Discrimination of Self and Non-Self Ribonucleic Acids

Anna Gebhardt,* Beatrice T. Laudenbach,* and Andreas Pichlmair

Most virus infections are controlled through the innate and adaptive immune system. A surprisingly limited number of so-called pattern recognition receptors (PRRs) have the ability to sense a large variety of virus infections. The reason for the broad activity of PRRs lies in the ability to recognize viral nucleic acids. These nucleic acids lack signatures that are present in cytoplasmic cellular nucleic acids and thereby marking them as pathogen-derived. Accumulating evidence suggests that these signatures, which are predominantly sensed by a class of PRRs called retinoic acid-inducible gene I (RIG-I)-like receptors and other proteins, are not unique to viruses but rather resemble immature forms of cellular ribonucleic acids generated by cellular polymerases. RIG-I-like receptors, and other cellular antiviral proteins, may therefore have mainly evolved to sense non-processed nucleic acids typically generated by primitive organisms and pathogens. This capability has not only implications on induction of antiviral immunity but also on the function of cellular proteins to handle self-derived RNA with stimulatory potential.

Keywords: ribonucleic acid sensing, antiviral mechanisms, interferon, MDA5, RIG-I, PRR

Introduction

THE HOST IMMUNE SYSTEM is constantly encountering pathogen invasion. Viruses, the most abundant pathogens on earth, can infect eukaryotes and prokaryotes and require the supportive environment of the cell to proliferate and spread. For this reason, organisms evolved barriers including the innate and adaptive immune system to suppress growth of pathogens. Protection against viral infections is to a large extent mastered by the innate immune system, which is able to sense incoming virus particles, viral proteins, viral replication products, and changes in the general integrity of the cell (Isaacs and Lindenmann 1957; Rassa and Ross 2003; Sancho and Reis e Sousa 2013). As a result, the organism mounts an appropriate antiviral response that impairs virus growth and allows virus clearance.

Activation of the antiviral innate immune system is characterized by secretion of antiviral cytokines, particularly type I interferons (IFN- α/β). These cytokines were identified by Isaacs and Lindenmann (1957) who demonstrated that cells secrete antiviral factors upon exposure to heat inactivated viruses. The main stimulatory agent was identified to be nucleic acids that are delivered through the viral infection process (Goubau and others 2013). Viral replication greatly enhances the abundance of stimulatory nucleic acids and thereby regulates the magnitude of the response (Rehwinkel and others 2010; Weber and others 2013). Both, viral RNA and DNA (vRNA/DNA) can stim-

ulate pattern recognition receptors (PRRs) and recently molecular principles underlying the basis for detecting viruses and immune-stimulatory nucleic acids were discovered (Schlee 2013; Ahmad and Hur 2015). Besides activation of the innate immune system, viral nucleic acids modulate the activity of general cellular machineries such as protein translation, RNA degradation, or cell death (Fig. 1). At the same time viral nucleic acids are directly targeted by cellular proteins with antiviral functions. Thus, it becomes more and more evident that the innate immune system is not only a blunt alarm system that reacts to a single stimulus. It rather consists of a highly sophisticated network of proteins that target pathogen-derived nucleic acids. Importantly, the innate immune system has dramatic impact on the cellular and organismal level and its activation has to be modulated in a very tight manner. In this regards, the discrimination between “pathogen derived” and “host” nucleic acids is of central importance.

In this review we will focus on the features of RNAs that are in place to discriminate between self and non-self nucleic acids. Viral nucleic acids can be sensed by their localization in nucleic acid free organelles (such as endosomes), and by their chemical modifications and secondary structures. Considering current knowledge, it appears that large parts of the cellular sensing mechanisms are targeting “missing-self” modifications rather than nucleic acids specific to a certain pathogen. This concept is reminiscent of the adaptive immune system that is also predominantly

Innate Immunity Laboratory, Max-Planck Institute of Biochemistry, Munich, Germany.

*Both these authors contributed equally to this work.

Ribosomal RNAs (rRNAs) are transcribed by Pol-I as a 45S-pre-rRNA with a 5' PPP-RNA structure. After cleavage of the precursor into 28S, 18S, and 8S, rRNA, a 5' monophosphate (5' P) is obtained on the individual RNAs (Fig. 2) (Drygin and others 2010). Similarly, Pol-III generates a subset of additional RNAs comprising transfer RNA (tRNA), some small nucleolar RNA (snoRNAs), 5S rRNA, 7SK RNA, and U6 snRNA (Hopper 2013; Kirchner and Ignatova 2015). Like Pol-I transcribed rRNAs, tRNAs bear a 5' P after cleavage of a 5' oligonucleotide. U6 snRNA and 7SK RNA are not cleaved but bear a 5' gamma-monomethyltriphosphate (5' P_mPP-RNA) after processing (Singh and Reddy 1989).

