IFI16 Promotes Innate Signaling during Herpes Simplex Virus Infection". *Proceedings of the National Academy of Sciences* 112 (14). <https://doi.org/10.1073/pnas.1424637112>.
- Orzalli, Megan H., Nicole M. Broekema, a David M. Knipe. 2016. „Relative Contributions of Herpes Simplex Virus 1 ICP0 and Vhs to Loss of Cellular IFI16 Vary in Different Human Cell Types". *Journal of Virology* 90 (18): 8351–59. <https://doi.org/10.1128/JVI.00939-16>.
- Orzalli, Megan H., Neal A. DeLuca, a David M. Knipe. 2012. „Nuclear IFI16 Induction of IRF-3 Signaling during Herpesviral Infection and Degradation of IFI16 by the Viral ICP0 Protein". *Proceedings of the National Academy of Sciences* 109 (44). <https://doi.org/10.1073/pnas.1211302109>.
- Ouyang, Songying, Xianqiang Song, Yaya Wang, Heng Ru, Neil Shaw, Yan Jiang, Fengfeng Niu, et al. 2012. „Structural and Functional Analysis of STING Sheds New Light on Cyclic di-GMP Mediated Immune Signaling Mechanism". *Immunity* 36 (6): 10.1016/j.immuni.2012.03.019. <https://doi.org/10.1016/j.immuni.2012.03.019>.
- Paola, Rosa Di, Nunzia Caporarello, Antonella Marucci, Claudia Dimatteo, Claudia Iadicicco, Silvia Del Guerra, Sabrina Prudente, et al. 2011. „ENPP1 Affects Insulin Action and Secretion: Evidences from In Vitro Studies". *PLOS ONE* 6 (5): e19462. <https://doi.org/10.1371/journal.pone.0019462>.
- Parker, Zachary M., Aisling A. Murphy, a David A. Leib. b.r. „Role of the DNA Sensor STING in Protection from Lethal Infection following Corneal and Intracerebral Challenge with Herpes Simplex Virus 1". *Journal of Virology* 89 (21): 11080–91. <https://doi.org/10.1128/JVI.00954-15>.
- Reinert, Line S., Katarína Lopusná, Henriette Winther, Chenglong Sun, Martin K. Thomsen, Ramya Nandakumar, Trine H. Mogensen, et al. 2016. „Sensing of HSV-1 by the CGAS–STING Pathway in Microglia Orchestrates Antiviral Defence in the CNS". *Nature Communications* 7 (1): 13348. <https://doi.org/10.1038/ncomms13348>.
- Rogowski, Krzysztof, Juliette van Dijk, Maria M. Magiera, Christophe Bosc, Jean-Christophe Deloulme, Anouk Bosson, Leticia Peris, et al. 2010. „A Family of Protein-Deglutamylating Enzymes Associated with Neurodegeneration". *Cell* 143 (4): 564–78. <https://doi.org/10.1016/j.cell.2010.10.014>.

- Sato, M., H. Suemori, N. Hata, M. Asagiri, K. Ogasawara, K. Nakao, T. Nakaya, et al. 2000. „Distinct and Essential Roles of Transcription Factors IRF-3 and IRF-7 in Response to Viruses for IFN-Alpha/Beta Gene Induction". *Immunity* 13 (4): 539–48. [https://doi.org/10.1016/s1074-7613\(00\)00053-4](https://doi.org/10.1016/s1074-7613(00)00053-4).
- Sato, Taku, Nobuyuki Onai, Hiroki Yoshihara, Fumio Arai, Toshio Suda, a Toshiaki Ohteki. 2009. „Interferon Regulatory Factor-2 Protects Quiescent Hematopoietic Stem Cells from Type I Interferon–Dependent Exhaustion". *Nature Medicine* 15 (6): 696–700. <https://doi.org/10.1038/nm.1973>.
- Seo, Gil Ju, Aerin Yang, Brandon Tan, Sungyoon Kim, Qiming Liang, Younho Choi, Weiming Yuan, Pinghui Feng, Hee-Sung Park, a Jae U. Jung. 2015. „Akt Kinase-Mediated Checkpoint of CGAS DNA Sensing Pathway". *Cell Reports* 13 (2): 440–49. <https://doi.org/10.1016/j.celrep.2015.09.007>.
