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## Latest advances in innate antiviral defence

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### Abstract

Recent identification of key components in the pattern recognition receptor pathway of retinoic acid-inducible gene-1-like receptors, coupled with characterisation of a new cytoplasmic DNA-sensing molecule, have led to a greater understanding of the role viral nucleic acids play in activating innate immunity. This activation of type I interferon is essential for both limiting viral infection and stimulating activation of the adaptive immune response.

### Introduction and context

Upon gaining access to a cell, a virus is recognised by the host's innate immune response, predominantly through the presence of foreign nucleic acids binding to pattern recognition receptors (PRRs). Key effectors of this response are Toll-like receptors (TLRs) and retinoic acid-inducible gene-1 (RIG-I)-like receptors (RLRs), which act by triggering signalling cascades, ultimately resulting in the production of type-I interferons (IFNs), the corresponding interferon-stimulated genes (ISGs), and activation of other key regulators of innate immunity, such as NF $\kappa$ B. This initial response is essential to limit viral infection and activate natural killer cells and dendritic cells, setting in train the adaptive immune response. While much has been learned about TLR signalling pathways, the mechanism by which RLRs lead to IFN activation has yet to be fully elucidated. The recent independent discovery by different groups of a key regulator named MPYS, stimulator of interferon genes (STING) or MITA has now identified a critical component of the pathway linking RLRs to type-I IFN production {1,2}. Moreover, other studies have revealed a key component in the mechanism of DNA-dependent activation of the IFN regulatory factors (IRFs) {3}.

RIG-I, melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2), are a small family of RNA helicases residing in the cytoplasm that contain RNA-binding domains capable of recognising foreign viral transcripts, such as those from negative strand RNA viruses {4}. While all three helicases function similarly, they differ in size-specific and sequence motif-specific recognition of viral transcripts {5,6}. RIG-I also contains a self-regulatory domain that recognises 5'-triphosphorylated single-stranded (ss) or double-stranded (ds) RNA to allow activation {7}. This affects the ability of each helicase to activate the innate immune system in response to different pathogens. The activation occurs through the tandem CARD domain in RIG-I and MDA5, which binds to the mitochondrial antiviral signalling protein (MAVS/IPS-1/CARDIF/VISA) {8-11}. Once activated, MAVS triggers activation of two protein complexes [tank-binding kinase 1 (TBK1)–IKK $\epsilon$ –IKK $\gamma$ –

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TRAF-family-member-associated NF $\kappa$ B activator (TANK) and IKK $\alpha$ -IKK $\beta$ -IKK $\gamma$ ] involved in activation of the IRFs and the NF $\kappa$ B transcription factor, respectively (Figure 1).

## Major recent advances

Two research groups have independently identified a trans-membrane protein, residing in both the endoplasmic reticulum and mitochondrial membrane, which is necessary for RIG-I-mediated IFN activation {1,2}. While differences in localisation were reported in the two papers, it was proposed that STING interacted with a RIG-I-SSR2/TRAP $\beta$  translocon complex, facilitating a link between viral RNA transcripts and IFN activation. Zhong et al {2} also showed a link for STING in RIG-I signalling, through MAVS, and its interaction with TBK1/IRF3. STING is encoded by the identical gene previously named MPYS, a plasma membrane tetraspanner implicated in mitochondrial and surface membrane presentation of antigens through an interaction with major histocompatibility complex type-II {12}. However, an unequivocal role for this molecule has been shown in pathogen associated molecular pattern recognition through the use of knockout mice. In mice deficient in STING or in cells derived from these mice, IRF3 activation via TBK1 is compromised and an increased susceptibility to viral infections is evident. However, there also appears to be a cell-type dependency of STING for production of a complete IFN response to viral RNA. Despite differences in the predicted mechanism of action, one via MAVS and the other via the translocon complex, both groups also showed an additional role for STING in non-CpG DNA-mediated induction of type I IFNs.