Besides chemical modifications of RNA it is important to note that most RNAs are bound by RNA-binding proteins under steady-state conditions. Proteins associating to RNA can either contribute to activation or inhibition of the innate immune system. Viral proteins such as the E3L protein of Vaccinia virus, the nonstructural protein 1 (NS1) protein of Influenza A virus (IAV) or B2 of Flock house virus are binding dsRNA and can reduce the potential of stimulatory RNA likely by restricting accessibility to PRRs (Lingel and others 2005; Avllon and Garcia-Sastre 2015). Similarly, the

FIG. 2. Stimulatory and nonstimulatory RNAs in cells. This schematic provides an overview of the main proportion of cellular RNAs and cytoplasmic RIG-I and MDA5 stimulatory RNAs. Host RNAs are synthesized by RNA polymerase I–III (Pol-I, Pol-II, and Pol-III) in the nucleus. The RNAs are generated as a precursor RNA bearing a 5' triphosphate group, which is extensively modified in the nucleus before getting transported to the cytoplasm where they perform their biological function. snRNAs are further modified in the cytoplasm and reimported to the nucleus. Nonstimulatory cytoplasmic cellular RNA (blue box) are not activating cytoplasmic PRRs. Specific viruses can introduce different types of stimulatory RNA during the infection process. These RNAs often resemble premature forms of cellular RNA and can be classified into RIG-I (green box) and MDA5 ligands (orange box). AAA, poly(A) tail; DI genomes, defective interfering genomes; dsRNA, double-stranded ribonucleic acid; MDA5, melanoma differentiation-associated protein 5; me, methyl group; mRNA, messenger RNA; P, phosphate group; PRR, pattern recognition receptor; RIG-I, retinoic acid-inducible gene I; rRNA, ribosomal RNA; snRNA, small nuclear RNA; tRNA, transfer RNA.

cellular RNA binding protein laboratory of genetics and physiology 2 (LGP2) reduces the stimulatory potential of certain RNAs (Venkataraman and others 2007), presumably by steric interference with activation of cellular PRRs (Yoneyama and others 2005; Saito and others 2007). However, LGP2 has also been shown to promote induction of IFN by a subset of viruses (Venkataraman and others 2007). Cellular RNA helicases can prepare viral ligands to be better sensed by effector proteins of the innate immune system (Ahmad and Hur 2015; Yao and others 2015). This is likely happening through displacement of other RNA binding proteins from the stimulatory RNA or due to changes in the secondary structure of the RNA. In recent years, a surprisingly high number of cellular helicases including DEAD-box protein (DDX) DDX1 (Zhang and others 2011a), DDX3 (Oshiumi and others 2010; Thulasi Raman and others 2016), DEAH-box helicase DHX9 (Zhang and others 2011b), DDX17 (Moy and others 2014), DDX60 (Miyashita and others 2011), and others, have been

shown to be involved in induction of type I IFN. These proteins mostly do not directly bind to signaling molecules of the canonical IFN pathway but appear to have important accessory functions to properly activate the innate immune system. However, the contribution of RNA binding proteins to modulate innate immune responses can be diverse and needs to be characterized on an individual level.

Features of vRNA

Viruses often use relatively simple replication machineries resulting in RNAs that are only partially processed and therefore resemble a premature state of cellular RNAs. A number of PRRs evolved to sense such unprocessed RNA. The simplest evidence for this is the ability of cells to sense 5' PPP-RNA, which constitutes the most basic product of RNA polymerases, through the cellular PRR retinoic acid-inducible gene I (RIG-I) (Hornung and others 2006; Pichlmair and others 2006). Delivery of 5' PPP-RNA into the

cytoplasm activates type I IFN expression in an RIG-I-dependent manner. Many negative strand RNA viruses such as orthomyxo-, paramyxo-, and most bunyaviruses generate 5' PPP-RNA constituting either the genomic RNA, replication by-products [eg, complementary RNA (cRNA)], or short subgenomic RNAs (Pichlmair and others 2009; Goubau and others 2013; Habjan and Pichlmair 2015). Interestingly, most viruses that generate 5' PPP-RNA express auxiliary factors (eg, NS1 of IAV, V protein of Measles virus, etc.) that actively impair activation of the innate immune system (Versteeg and Garcia-Sastre 2010). Viruses that do not express such dedicated viral proteins, commonly process the 5' RNA terminus to escape immune surveillance. Bornaviruses and a subset of Bunyaviruses including Crimean–Congo hemorrhagic fever virus, for instance, employ a 5' trimming event as part of their replication strategy (Schneider and others 2007; Habjan and others 2008). Thereby the terminal nucleotides are cleaved by a viral nuclease leaving a 5' P on the genomic RNA. Picorna- and caliciviruses shield their genomic 5' PPP-end with the covalently linked VPg protein (Flanagan and others 1977; Lee and others 1977; Habjan and others 2008), which impairs binding of cellular PRRs to the 5'-end of vRNAs.

