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Viruses are the most abundant pathogens on earth. A fine-tuned framework of intervening pathways is in place in mammalian cells to orchestrate the cellular defence against these pathogens. Key for this system is sensor proteins that recognise specific features associated with nucleic acids of incoming viruses. Here we review the current knowledge on cytoplasmic sensors for viral nucleic acids. These sensors induce expression of cytokines, affect cellular functions required for virus replication and directly target viral nucleic acids through degradation or sequestration. Their ability to respond to a given nucleic acid is based on both the differential specificity of the individual proteins and the downstream signalling or adaptor proteins. The cooperation of these multiple proteins and pathways plays a key role in inducing successful immunity against virus infections.

**Addresses**
Innate Immunity Laboratory, Max-Planck Institute of Biochemistry, Martinsried/Munich, Germany

Corresponding author: Pichlmair, Andreas (apichl@biochem.mpg.de)

**General properties of virus sensors**
Almost all cells express germ-line encoded sensors with the ability to recognise virus infections and to initiate defence systems necessary to limit virus spread and pathogenicity. In technical terms, a sensor is ‘a device that detects events or changes in quantities and provides a corresponding output without affecting the original trigger’. Sensors follow certain rules that include selective sensitivity to a specific measured property and insensitivity to other properties likely to be encountered. In analogy to technical terms, virus sensors convert a signal (virus infection) to an output that instructs the cell to take further actions. The magnitude of its activation is characterised by properties related to the exact nature and the quantity of the trigger. The targets of these sensors can be incoming virus particles [1], particular viral proteins [2] as well as general integrity of the cell [3]. However, the yet best understood sensors involved in antiviral defence are activated by viral nucleic acids [4]. Endosomal Toll-like receptors sample the extracellular milieu or cytoplasmic contents that are delivered into endosomes through autophagy. In this review we concentrate on intracellular nucleic acid sensors and effector proteins that evolved to mediate specialised tasks including, firstly, expression of cytokines such as type I interferons (IFN-α/β); secondly, modulation of cellular machineries required for virus replication and thirdly, direct inhibition of virus growth (Figure 1). Induction of cytokines utilises at least two distinct pathways either involving the adaptor proteins mitochondrial antiviral-signalling protein (MAVS) or stimulator of interferon genes (STING). Activation of either pathway regulates transcription of cytokines, which are key signals to shape adaptive immunity to induce an intracellular ‘antiviral state’ characterised by expression of antiviral defence proteins. Some of the latter proteins are activated by viral nucleic acids and in turn re-wire cellular machineries to limit virus spread. Other proteins directly bind viral nucleic acid and impair functionality through steric hindrance or degradation.

**Differences between cellular and viral nucleic acids**
To understand how viral nucleic acids are sensed by the innate immune system it is important to consider the different types of nucleic acids generated after virus infection. Viruses are intracellular pathogens that require cellular translation and host metabolism, but provide their own replication machinery. Independence of the host for multiplication of viral genomes allows high replication rates, which is often associated with pathogenicity [5]. 24–48 hours after infection approximately 25% of RNA can be of viral origin (P. Hubel and A. Meiler, unpublished). Viral nucleic acids accumulate in compartments typically devoid of cellular nucleic acids and often possess or lack modifications or physical properties that are not normally associated with cellular RNA or DNA (Figure 2). RNA polymerases commonly generate RNA with a 5’ triphosphate group (PPP-RNA). Cellular RNA polymerases cotranscriptionally modify newly synthesised RNA at the 5’ terminus. In case of mRNA an inverted guanine nucleotide cap is added and methylated at the N7-position as well as the 2’O position of the first ribose of the RNA strand (Cap1 mRNA) (Figure 2) [6]. These modifications are necessary to mark mRNA for further processing and export into the cytoplasm, where translation takes place. Other cellular RNAs are cleaved and have a 5’ monophosphate in case of transfer (t)RNA, most ribosomal (r)RNAs and small nucleolar (sno) RNAs [7]. Some small RNAs bear a terminally methylated 5’ triphosphate (U6 snRNA, 7SK RNA) or are further processed to a...
Viral nucleic acid sensor and effector proteins and their primary antiviral properties. Engagement of a particular set of nucleic acid sensors (Class I sensors, red) results in signal transduction events, leading to expression of the type I interferons IFN-α/β and other cytokines. These in turn upregulate additional sensors (Class II sensors, green) with the ability to modulate the cellular machinery. In addition cytokines induce expression of effector proteins (blue) directly targeting viral nucleic acids. Transmission of signals in some pathways occurs through second messengers (yellow). Class II sensors include PKR, OAS and AIM2. PKR phosphorylates translation initiation factor eIF2α and consequently inhibits translation. Activated OAS synthesises the second messenger 2′,5′-oligoA, which then binds to and activates the latent endoribonuclease RNASEL. Activation of the inflammasome, a large multimeric complex including pro-caspase-1, is mediated by the DNA sensor AIM2. Caspase-1 cleaves its substrates pro-IL-1β and IL-18 for extracellular release. For signal transduction, MDA5 and RIG-I (either activated directly or through binding of RNAPIII-synthesised PPP-RNA) engage the adaptor protein MAVS. cGAS and IFI16 transmit their signal to the adaptor STING. Both pathways culminate in phosphorylation and dimerization of IFR-3 as well as release of active NFκB into the nucleus, where they cooperate to form an enhanceosome to turn on transcription of cytokine genes.