- Shaikh, Mushfiq H., Vuk Bortnik, Nigel A.J. McMillan, a Adi Idris. 2019. „CGAS-STING Responses Are Dampened in High-Risk HPV Type 16 Positive Head and Neck Squamous Cell Carcinoma Cells". *Microbial Pathogenesis* 132 (červenec): 162–65. <https://doi.org/10.1016/j.micpath.2019.05.004>.
- Shang, Guijun, Conggang Zhang, Zhijian J. Chen, Xiao-chen Bai, a Xuewu Zhang. 2019. „Cryo-EM structures of STING reveal its mechanism of activation by cyclic GMP–AMP". *Nature* 567 (7748): 389–93. <https://doi.org/10.1038/s41586-019-0998-5>.
- Shang, Guijun, Deyu Zhu, Ning Li, Junbing Zhang, Chunyuan Zhu, Defen Lu, Cuilan Liu, et al. 2012. „Crystal Structures of STING Protein Reveal Basis for Recognition of Cyclic Di-GMP". *Nature Structural & Molecular Biology* 19 (7): 725–27. <https://doi.org/10.1038/nsmb.2332>.
- Shi, Chengrui, Xikang Yang, Ye Liu, Hongpeng Li, Huiying Chu, Guohui Li, a Hang Yin. 2022. „ZDHHC18 Negatively Regulates CGAS-Mediated Innate Immunity through Palmitoylation". *The EMBO Journal* 41 (11): e109272. <https://doi.org/10.15252/embj.2021109272>.
- Shu, Chang, Guanghui Yi, Tylan Watts, C. Cheng Kao, a Pingwei Li. 2012. „Structure of STING bound to c-di-GMP Reveals the Mechanism of Cyclic Dinucleotide Recognition by the Immune System". *Nature structural & molecular biology* 19 (7): 722–24. <https://doi.org/10.1038/nsmb.2331>.
- Singleton, B. K., M. I. Torres-Arzayus, S. T. Rottinghaus, G. E. Taccioli, a P. A. Jeggo. 1999. „The C Terminus of Ku80 Activates the DNA-Dependent Protein Kinase Catalytic Subunit". *Molecular and Cellular Biology* 19 (5): 3267–77.
- Srikanth, Sonal, Jin Seok Woo, Beibei Wu, Yasser M. El-Sherbiny, Jennifer Leung, Koollawat Chupradit, Laura Rice, et al. 2019. „The Ca²⁺ Sensor STIM1 Regulates the Type I Interferon Response by Retaining the Signaling Adaptor STING at the Endoplasmic Reticulum". *Nature Immunology* 20 (2): 152–62. <https://doi.org/10.1038/s41590-018-0287-8>.
- Stetson, Daniel B., a Ruslan Medzhitov. 2006. „Recognition of Cytosolic DNA Activates an IRF3-Dependent Innate Immune Response". *Immunity* 24 (1): 93–103. <https://doi.org/10.1016/j.immuni.2005.12.003>.
- Stratmann, Sarah A, Seamus R Morrone, Antoine M van Oijen, a Jungsan Sohn. 2015. „The innate immune sensor IFI16 recognizes foreign DNA in the nucleus by scanning along the duplex". Editoval David M Knipe. *eLife* 4 (prosinec): e11721. <https://doi.org/10.7554/eLife.11721>.
- Su, Chenhe, a Chunfu Zheng. 2017. „Herpes Simplex Virus 1 Abrogates the cGAS/STING-Mediated Cytosolic DNA-Sensing Pathway via Its Virion Host Shutoff Protein, UL41". *Journal of Virology* 91 (6): e02414-16. <https://doi.org/10.1128/JVI.02414-16>.
- Sui, Hongyan, Ming Zhou, Hiromi Imamichi, Xiaoli Jiao, Brad T. Sherman, H. Clifford Lane, a Tomozumi Imamichi. 2017. „STING Is an Essential Mediator of the Ku70-Mediated Production of IFN- λ 1 in Response to Exogenous DNA". *Science Signaling* 10 (488): eaah5054. <https://doi.org/10.1126/scisignal.aah5054>.

- Sun, He, Qiang Zhang, Ying-Ying Jing, Man Zhang, Hai-Ying Wang, Zeng Cai, Tianzi Liuyu, et al. 2017. „USP13 Negatively Regulates Antiviral Responses by Deubiquitinating STING". *Nature Communications* 8 (květen): 15534. <https://doi.org/10.1038/ncomms15534>.