Viruses, such as flavi-, corona-, pox-, and reoviruses encode their own capping enzymes to generate 5' capped mRNA (Decroly and others 2012). In addition to capping, these viruses independently evolved proteins that methylate the first ribose at the 2'O-position to generate RNA that resembles cellular mRNA modifications, indicating a strong selective pressure to favor this modification. Interestingly, viruses that lack the ability to methylate the 2'O position on the first ribose are sensed by the innate immune system (Daffis and others 2010; Habjan and others 2013; Schubert-Wagner and others 2015). Orthomyxo- and bunyaviruses employ an alternative approach to gain a fully processed cap structure called "cap-snatching." The first 10–13 nucleotides of a cellular mRNA are fused to the 5' end of a vRNA, which thereby acquire cellular marks that allow evasion from host sensing mechanisms (Dias and others 2009; Decroly and others 2012; Reich and others 2014; Koppstein and others 2015).

In addition to 5'-end modifications vRNA has structural properties that allow discrimination from cellular RNAs. In particular, RNA double-strandedness of certain length is a feature that is often sensed by PRRs. Such long dsRNAs are generated by genome replication of dsRNA viruses or as replication by-products of many single-stranded (ss) RNA viruses. Even DNA viruses such as pox- and herpes viruses generate dsRNA through convergent transcription (Weber and others 2006; Feng and others 2012).

In the last years, genomic viral secondary structure elements generating portions of double-strandedness on ssRNA genomes like panhandle structures or stem loops have been shown to be recognized by antiviral mechanisms (Schlee and others 2009; Resa-Infante and others 2011; Moy and others 2014; Xu and others 2015). Although the underlying data are very compelling, it is still challenging to explain how the cell distinguishes viral from cellular dsRNA, particularly given the well-documented existence of natural dsRNA (Portal and others 2015) and high frequency of stem loops commonly found in RNAs of human origin. Different explanations for the increased immunogenicity of viral nucleic acids are possible: A certain threshold of dsRNA may

have to be reached to activate the innate immune system. The abundance of vRNAs present in infected cells by far exceeds the abundance of cellular dsRNA. A threshold model is also supported by experiments using the poxvirus Modified Vaccinia Virus Ankara (MVA): Although poxviruses naturally generate dsRNA, a genetically engineered MVA strain that expresses increased levels of dsRNA shows superior immunogenicity (Wolferstatter and others 2014). Another explanation could be the subcellular localization of dsRNA since the majority of cellular dsRNA should remain in the nucleus or membrane covered cytoplasmic virus factories (Mackenzie 2005; Paul and Bartenschlager 2015), which cannot be surveyed by PRRs. Additionally, specific sequences associated to viral dsRNAs may contribute to signaling strength. Interestingly, the presence of double-strand portions within RNA is not always beneficial for virus sensing. Alphaviruses exhibit a secondary structural motif within the 5' UTR, which prevents binding and activation of innate immune restriction proteins (Hyde and others 2014). Mechanistically, this high affinity dsRNA portion may not be accessible to cellular proteins and therefore most likely evolved as virus countermeasure against immune surveillance by the immune system (Hyde and others 2014).

Sensors of Viral or Nonprocessed RNA

A number of germline-encoded receptors have the ability to sense the presence of viral nucleic acids and initiate a variety of downstream events aiming at clearance of the invading pathogen. These receptors can be grouped in respect to their subcellular localization (endosomal or cytoplasmic), their ligand specificity (DNA, dsRNA, and ssRNA) and their effect after vRNA engagement (regulators of transcription, translation, or direct effect on vRNA). Here, we focus on cytoplasmic sensors of viral-derived RNAs and refer to other excellent reviews dealing with sensing in endosomes or of other viral ligands (O'Neill and others 2013; Cai and others 2014; Horning and others 2014; Pelka and others 2016; Roers and others 2016).

RIG-I-Like Receptor-Mediated Recognition

The discovery of the PRR RIG-I by Takashi Fujita's laboratory opened a new era in the understanding of RNA virus sensing (Yoneyama and others 2004) and led to the identification of a class of receptors named RIG-I-like receptors (RLRs) (Onoguchi and others 2011). The highly conserved family of RLRs is comprised of three structurally related proteins named RIG-I, melanoma differentiation-associated protein-5 (MDA5), and LGP2 (Yoneyama and others 2005; Onoguchi and others 2011). All members belong to the Asp-Glu-Ala-Asp (DEAD) box family and consist of a RNA helicase domain with ATPase activity and a C-terminal domain (CTD), which is important to mediate specificity to virus-derived nucleic acid sensing (Takahashi and others 2008). RIG-I and MDA5 accommodate two N-terminal caspase activation and recruitment domains (CARDs), which initiate downstream signaling through CARD-dimerization with the MAVS protein.