Abbreviations: OAS, 2′,5′-oligoadenylate synthetase; PKR, dsRNA-dependent protein kinase; AIM2, absent in melanoma 2; eIF2α, eukaryotic initiation factor 2 alpha subunit; RNASEL, 2′-5′A-dependent ribonuclease; MDA5, melanoma differentiation-associated protein 5; RIG-I, retinoic acid inducible gene I; RNAPIII, RNA polymerase III; cGAS, cyclic GMP–AMP synthase; IFI16, interferon gamma-inducible protein 16; MAVS, mitochondrial antiviral-signalling protein; STING, stimulator of interferon genes; IRF-3, interferon regulatory factor 3; NFκB, nuclear factor κ-light-chain enhancer of activated B cells; IFIT, interferon-induced protein with tetratricopeptide repeats; APOBEC3, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3; ADAR1, RNA-specific adenosine deaminase 1; TREX1, three prime repair exonuclease 1; PPP-RNA, 5′ triphosphorylated RNA.

Figure 1

hypermethylated 2,2,7-trimethylguanosine cap (TMG) cap (snRNAs). In addition, more than 100 modifications on internal nucleotides of cellular RNAs have been described, some of which are critical to tame activation of the innate immune system. Total cellular RNA isolated from cells and transfected into indicator cells does not activate the innate immune system, whereas the products of most viral RNA polymerases are strong stimuli of antiviral responses [8]. Negative strand RNA viruses such as orthomyxo-viruses, paramyxoviruses and bunyaviruses commonly generate full-length genomic PPP-RNA and short 5′ PPP subgenomic RNA, which have strong immunostimulatory potential [4]. To avoid the cellular defence system many viruses mimic cellular mRNA-like cap structures by encoding capping enzymes (e.g. Flaviviruses, Coronaviruses, Poxviruses, and Reoviruses), ‘steal’ cap structures from cellular mRNAs for their transcripts (e.g. Orthomyxoviruses, Bunyaviruses) or trim their genomic RNA to display only monophosphorylated termini (Bunyaviruses, Bornaviruses) [9,10]. Picornaviruses and Caliciviruses mask their RNA with a covalently 5′ genome-linked viral protein (VpG). In addition to the cap itself, 2′O methylation of the first ribose of mRNAs is an additional modification that is highly
Differences between cellular and viral nucleic acids. Synthesis of host RNA (red) from nuclear dsDNA (blue) is achieved by three cellular RNA polymerases. RNA polymerase II synthesises mRNA, ncRNA and some snRNAs, whereas RNA polymerase III generates tRNA and 5S rRNA. ssRNAs are produced by RNA polymerase I. Virus-derived DNA and RNA are present in the cell either as genomes, transcripts or replication by-products. Indicated are particular differences at the RNA 5' and 3' end, such as cap structures and methylations (e.g. cellular mRNA harbouring an N7-methylated guanine cap structure and 2'O-methylation at the first and/or second ribose). *5S rRNA harbours a 5' triphosphate group; **U6 and 7SK RNA both have a 5' gamma-monomethyl phosphate, and SRP RNA has a 5' triphosphate. Abbreviations: ds, double-stranded; mRNA, messenger RNA; ssRNA, ribosomal RNA; tRNA, transfer RNA; snRNA, small nuclear RNA; ncRNA, non-coding RNA; m7G, N7-methylated guanine cap; m, 2'O-methylation; p, phosphate group; TMG, hypermethylated 2,2,7-trimethylguanosine cap; VPg, viral protein genome-linked; A(n), poly(A) tail.