- Sun, Chenglong, Stefan A. Schattgen, Prapaporn Pisitkun, Joan P. Jorgensen, Adam T. Hilterbrand, Lucas J. Wang, John A. West, et al. 2015. „Evasion of Innate Cytosolic DNA Sensing by a Gammaherpesvirus Facilitates Establishment of Latent Infection". *Journal of Immunology (Baltimore, Md.: 1950)* 194 (4): 1819–31. <https://doi.org/10.4049/jimmunol.1402495>.
- Sun, Lijun, Jiayi Wu, Fenghe Du, Xiang Chen, a Zhijian J. Chen. 2013. „Cyclic GMP-AMP Synthase Is a Cytosolic DNA Sensor That Activates the Type I Interferon Pathway". *Science* 339 (6121): 786–91. <https://doi.org/10.1126/science.1232458>.
- Sun, Xiaona, Ting Liu, Jun Zhao, Hansong Xia, Jun Xie, Yu Guo, Li Zhong, et al. 2020. „DNA-PK Deficiency Potentiates CGAS-Mediated Antiviral Innate Immunity". *Nature Communications* 11 (1): 6182. <https://doi.org/10.1038/s41467-020-19941-0>.
- Takaoka, Akinori, Zhichao Wang, Myoung Kwon Choi, Hideyuki Yanai, Hideo Negishi, Tatsuma Ban, Yan Lu, et al. 2007. „DAI (DLM-1/ZBP1) Is a Cytosolic DNA Sensor and an Activator of Innate Immune Response". *Nature* 448 (7152): 501–5. <https://doi.org/10.1038/nature06013>.
- Takeshige, K., M. Baba, S. Tsuboi, T. Noda, a Y. Ohsumi. 1992. „Autophagy in Yeast Demonstrated with Proteinase-Deficient Mutants and Conditions for Its Induction". *The Journal of Cell Biology* 119 (2): 301–11. <https://doi.org/10.1083/jcb.119.2.301>.
- Tan, Yee Sun, Kanokwan Sansanaphongpricha, Yuying Xie, Christopher R. Donnelly, Xiaobo Luo, Blake R. Heath, Xinyi Zhao, et al. 2018. „Mitigating SOX2-potentiated Immune Escape of Head and Neck Squamous Cell Carcinoma with a STING-inducing Nanosatellite Vaccine". *Clinical Cancer Research* 24 (17): 4242–55. <https://doi.org/10.1158/1078-0432.CCR-17-2807>.
- Thomas, Paul G., Pradyot Dash, Jerry R. Aldridge, Ali H. Ellebedy, Cory Reynolds, Amy J. Funk, William J. Martin, et al. 2009. „The Intracellular Sensor NLRP3 Mediates Key Innate and Healing Responses to Influenza A Virus via the Regulation of Caspase-1". *Immunity* 30 (4): 566–75. <https://doi.org/10.1016/j.immuni.2009.02.006>.
- Tsukada, M., a Y. Ohsumi. 1993. „Isolation and Characterization of Autophagy-Defective Mutants of *Saccharomyces Cerevisiae*". *FEBS Letters* 333 (1–2): 169–74. [https://doi.org/10.1016/0014-5793\(93\)80398-e](https://doi.org/10.1016/0014-5793(93)80398-e).
- Unterholzner, Leonie, Sinead E. Keating, Marcin Baran, Kristy A. Horan, Søren B. Jensen, Shruti Sharma, Cherilyn M. Sirois, et al. 2010. „IFI16 is an innate immune sensor for intracellular DNA". *Nature immunology* 11 (11): 997–1004. <https://doi.org/10.1038/ni.1932>.
- Verrier, Eloi R., Seung-Ae Yim, Laura Heydmann, Houssein El Saghire, Charlotte Bach, Vincent Turon-Lagot, Laurent Mailly, et al. 2018. „Hepatitis B Virus Evasion From Cyclic Guanosine Monophosphate–Adenosine Monophosphate Synthase Sensing in Human Hepatocytes". *Hepatology* 68 (5): 1695–1709. <https://doi.org/10.1002/hep.30054>.