Genetic deletion of RIG-I and MDA5 showed that RIG-I and MDA5 exhibit specificity for certain viruses indicating that distinct nucleic acids are sensed by these receptors. RIG-I is able to detect viruses of the rhabdoviridae family

such as Vesicular stomatitis virus and viruses of the paramyxoviridae family including Newcastle disease virus, Sendai virus (SeV), and Measles virus. MDA5 detects viruses of the Picornaviridae family such as Encephalomyocarditis virus and Polio virus (Gitlin and others 2006; Kato and others 2006). Some flaviviruses (eg, Dengue virus, West Nile virus, and Semliki forest virus) activate both, RIG-I and MDA5 (Fredericksen and others 2008; Akhrymuk and others 2016).

In contrast to RIG-I and MDA5, the role of LGP2 is less well understood particularly since this protein is missing a CARD. Depending on the experimental system, it was shown that LGP2 could serve as a negative regulator of RLR signaling (Saito and others 2007; Venkataraman and others 2007) or activate induction of IFN (Venkataraman and others 2007). However, more recent studies strengthen the finding that LGP2 operates as a positive regulator of MDA5 (Sato and others 2010; Bruns and others 2014; Uchikawa and others 2016).

An important property of all RLRs is their ability to interact with dsRNA. However, although it became evident that binding to dsRNA is important to activate RLRs, RNA double-strandedness is not always sufficient to induce signaling. Additional requirements on stimulatory RNA, particularly chemical modifications, may serve as safeguard to reduce accidental activation of type I IFN signaling, particularly if the double-stranded stretch on RNA is only short.

Activation and Downstream Signaling of RLRs

Activation of RIG-I requires ligand binding, a cascade of regulatory post-translational modifications and binding of proteins resulting in the exposure of the CARDS. In uninfected cells, RIG-I CARDS and the CTD are constitutively phosphorylated at Ser8 and Thr170 by Protein Kinase C α/β and Casein Kinase II (CKII) (Sun and others 2011; Maharaj and others 2012). In the phosphorylated state RIG-I adopts a “closed” conformation sequestering the CARDS from signaling due to interactions with the C-terminal repressor domain. The ATPase activity of the helicase domain allows RIG-I to constantly scan RNAs for the presence of viral motifs and was shown to be a key element to prevent recognition of self-RNA (Lassig and others 2015). ATP hydrolysis facilitates the release of RIG-I from self-RNA, while presence of viral motifs detected through the CTD leads to stalling and activation of RIG-I. Subsequently, the constitutive Ser/Thr phosphorylation sites in the CARDS are removed by serine/threonine-protein phosphatase α and γ (PP1 α and PP1 γ) resulting in Riplet-mediated ubiquitination of the CTD. This is followed by dimerization and a conformational rearrangement of RIG-I leading to the exposure of CARDS (Oshiumi and others 2013; Peisley and others 2013), binding of ubiquitin/ISG15-conjugating enzyme (TRIM25) to CARD1, and subsequent K63-linked ubiquitination in CARD2 (Gack and others 2007). After activation, the CARDS of RIG-I form a helical tetramer in a lock washer conformation. Multiple lock washer tetramers form a helical trajectory that allows MAVS filament assembly along this structure (Wu and others 2014). This results in clustering of multiple MAVS molecules and activation of downstream signaling.

In contrast to RIG-I, MDA5 oligomerizes as filaments in a head-to-tail fashion along dsRNA to reach high-affinity in-

teractions with long dsRNA ligands (Peisley and others 2011; Berke and others 2012). After oligomerization, the CARDS of MDA5 point outward of the filament and oligomerize in structures that can bind to MAVS and activate downstream signaling (Wu and others 2013). Interestingly, MDA5 activation is significantly increased by LGP2, which does not contain a CARD itself. Structural analysis showed that LGP2 binds the end of dsRNAs (very much like RIG-I) and thereby initiates MDA5 filament oligomerization on dsRNA (Uchikawa and others 2016). LGP2 and MDA5 have been shown to bind similar stimulatory RNAs, which is in line with cooperative activity of LGP2 in MDA5 activation. LGP2-precipitated RNAs induce IFN- α/β in a MDA5-dependent manner providing a functional link between LGP2 and MDA5 (Deddouche and others 2014).

A key uniting feature of all RLRs is signaling through MAVS. Under physiological conditions, MAVS is kept inactive by an autoinhibitory mechanism. Upon binding to oligomerized CARDS of RLRs, the regions responsible for downstream signaling including TANK-binding kinase 1 (TBK1)/Interferon regulatory factor 3 (IRF3) and I κ B kinase (IKK)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) activation sites are exposed and induce signaling events that result in expression of type I IFNs and proinflammatory cytokines (Shi and others 2015).