In addition to activating an RNA-sensing mechanism, virus DNA can induce a protective innate immune response through other cellular sensors. This recognition was believed to be through the action of TLR9 recognising CpG-rich DNA {13,14}. However, there is also evidence indicating a TLR9-independent activator of ISGs {15}. Recent work has revealed DNA-dependent activator of interferon regulatory factors (DAI)/DLM-1/ZBP1 as a key component in the recognition of DNA through its three DNA-binding domains. DNA binding by DAI activates dimer formation enabling interaction with TBK1 and IRF3, enhancing activation of IRF3 and possibly IRF7 in response to foreign, cytoplasmic, DNA. This interaction is dependent upon DNA to maintain the interaction of DAI with TBK1 {16}.

Interestingly, the ability of DAI to induce IRF activation in response to pathogenic or host DNA residing in the cytoplasm is cell-type dependent {16}. In mouse embryonic fibroblasts with depleted DAI, there was only a minimal reduction to non-CpG (B-form)-mediated DNA induction of DNA. This, along with previous evidence, suggests other DNA-sensing molecules required for recognition of DNA present in the cytoplasm remain to be identified. Nonetheless, reduction of DAI in the macrophage cell lines showed a decrease in IFN induction upon viral infection.

The characterisation of further components in the RLR signalling pathway has identified an important link between cytoplasmic RNA sensors and the TBK1/IRF protein complex for activation of IFN. The DNA cytoplasmic sensor, DAI, also interacts with TBK1/IRF. This major component of IFN induction plays a role in both RNA and DNA cytoplasmic sensing and emphasises a convergence of two different PRR pathways.

Important to note is the abundance of viral proteins aimed at inhibiting either RLR sensing, for example, influenza virus NS1 {17,18} and human metapneumovirus G protein {19}, or DNA sensing, inhibited by Vaccinia virus E3L {20} and porcine circovirus type 2 {21}. Different viral proteins have also evolved to target the convergence of the two pathways, and inhibit TBK1 and IRF3 phosphorylation. These include Ebola virus VP35 {22}, Herpes simplex virus 1  $\gamma$ 34.5 {23}, rabies virus P protein {24} and hantavirus G1 {25}, among many others (reviewed in {26}).

## Future directions

These recent findings provide new insights into the signalling pathways triggered by viral nucleic acids, but still leave many unanswered questions. The exact mechanism of action for STING/MITA remains to be determined, along with its localisation and role in either MAVS activation or the translocon complex. The latter raises questions about the way viral nucleic acids are processed and presented to activate the cell's innate immune response.

Further investigation should also lead to identification of other cytoplasmic DNA sensors and elucidate which sensors are required in each cellular compartment, or cell type. This will provide a better understanding of host response to different pathogens and of the particular cell type engaged by the host to recognise the infection. Careful characterisation of all components required for recognising cellular pathogens will lead to a greater understanding of the innate immune response and ideally provide new therapeutic targets to help treat infections.