- Walker, J. R., R. A. Corpina, a J. Goldberg. 2001. „Structure of the Ku Heterodimer Bound to DNA and Its Implications for Double-Strand Break Repair". *Nature* 412 (6847): 607–14. <https://doi.org/10.1038/35088000>.
- Wang, Lei, Mingyue Wen, a Xuetao Cao. 2019. „Nuclear HnRNPA2B1 Initiates and Amplifies the Innate Immune Response to DNA Viruses". *Science* 365 (6454): eaav0758. <https://doi.org/10.1126/science.aav0758>.

- Wang, Qiang, Liyuan Huang, Ze Hong, Zhongshi Lv, Zhaomin Mao, Yijun Tang, Xiufang Kong, et al. 2017. „The E3 Ubiquitin Ligase RNF185 Facilitates the CGAS-Mediated Innate Immune Response". *PLoS Pathogens* 13 (3): e1006264. <https://doi.org/10.1371/journal.ppat.1006264>.
- Wang, Yanming, Qiaoshi Lian, Bo Yang, Shanshan Yan, Haiyan Zhou, Lan He, Guomei Lin, Zhexiong Lian, Zhengfan Jiang, a Bing Sun. 2015. „TRIM30 α Is a Negative-Feedback Regulator of the Intracellular DNA and DNA Virus-Triggered Response by Targeting STING". *PLOS Pathogens* 11 (6): e1005012. <https://doi.org/10.1371/journal.ppat.1005012>.
- Wang, Yutao, Xiaohan Ning, Pengfei Gao, Shuxian Wu, Mengyin Sha, Mengze Lv, Xiang Zhou, et al. 2017. „Ve". *Immunity* 46 (3): 393–404. <https://doi.org/10.1016/j.immuni.2017.02.011>.
- West, A. Phillip, William Khoury-Hanold, Matthew Staron, Michal C. Tal, Cristiana M. Pineda, Sabine M. Lang, Megan Bestwick, et al. 2015. „Mitochondrial DNA Stress Primes the Antiviral Innate Immune Response". *Nature* 520 (7548): 553–57. <https://doi.org/10.1038/nature14156>.
- Wilkinson, Keith D. 2000. „Ubiquitination and Deubiquitination: Targeting of Proteins for Degradation by the Proteasome". *Seminars in Cell & Developmental Biology* 11 (3): 141–48. <https://doi.org/10.1006/scdb.2000.0164>.
- Wilson, Anne, Elisa Laurenti, Gabriela Oser, Richard C. van der Wath, William Blanco-Bose, Maïke Jaworski, Sandra Offner, et al. 2008. „Hematopoietic Stem Cells Reversibly Switch from Dormancy to Self-Renewal during Homeostasis and Repair". *Cell* 135 (6): 1118–29. <https://doi.org/10.1016/j.cell.2008.10.048>.
- Wu, Jian-jun, Wenwei Li, Yaming Shao, Denis Avey, Bishi Fu, Joseph Gillen, Travis Hand, et al. 2015. „Inhibition of CGAS DNA Sensing by a Herpesvirus Virion Protein". *Cell Host & Microbe* 18 (3): 333–44. <https://doi.org/10.1016/j.chom.2015.07.015>.
- Wu, Lizhen, Jian Cao, Wesley L. Cai, Sabine M. Lang, John R. Horton, Daniel J. Jansen, Zongzhi Z. Liu, et al. 2018. „KDM5 Histone Demethylases Repress Immune Response via Suppression of STING". *PLOS Biology* 16 (8): e2006134. <https://doi.org/10.1371/journal.pbio.2006134>.
- Wu, Min-Zu, Wei-Chung Cheng, Su-Feng Chen, Shin Nieh, Carolyn O'Connor, Chia-Lin Liu, Wen-Wei Tsai, et al. 2017. „MiR-25/93 Mediates Hypoxia-Induced Immunosuppression by Repressing CGAS". *Nature Cell Biology* 19 (10): 1286–96. <https://doi.org/10.1038/ncb3615>.
- Wu, Shiyong, Qian Zhang, Fei Zhang, Fansen Meng, Shengduo Liu, Ruyuan Zhou, Qingzhe Wu, et al. 2019. „HER2 Recruits AKT1 to Disrupt STING Signalling and Suppress Antiviral Defence and Antitumour Immunity". *Nature Cell Biology* 21 (8): 1027–40. <https://doi.org/10.1038/s41556-019-0352-z>.