Sensing of 5' Modifications by RIG-I

RIG-I activation requires two features that co-occur on one RNA molecule. The first essential feature for RIG-I activation is a chemical modification on the 5' terminus of the RNA. The best described 5' modifications that activate RIG-I are 5' tri- and 5' di-phosphates (Hornung and others 2006; Pichlmair and others 2006; Goubau and others 2014). However, more recently it has been shown that RIG-I is also activated by capped RNA that lack a methylation mark at the 2'O ribose position of the first nucleotide (Schuberth-Wagner and others 2015; Devarkar and others 2016). A conserved residue (Histidine 830) within the CTD of RIG-I sterically prevents binding of cellular 2'O-methylated RNA, and therefore serves as molecular gatekeeper to certain activation by cellular mRNAs. Silencing of the endogenous cap-specific mRNA (nucleoside-2'-O)-methyltransferase 1 (MT1) converts nonstimulatory into stimulatory mRNA and triggers a spontaneous RIG-I-dependent type I IFN response (Schuberth-Wagner and others 2015). The second essential feature for RIG-I activation is a short stretch of blunt-ended dsRNA. While chemically synthesized 5' PPP-ssRNA is not sufficient to prominently activate RIG-I, 5' PPP-dsRNA molecules with the same sequence show very strong activity (Schlee and others 2009; Schmidt and others 2009). Reports regarding the minimum length of this dsRNA stretch range from at least 10 base pairs (bp) (Schmidt and others 2009; Kohlway and others 2013) to 19 bp (Schlee and others 2009). Experiments transfecting differently modified RNA clearly show that 3' overhangs at the 5'-end are sufficient to impair activation of RIG-I (Schlee and others 2009) and may even serve as dominant negative decoy substrate (Marq and others 2011).

Besides dsRNA with 5' PPP modifications, short 5' hydroxyl (5' OH) and 3' monophosphoryl (3' P) dsRNA cleavage products of 2-5A-dependent ribonuclease (RNAseL) have been proposed to serve as RIG-I ligands (Malathi

and others 2007, 2010). Although this notion is supported by many functional data, the exact mechanism of RIG-I activation is not clear to date, particularly since this appears to happen in a cell type-dependent manner (Banerjee and others 2014).

Sensing of RNA in Virus-Infected Cells

Despite formidable progress that allowed defining optimal activation of RIG-I by synthetic ligands much less is known about the nature of the physiological ligand generated during virus infections. Many elegant studies aimed to define the physiological RIG-I stimulus. Compelling evidence suggests that viral genomic RNA delivered by virus infection activates RIG-I. Here, we discuss data that support this notion and also consider questions that still remain to be answered. Evidence for vRNA being the physiological ligand for RIG-I was already provided by Isaacs and Lindenmann (1957), who found that cells treated with high amounts of heat-inactivated viruses activate type I IFN. More recently this was supported by data that show that delivery of replication incompetent viral particles activate RIG-I (Weber and others 2013). Furthermore, shortly after infection, vRNA of infectious viral particles closely associates with mitochondria, which are serving as signaling hubs for the induction of type I IFN (Liedmann and others 2014). However, the magnitude of an antiviral response triggered by incoming vRNA appears to be relatively low and viral replication appears to be required to elicit high amounts of IFN (Crotta and others 2013; Killip and others 2014). One reason could be insufficient abundance of stimulatory RNAs, which questions whether incoming viral nucleic acids are significantly contributing to type I IFN production under physiological conditions.

Genomic vRNA isolated from virus particles and transfected into cells potently stimulates RIG-I (Hornung and others 2006; Pichlmair and others 2006). This is in agreement with the notion that vRNA isolated from IAV particles spontaneously forms the so-called panhandle structure. This structure is formed due to base-pairing of terminal ssRNA sequences of many negative ssRNA viruses and serves as promoter for the viral polymerase complex (Hsu and others 1987; Fodor and others 1994; Tiley and others 1994). The panhandle structure resembles *in vitro* synthesized blunt ended 5' PPP RNA. In line with RIG-I activation by synthetic ligands, the panhandle thus constitutes a perfect stimulus for RIG-I activation and explains the very strong stimulatory activity of isolated vRNA. Interestingly, mismatches in the panhandle structure of some IAV strains disrupt RNA complementarity and results in reduced activation of RIG-I (Anchisi and others 2016). Such an adaptation may therefore represent a viral strategy to evade RIG-I activation (Anchisi and others 2016). Supporting evidence that viral genomic RNA stimulates RIG-I comes from experiments using minireplicon systems. vRNA of defined length generated by the IAV polymerase complex activates RIG-I (Rehwinkel and others 2010). Interestingly, PPP-RNA is not only generated by viruses but also by bacteria. mRNA in bacteria is not capped and intracellular bacteria such as *Listeria* and *Legionella* have been shown to activate RIG-I (Monroe and others 2009; Abdullah and others 2012).