Conserved between viruses and their hosts, evidenced by the presence of dedicated viral proteins that catalyse this reaction [10]. Lack of 2'O methylation renders viruses highly vulnerable to the antiviral activity of the interferon system [11,12].

A type of RNA often associated with viral replication is double-stranded RNA (dsRNA). dsRNA could be either the result of replication intermediates (for RNA viruses), generation of genomic RNA (for dsRNA viruses), convergent transcription (for DNA viruses), or of the presence of secondary structures found in viral RNAs (e.g. the IRES structure of ssRNA viruses) [5]. However, the definition and exact nature of dsRNA still remains enigmatic. Using an antibody raised against dsRNA, it was found that such RNA is produced in cells infected with DNA viruses as well as some RNA viruses, such as Flavi and Picornaviruses [8]. However, although double-strandedness is an important feature recognised by virus sensors, it does not seem to be the only important determinant to stimulate fulminating antiviral responses since different double-stranded homopolymers vary considerably in their ability to induce IFN-α/β. Furthermore, dsRNA is commonly generated by convergent transcription of cellular RNA polymerases and is involved in transcriptional and post-transcriptional gene silencing [13*,14]. Despite the presence of cell-generated dsRNA no spontaneous synthesis of IFN-α/β is apparent nor is transcription of total cellular RNA containing detectable dsRNA molecules or plasmid-based convergent transcription capable to induce significant levels of IFN-α/β [13*]. A possible explanation for the lack of stimulatory activity of cellular RNA may be insufficient concentration of dsRNA as proposed by a recent study showing that nuclear dsRNA is digested by the endonuclease Dicer [15]. It may be that the latter function is used by orthomyxoviruses that replicate in the nucleus to reduce the abundance of viral dsRNA in the cytoplasm.

Since most virus sensors and signalling molecules are localised in the cytoplasm, the cellular nucleus is considered not to promote sensing and signalling of virus infection. Indeed, cellular DNA, present in the nucleus does not elicit IFN-α/β whereas double-stranded DNA (dsDNA) introduced into the cytoplasm through transfection or virus infection induces an innate immune response [4]. However, simple compartmentalisation is insufficient to explain the ability of the innate immune system to recognise DNA viruses that replicate the
nucleus [16]. It is therefore likely that additional yet unknown features of viral DNA can be sensed by the innate immune system.

**Sensors that drive expression of cytokines**

Among the best characterised cytoplasmic proteins involved in virus sensing are RIG-I-like receptors (RLRs), a family of DEAH-box helicases which specifically identify viral RNAs and have the ability to stimulate expression of IFN-α/β and other cytokines (Figure 3) [4,17]. The founding member of this family, Retinoic acid inducible gene-I (RIG-I) bears two N-terminal Caspase activation and recruitment domains (CARDs) required for signalling, a central helicase domain that mediates binding to dsRNA and a C-terminal repressor domain, which binds 5’ tri-phosphorylated, di-phosphorylated or dephosphorylated RNA ends [18–20]. RIG-I forms oligomers along the bound RNA in an ATP-dependent manner, the CARDs oligomerize and allow signalling through CARD–CARD interactions with MAVS [21,22]. Activation in addition requires dephosphorylation and ubiquitination of RIG-I [23]. Optimal RIG-I ligands are consisting of blunt dsRNA formed by two complementary RNAs (e.g. Reovirus) or generated by intramolecular base pairing as is proposed for the sensing of influenza A virus ribonucleoprotein complexes [20].