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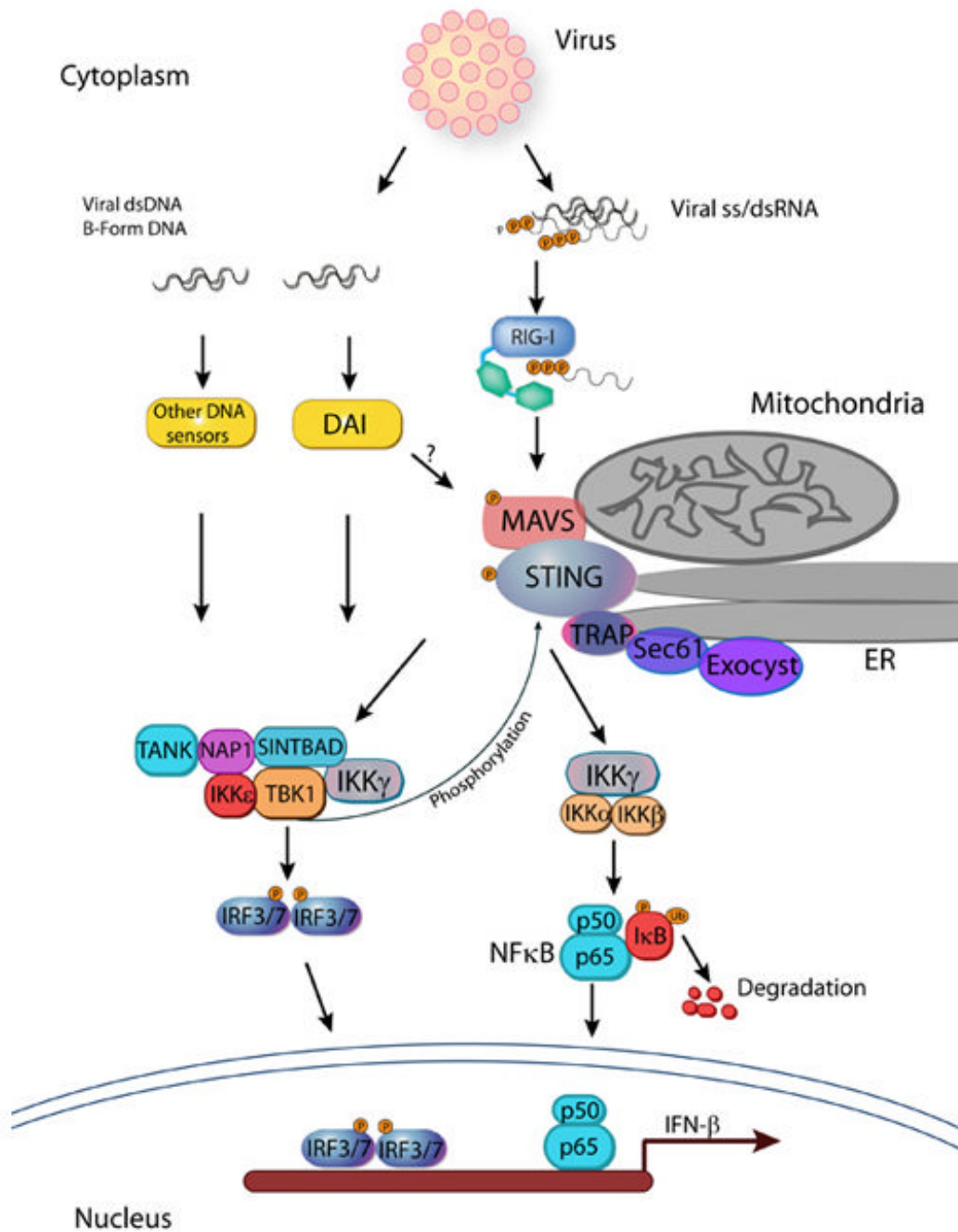
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## Abbreviations

DAI	DNA-dependent activator of interferon regulatory factors
ds	double-stranded
IFN	interferon (type I)
IRF	interferon regulatory factor
ISG	interferon-stimulated gene

LGP2	laboratory of genetics and physiology 2
MAVS	mitochondrial antiviral signalling protein
MDA5	melanoma differentiation-associated gene 5
NAP1	NF $\kappa$ B-activating kinase-associated protein 1
PRR	pattern recognition receptors
RIG-I	retinoic acid-inducible gene-1
RLR	RIG-I-like receptor
SINTBAD	similar to NAP1 TBK1 adaptor
ss	single-stranded
STING	stimulator of interferon genes
TANK	TRAF-family-member-associated NF $\kappa$ B activator
TBK1	tank-binding kinase 1
TLR	Toll-like receptor
TRAP	translocon-associated protein



**Figure 1. Cytoplasmic recognition of viral nucleic acids**

Stimulation of DAI and possibly other DNA sensors by viral dsDNA (or B-form DNA) activates the TANK-NAP1-SINTBAD-IKK $\gamma$ -IKK $\epsilon$ -TBK1 kinase complex and stimulates phosphorylation of IRF3 and probably IRF7. This process could be the method of STING/ MITA/MPYS's role in DNA sensing. Viral ss/dsRNA recognition through RIG-I-MAVS interaction involves STING and the TRAP-Sec61-Exocyst complex to stimulate the same kinase complex as DNA sensing, as well as activating the IKK $\gamma$ -IKK $\alpha$ -IKK $\beta$  complex and triggering NF $\kappa$ B activation.