Although the notion that genomic vRNA is the major ligand activating RIG-I in virally infected cells is elegant, a

number of additional aspects linked to the replication process of viruses complicate this simple model: During viral replication, vRNA is constantly bound by viral proteins, which theoretically prevent activation of RIG-I. In case of many negative strand RNA viruses the viral polymerase complex is located at the 5'-end of vRNAs potentially shielding the terminal PPP group from being sensed by RIG-I. Indeed, for IAV it has been shown that association of the polymerase complex to the vRNA polymerase protects from innate sensing by RIG-I (Weber and others 2013; Liedmann and others 2014). Variants of the viral polymerase complex featuring weaker affinity for viral genomic RNAs have reduced ability to impair activation of the innate immune system (Weber and others 2013) suggesting that under certain conditions vRNAs can be a physiological activator of RIG-I and that the presence of proteins actively impair RIG-I activation. Besides a potentially inaccessible 5'-end, the relative contribution of the panhandle structure to provide a dsRNA platform for RIG-I binding is not so clear: Although isolated genomic RNA of IAV clearly generates blunt double-stranded ends, the structure of the panhandle bound to the viral polymerase complex, as it exists in virally infected cells, adopts a partially single-stranded conformation (Tiley and others 1994). This notion is supported by low affinity binding of the negative strand RNA virus polymerases to the double-stranded panhandle structure compared to binding to ssRNA (Pflug and others 2014; Gerlach and others 2015). Thus, the panhandle structure most likely either exists as a "corkscrew" (Neumann and Hobom 1995; Flick and others 1996) or as a "fork" (Fodor and others 1994, 1995; Kim and others 1997) structure in virally infected cells. Both structures presumably only allow suboptimal activation of RIG-I and it is therefore still not finally solved what type of RNA prominently activates RIG-I in virus infected cells.

A possibility is the activation of RIG-I by viral replication intermediates or by defective interfering (DI) particles, which are commonly generated during infections with viruses including SeV, IAV, and Measles virus (Strahle and others 2006; Baum and others 2010; Runge and others 2014). Although DI particles are not replicating, they prominently activate the innate immune system (Killip and others 2012).

Besides generating stimulatory nucleic acids from viral templates, RNA-dependent RNA polymerases (RdRP) also have the ability to use cellular RNA as templates resulting in the generation of host-derived stimulatory RNAs. Expression of Simian foamy virus RdRP, for instance, leads to induction of IFN- β in the absence of viral templates (Nikonov and others 2013). In addition, expression of RdRP in transgenic animals induces a constant IFN response that leads to increase virus resistance in these animals (Painter and others 2015). The ability of viral polymerases to generate cellular RNA copies with IFN inducing capability indicates that RdRP transcripts feature stimulatory modifications rather than the actual sequence being sensed by innate immune sensors. Interestingly, the ability to generate stimulatory RNA from cellular templates has so far only been shown for RdRPs that are active in the cytoplasm. A possible explanation for this could be the absence of RNA-modifying enzymes in the cytoplasm, which does not allow RNA processing to minimize the stimulatory potential of RdRP generated RNA.

Sensing of dsRNAs by MDA5

In contrast to ligands for RIG-I, requirements for MDA5 activation are less well understood. MDA5 recognizes long dsRNA (greater than ~ 0.5 kb) that is normally not present in the cytoplasm of uninfected cells (Kato and others 2008). A commonly used synthetic activator for MDA5 is poly-I:C, which is generated through annealing of enzymatically synthesized poly-I and poly-C homopolymers of undefined length. Although poly-I:C is regarded as dsRNA analog, the structure of poly-I:C is most likely not a uniform double-strand but may rather adopt a web-like structure (Pichlmair and others 2009). Interestingly, similarly synthesized poly-A:U or poly-G:C have very little stimulatory potential suggesting features associated with poly-I:C that are not yet well understood.

The best characterized viruses leading to MDA5 activation are picornaviruses [eg, Encephalomyocarditis virus (EMCV), Theiler's murine encephalitis virus, Poliovirus, or Norovirus] featuring a positive ssRNA genome (Gitlin and others 2006; Kato and others 2006; Loo and others 2008; McCartney and others 2008). While RIG-I activating viruses have the ability to induce PRR signaling through viral genome recognition, activation of MDA5 by EMCV strictly requires transcription of the viral genome and generation of a dsRNA intermediate resulting in a 7.5 kb replicative form of EMCV (Feng and others 2012; Triantafilou and others 2012). Furthermore, it is likely that additional replication intermediates representing higher order structural RNAs are generated and sensed by MDA5 (Pichlmair and others 2009).