Other proteins belonging to RLR helicases are MDA5 and LGP2. LGP2 lacks functional CARD domains and therefore cannot induce signalling. However, LGP2 appears to be an important co-factor to facilitate sensing of some viruses [24]. Recently, L-antisense RNA expressed by encephalomyocarditis virus (EMCV) has been identified to associate with LGP2 [25]. L-antisense RNA activates MDA5, raising the possibility that LGP2 prepares ligands for sensing through other RLRs. MDA5 appears to be activated by structural properties of viral RNAs, but there is no unifying feature known that could generally explain MDA5 activation. Instead, a number of different RNAs are proposed to activate MDA5. Firstly, long synthetic dephosphorylated dsRNA stimulates MDA5 whereas shorter dsRNA loses this ability [26,27]. Secondly, replication intermediates consisting of dsRNA and generated by picornaviruses [28,29]. Thirdly, high molecular weight RNA generated during replication and likely bearing branched RNA molecules activates MDA5 [8]. The most commonly used synthetic MDA5 stimulus, poly-I:C would most likely form such

![](current-opinion-in-virology-2015-11-31-37.png)

Cellular proteins involved in sensing and engagement of viral nucleic acids. Antiviral nucleic acid sensors operate in three layers, including viral sensing leading to signal transduction (red), modulation of the cellular machinery (green), and direct targeting of viral nucleic acids (blue). The exact mechanisms of particular cellular proteins engaging specific viral RNA and DNA structures are described in detail in the text. Abbreviations: ss, single-stranded; ds, double-stranded; Cap0, m7G cap structure lacking 2’-O-methylation at the first and/or second ribose; PPP-RNA, 5’ triphosphorylated RNA; PP-RNA, 5’ dephosphorylated RNA; cccDNA, covalently closed circular DNA.
structures. Fourthly, a specific sequence in the L-region present on the antisense single-stranded genomic RNA of EMCV appears to stimulate MDA5 [25]. Fifthly, for measles virus a sequence bias towards AU-rich regions was proposed to be associated with MDA5 activating activity [30]. Sixthly, mutant coronaviruses that generate RNA lacking 2’O methylation on the first ribose are a stronger MDA5 agonists than corresponding wild-type viruses highlighting the possibility that MDA5 may sense a chemical modification on the RNA 5’ end [31]. Similarly to RIG-I, N-terminal CARDs of MDA5 are required for downstream signalling but unlike RIG-I, MDA5 oligomerizes along dsRNA in a head to tail manner and positions the CARDs in an elongated structure that activates signalling through MAVS [32].

In addition to cytoplasmic RNA sensors, cells are equipped with sensors of cytoplasmic DNA. DNA sensing shows considerable cell-type specificity but in general two different concepts of DNA sensing seem to emerge: firstly, direct activation of IFN-α/β and secondly, generation of second messengers that are activating other proteins to induce IFN-α/β. Proteins directly activating IFN-α/β via the STING pathway are DNA-dependent activator of IRFs (DAI) [33*,34] and the more recently identified interferon-inducible protein 16 (IFI16) [34]. IFI16 belongs to the family of PYHIN proteins and contains a pyrin domain and two DNA-binding HIN domains. IFI16 is able to induce IFN-α/β after infection with Herpes simplex virus 1 (HSV-1) and Human immunodeficiency virus 1 (HIV-1) as well as transfected DNA [34,35]. Proteins generating a second messenger include RNA polymerase-III (RNAPIII) and cyclic GMP–AMP synthase (cGAS). RNAPIII binds AT-rich regions in viral DNA genomes to produce PPP-RNA, serving as ligand for RIG-I [36,37]. cGAS belongs to the nucleotidyltransferase family and upon dsDNA-binding generates cyclic 2’–5’ GMP–AMP (cGAMP) from ATP and GTP [38,39**,40–42]. cGAMP binds and directly activates STING and can also cross cell barriers to activate innate immune responses in adjacent cells [43]. Although the exact viral ligand has not yet been defined, lack of cGAS in human or mouse cells impairs interferon responses to DNA viruses and transfected DNA [44**].