- Xia, Pengyan, Shuo Wang, Buqing Ye, Ying Du, Chong Li, Zhen Xiong, Yuan Qu, a Zusen Fan. 2018. „A Circular RNA Protects Dormant Hematopoietic Stem Cells from DNA Sensor CGAS-Mediated Exhaustion". *Immunity* 48 (4): 688–701.e7. <https://doi.org/10.1016/j.immuni.2018.03.016>.
- Xia, Pengyan, Buqing Ye, Shuo Wang, Xiaoxiao Zhu, Ying Du, Zhen Xiong, Yong Tian, a Zusen Fan. 2016. „Glutamylation of the DNA Sensor CGAS Regulates Its Binding and Synthase Activity in Antiviral Immunity". *Nature Immunology* 17 (4): 369–78. <https://doi.org/10.1038/ni.3356>.
- Xia, Tian, Xue-Mei Yi, Xin Wu, Jun Shang, a Hong-Bing Shu. 2019. „PTPN1/2-mediated dephosphorylation of MITA/STING promotes its 20S proteasomal degradation and attenuates innate antiviral response". *Proceedings of the National Academy of Sciences* 116 (40): 20063–69. <https://doi.org/10.1073/pnas.1906431116>.
- Xie, Wei, Lodoë Lama, Carolina Adura, Daisuke Tomita, J. Fraser Glickman, Thomas Tuschl, a Dinshaw J. Patel. 2019a. „Human cGAS catalytic domain has an additional DNA-binding interface that

- enhances enzymatic activity and liquid-phase condensation". *Proceedings of the National Academy of Sciences* 116 (24): 11946–55. <https://doi.org/10.1073/pnas.1905013116>.
- Xie, Zhiping, Usha Nair, a Daniel J. Klionsky. 2008. „Atg8 Controls Phagophore Expansion during Autophagosome Formation". *Molecular Biology of the Cell* 19 (8): 3290–98. <https://doi.org/10.1091/mbc.e07-12-1292>.
- Xing, Junji, Ao Zhang, Hua Zhang, Jin Wang, Xian Chang Li, Mu-Sheng Zeng, a Zhiqiang Zhang. 2017. „TRIM29 Promotes DNA Virus Infections by Inhibiting Innate Immune Response". *Nature Communications* 8 (1): 945. <https://doi.org/10.1038/s41467-017-00101-w>.
- Yang, Pengyuan, Huazhang An, Xingguang Liu, Mingyue Wen, Yuanyuan Zheng, Yaocheng Rui, a Xuetao Cao. 2010. „The Cytosolic Nucleic Acid Sensor LRRFIP1 Mediates the Production of Type I Interferon via a Beta-Catenin-Dependent Pathway". *Nature Immunology* 11 (6): 487–94. <https://doi.org/10.1038/ni.1876>.
- Ye, Liya, Qiang Zhang, Tianzi Liuyu, Zhigao Xu, Meng-Xin Zhang, Min-Hua Luo, Wen-Bo Zeng, Qiyun Zhu, Dandan Lin, a Bo Zhong. 2019. „USP49 Negatively Regulates Cellular Antiviral Responses via Deconjugating K63-Linked Ubiquitination of MITA". *PLOS Pathogens* 15 (4): e1007680. <https://doi.org/10.1371/journal.ppat.1007680>.
- Yu, Xiaoyu, Liyuan Zhang, Jingxiang Shen, Yanfang Zhai, Qifei Jiang, Mengran Yi, Xiaobing Deng, et al. 2021. „The STING Phase-Separator Suppresses Innate Immune Signalling". *Nature Cell Biology* 23 (4): 330–40. <https://doi.org/10.1038/s41556-021-00659-0>.
- Zhang, Conggang, Guijun Shang, Xiang Gui, Xuewu Zhang, Xiao-chen Bai, a Zhijian J. Chen. 2019. „Structural Basis of STING Binding with and Phosphorylation by TBK1". *Nature* 567 (7748): 394–98. <https://doi.org/10.1038/s41586-019-1000-2>.