Unbiased Approaches to Identify PRR-Associated vRNA

More recently, next generation sequencing was used to characterize the RNA ligand bound to MDA5 or RIG-I. An issue with such approaches is that the helicase domain of RLRs can generally associate with dsRNA and that this affinity does not directly lead to activation of RIG-I or MDA5. However, next generation sequencing helped to identify commonalities and differences in RNA binding between MDA5 and RIG-I. In Measles virus-infected cells, for instance, both, MDA5 and RIG-I preferentially bind to viral AU-rich sequences, particularly in the Measles virus L-region (Runge and others 2014). However, MDA5 shows superior enrichment for the Measles virus (+) sense RNA, while RIG-I preferentially bind to RNA of negative polarity. In a similar approach, Sanchez David and colleagues used next-generation sequencing analysis to investigate RNA precipitated with RIG-I, MDA5, and LGP2 in Measles and Chikungunya virus-infected cells. The authors showed that each of the RLRs binds distinct regions of the viral genome. RIG-I bound specifically the 3' UTR of the Chikungunya virus genome and DI genomes of Measles virus, whereas, MDA5 and LGP2 sensed nucleoprotein coding regions of Measles virus (Sanchez David and others 2016). The shared RNAs targeted by MDA5 and LGP2 strongly support a functional relationship between these two PRRs, which is also confirmed by alternative functional and structural approaches (Goubau and others 2014) (Uchikawa and others 2016).

In sum, it is evident that distinct RNA species trigger RIG-I and MDA5 activation. These RNA species may even be expressed at different time points during the infection process. Indeed, it was shown that vRNA products generated at dif-

ferent time points after West Nile virus infection sequentially stimulate RIG-I and MDA5, whereby, RIG-I triggers an early and MDA5 a later response (Errett and others 2013).

Direct Effectors of Viral Nucleic Acids

Besides RLRs, which have the ability to sense nucleic acids and regulate transcriptional programs, a set of additional proteins exist that are binding to specific viral nucleic acids (Habjan and Pichlmair 2015). These proteins either have the ability to regulate cellular processes that are unrelated to transcription or directly bind and thereby impair the activity of viral nucleic acids. Often these additional vRNA binding proteins are inducible by type I IFNs underlining their involvement in antiviral processes. This concept of multiple cellular proteins associating to a given type of stimulatory RNA is best illustrated by dsRNA binding proteins (Fig. 3). dsRNA activates the PRR MDA5, which is activating a transcriptional program culminating in expression of type I IFN. Besides MDA5, dsRNA-dependent Protein Kinase R (PKR) and 2'-5'-oligoadenylate synthetase (OAS1) directly bind dsRNAs. PKR regulates a plethora of antiviral processes after binding to dsRNA including inhibition of translation through continuous phosphorylation of eukaryotic initiation factor 2 alpha (eIF2- α), induction of apoptosis and autophagy (Kang and Tang 2012), and activation of NF- κ B through interacting with the IKK complex (Zamanian-Daryoush and others 2000). PKR has additionally been linked to regulation of type I IFN expression (Diebold and others 2003; Gilfoy and Mason 2007). While this accessory function of PKR is dispensable for RIG-I-dependent responses, IFN induction by viruses that activate MDA5 appears to be critically relying on additional activity by PKR (Schulz and others 2010; Wolferstatter and others 2014; Pham and others 2016). After binding to dsRNA OAS1 generates 2'-5'-linked oligoadenylates (2'-5'-OA) that serve as second messengers and have the ability to activate the latent RNaseL. Only after activation, RNaseL cleaves RNA eventually resulting in cell death (Chakrabarti and others 2011). Furthermore, vRNAs bind to a number of DEAD- and DEAH-box helicases, which often appear to have auxiliary functions to regulate IFN- α/β expression and also bear direct virus inhibitory function. DDX3, for instance, has been shown to be important for activation of the IFN signaling pathway (Oshiumi and others 2010; Gu and others 2013). DDX3 was also shown to restrict HBV replication and, thereby, acting as an antiviral effector protein (Ko and others 2014).

As for dsRNA, 5' PPP-RNA, which activates RIG-I, can associate with additional cellular proteins. Unbiased AP-MS experiments and follow-up studies using mutational approaches identified the interferon-induced protein with tetratricopeptide repeats (IFIT) 1 and -5, which directly associate with 5' PPP-RNA (Pichlmair and others 2011; Fensterl and Sen 2015). Depending on the exact experimental setup, IFIT1-deficient mice appear to be more susceptible to 5' PPP-RNA generating viruses such as Vesicular stomatitis virus (Pichlmair and others 2011). However, while RIG-I activation requires dsRNA, IFIT proteins specifically bind only ssRNAs in a helical positively charged binding cleft (Abbas and others 2013). Besides associating with 5' PPP-RNA, IFIT1 has the ability to directly associate with capped RNA that lacks a methylation mark at the first ribose (2'O N1 unmethylated RNA) (Habjan and others 2013; Kumar and