**Nucleic acid sensors with direct effects on the cellular machinery**

Another set of nucleic acid sensors that activate transcription another subset of sensors directly affects cellular machineries to impair virus growth. These proteins include 2’5’ oligoadenylate synthetase (OAS), dsRNA-dependent protein kinase R (PKR) and absent in melanoma 2 (AIM2). dsRNA binding to OAS catalyses the conversion of ATP to 2’5’-linked oligoadenylates, which activate the latent ribonuclease RNASEL to degrade cellular and viral RNAs [42]. RNASEL cleavage products have been demonstrated to stimulate the MAVS pathway but the exact mechanism is not known. PKR is a serine/threonine kinase that is activated either by dsRNA of at least 30 bp in length or by PPP-RNA and suppresses general translation by phosphorylating eukaryotic initiation factor 2 alpha (eIF2-α) [45,46]. In addition PKR induces apoptosis and regulates cytokine expression, most likely by modulating mRNA stability. Some nucleic acid binding proteins, such as the PYHIN family member AIM2, regulate post-translational processing and cell death [47]. AIM2 binds DNA and triggers the activation of the inflammasome, a molecular platform responsible for the maturation of interleukin 1β (IL-1β) and IL18 as well as triggering cell death. The RNA-binding helicases RIG-I [48] and DHX33 [49] have also been implicated in inflammasome activation, but the precise molecular details remain to be determined.

**Cellular effector proteins directly targeting viral nucleic acids**

Innate sensing leads to expression of effector proteins with the ability to sequester, modify or degrade viral nucleic acid (Figure 1). Sequestration of viral RNAs can be achieved by interferon-induced proteins with tetratricopeptide repeats (IFITs). Although combinations of IFITs are expressed in a species-specific manner most IFITs are highly induced in expression after virus infection. IFITs bind viral RNA through a deep binding cleft formed by a complex arrangement of tetratricopeptide repeats [50,51]. IFIT1 preferentially binds single-stranded capped non-2’O-methylated (Cap0) or 5’ triphosphorylated (PPP) RNA, IFIT5 exclusively binds PPP-RNA [12,52*]. IFITs compete with the function of other RNA-binding proteins, such as cellular translation initiation factors and/or viral proteins. Since RNA-binding by IFITs is highly specific, translation or localisation of cellular mRNAs is not affected by IFIT proteins [12].

An alternative strategy to directly target viral nucleic acids is to modify or degrade them. RNA-specific adenosine deaminase 1 (ADAR1) or members of the DNA-specific apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) family deaminate nucleotides to introduce mutations, which potentially impacts RNA secondary structure, stability and protein-coding capacity [45,53]. APOBEC3A and 3B recognise the Hepatitis B virus core protein and target core-associated DNA to impair virus growth [54]. Viral nucleic acids are directly targeted for degradation by 2’5’oligoadenylate-activated ribonuclease RNASEL [45] and by Zinc-finger antiviral protein (ZAP), which specifically targets viral mRNAs for degradation through recruitment of the cellular exosome machinery [55*]. DNA degradation through Three prime repair exonuclease 1 (TREX1) is required to restrict endogenous retroviruses [56].

Mutations in TREX1 have been linked to autoimmune diseases, clearly highlighting the importance of nucleic
acid metabolising enzymes to reduce the abundance of stimulatory nucleic acids. More recently it has also been shown that the SKIVL2 exosome is important to reduce stimulatory RNA [57*].

Concluding remarks

Virus infection activates a restricted set of sensor and effector proteins that modulate cellular pathways and directly target viral nucleic acid, thereby shaping the innate immune response. Despite remarkable progress in the last few years to uncover modifications that are sensed by the innate immune system, many questions still remain to be answered. The natural ligand of cytoplasmic sensors, for instance, is often not well understood, nor do we know the exact localisation of virus sensing in the cytoplasm. Furthermore, numerous cellular pathways and second messengers contribute to innate immunity to viral pathogens and cell biological processes are similarly prominent in contributing to virus defence. We thus anticipate that even more entangled relationships between viruses and hosts are likely to be uncovered in the future.

Acknowledgements

The authors want to thank Friedemann Weber, Gareth Brady and the members of the Innate Immunity Laboratory for critical input and suggestions. MH is funded by an Alexander von Humboldt fellowship. AP is funded by the Max-Planck Free Floater programme, an ERC starting grant (VIP), Infect-ERA (ERASE) and the German Research Foundation (PI 1084/2-1).

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