- Zhang, Guigen, Baca Chan, Naira Samarina, Bizunesh Abere, Magdalena Weidner-Glunde, Anna Buch, Andreas Pich, Melanie M. Brinkmann, a Thomas F. Schulz. 2016. „Cytoplasmic Isoforms of Kaposi Sarcoma Herpesvirus LANA Recruit and Antagonize the Innate Immune DNA Sensor CGAS". *Proceedings of the National Academy of Sciences of the United States of America* 113 (8): E1034–1043. <https://doi.org/10.1073/pnas.1516812113>.
- Zhang, Lu, Jinyao Mo, Karen V. Swanson, Haitao Wen, Alex Petrucelli, Sean M. Gregory, Zhigang Zhang, et al. 2014. „NLRC3, a Member of the NLR Family of Proteins, Is a Negative Regulator of Innate Immune Signaling Induced by the DNA Sensor STING". *Immunity* 40 (3): 329–41. <https://doi.org/10.1016/j.immuni.2014.01.010>.
- Zhang, Xiao-Ou, Rui Dong, Yang Zhang, Jia-Lin Zhang, Zheng Luo, Jun Zhang, Ling-Ling Chen, a Li Yang. 2016. „Diverse Alternative Back-Splicing and Alternative Splicing Landscape of Circular RNAs". *Genome Research* 26 (9): 1277–87. <https://doi.org/10.1101/gr.202895.115>.
- Zhang, Xing, Terrence W. Brann, Ming Zhou, Jun Yang, Raphael M. Oguariri, Kristy B. Lidie, Hiromi Imamichi, et al. 2011. „Cutting Edge: Ku70 Is a Novel Cytosolic DNA Sensor That Induces Type III Rather than Type I IFN". *Journal of Immunology (Baltimore, Md.: 1950)* 186 (8): 4541–45. <https://doi.org/10.4049/jimmunol.1003389>.
- Zhang, Zhiqiang, Bin Yuan, Musheng Bao, Ning Lu, Taeil Kim, a Yong-Jun Liu. 2011. „The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells". *Nature immunology* 12 (10): 959–65. <https://doi.org/10.1038/ni.2091>.
- Zhao, Mengmeng, Fei Wang, Juehui Wu, Yuanna Cheng, Yajuan Cao, Xiangyang Wu, Mingtong Ma, et al. b.r. „CGAS is a micronucleophagy receptor for the clearance of micronuclei". *Autophagy* 17 (12): 3976–91. <https://doi.org/10.1080/15548627.2021.1899440>.

- Zhong, Bo, Yan Yang, Shu Li, Yan-Yi Wang, Ying Li, Feici Diao, Caoqi Lei, et al. 2008. „The Adaptor Protein MITA Links Virus-Sensing Receptors to IRF3 Transcription Factor Activation". *Immunity* 29 (4): 538–50. <https://doi.org/10.1016/j.immuni.2008.09.003>.
- Zhong, Bo, Lu Zhang, Caoqi Lei, Ying Li, Ai-Ping Mao, Yan Yang, Yan-Yi Wang, Xiao-Lian Zhang, a Hong-Bing Shu. 2009. „The Ubiquitin Ligase RNF5 Regulates Antiviral Responses by Mediating Degradation of the Adaptor Protein MITA". *Immunity* 30 (3): 397–407. <https://doi.org/10.1016/j.immuni.2009.01.008>.
- Zhong, Li, Ming-Ming Hu, Li-Jun Bian, Ying Liu, Qiang Chen, a Hong-Bing Shu. 2020. „Phosphorylation of CGAS by CDK1 Impairs Self-DNA Sensing in Mitosis". *Cell Discovery* 6 (1): 1–12. <https://doi.org/10.1038/s41421-020-0162-2>.
- Zhou, Wen, Aaron T. Whiteley, Carina C. de Oliveira Mann, Benjamin R. Morehouse, Radosław P. Nowak, Eric S. Fischer, Nathanael S. Gray, John J. Mekalanos, a Philip J. Kranzusch. 2018. „Structure of the Human CGAS-DNA Complex Reveals Enhanced Control of Immune Surveillance". *Cell* 174 (2): 300-311.e11. <https://doi.org/10.1016/j.cell.2018.06.026>.
- Zierhut, Christian, Norihiro Yamaguchi, Maria Paredes, Ji-Dung Luo, Thomas Carroll, a Hironori Funabiki. 2019. „The cytoplasmic DNA sensor cGAS promotes mitotic cell death". *Cell* 178 (2): 302-315.e23. <https://doi.org/10.1016/j.cell.2019.05.035>.