FIG. 3. Cellular sensor and effector proteins binding viral nucleic acids. **(A)** Sensing of dsRNAs by MDA5 results in expression of IFN- α/β . These in turn upregulate additional sensors including dsRNA-dependent PKR and OAS1. Binding of PKR to dsRNA phosphorylates the translation initiation factor eIF2-leading to an inhibition of translation and an induction of apoptosis. OAS1 synthesizes 2'-5'-oligoadenylates activating RNASEL. Activation of RNASEL results in RNA degradation and apoptosis. DDX60, ATP-dependent RNA helicase SKIV2L2 (MTR4) and ZCCHC7 promote vRNA degradation via the exosome complex upon virus infection. DDX3 activates IFN signaling and restricts virus replication. **(B)** Engagement of 5'-triphosphorylated-RNA by RIG-I leads to the expression of type I IFNs, thereby inducing the expression of the effector proteins IFIT1 and IFIT5. Sensing of PPP-RNA by IFIT1 and IFIT5 leads to decreased viral translation and restriction of virus, respectively. vRNA can be recognized and degraded by the exosome cofactors DDX60, MTR4, and ZCCHC7. DDX3, DEAD box protein 3; DDX60, DEAD box protein 60; eIF2, eukaryotic initiation factor 2; IFIT, interferon-induced protein with tetratricopeptide repeats; IFN, interferon; IRFs, interferon regulatory factors; MTR4, ATP-dependent RNA helicase SKIV2L2; OAS1, 2'-5'-oligoadenylate synthetase 1; PKR, double-stranded RNA-dependent protein Kinase R; PPP-RNA, triphosphorylated RNA; vRNA, viral RNA; ZCCHC7, Zinc finger CCHC domain-containing protein 7.

others 2014). Viruses that fail to methylate the 2'O position of the first ribose are attenuated in wild-type mice but are highly pathogenic in IFIT-deficient animals (Daffis and others 2010; Leung and Amarasinghe 2016). It is yet not entirely clear how IFIT1 and -5 impair virus growth but the high amounts of IFIT proteins expressed after IFN treatment suggests stoichiometric interference with viral nucleic acids rather than enzymatic activity of IFIT proteins (Habjan and others 2013; Kumar and others 2014). Pathogenic alphaviruses that generate high affinity dsRNA secondary structures on the 5'-end of their genome evade surveillance by IFIT proteins (Hyde and others 2014). IFIT1 appears to show surprisingly little efficiency against negative strand RNA viruses *in vivo* despite that these viruses are known to generate 5' PPP-RNA (Pinto and others 2015). Potential explanations may be eva-

sion strategies of these viruses, including replication in the nucleus that is not surveyed by IFIT proteins (eg, for IAV) or generation of secondary structures of the RNA 5'-end as has been shown for alphaviruses (Pinto and others 2015). IFITs have also been proposed to be (Berchtold and others 2008; Zhang and others 2013; Imaizumi and others 2016) or not to be (Pichlmair and others 2011; Habjan and others 2013) involved in regulation of antiviral gene expression. Thus, the activity of IFIT proteins is not yet fully understood and highlights the importance of functional studies that will give further mechanistic insights.

While the induction of type I IFNs and cellular restriction mechanisms are partially well understood, relatively little is known about the cellular machinery that specifically degrades vRNA. A prominent machinery responsible

for RNA degradation is the exosome, a large molecular complex with 3'-5' exonuclease activity. However, this complex requires accessory factors that mark RNA for degradation. Recently, the Ski2-like protein DDX60 was identified as cellular protein that promotes degradation of vRNA of diverse viruses through the exosome (Oshiumi and others 2015). Furthermore, DDX60 has been proposed to support RIG-I and MDA5-dependent induction of type I IFNs (Oshiumi and others 2015). However, the role of DDX60 appears complex since other laboratories found little effect of DDX60 in antiviral immunity (Goubau and others 2015; Grunvogel and others 2015). More recently, superkiller viralicidal activity 2-like 2 (SKIV2L2; also called Mtr4) and Zinc finger CCHC domain-containing protein 7 (ZCCHC7), components of the Trf4/Air2/Mtr4p polyadenylation (TRAMP) complex, were identified to colocalize with vRNA and the exosome in the cytoplasm of infected cells (Mollestone and others 2016) and the helicase SKI2W (SKIV2L) RNA exosome has been shown to prevent autoimmunity by regulating the abundance of RIG-I ligands (Eckard and others 2014) suggesting that vRNAs are specifically targeted for decay.

Concluding Remarks

The knowledge on virus sensing and restriction has dramatically increased in recent years. It became evident that the innate immune system particularly senses RNA that is insufficiently processed and therefore lacks motifs commonly found on cellular RNA. The ability of cytoplasmic PRRs to sense such missing-self motifs allows them to be broadly active and to detect nucleic acids generated by viruses and other pathogens such as bacteria. In case of failure to properly process cellular RNA this ability also bears the risk of inducing unwanted immune responses that can lead to autoimmune disorders. Besides nucleic acid sensors, a set of cellular proteins exists that directly restricts viruses or leads to changes in cellular machineries ranging from translational control to cell death. More mechanistic insights into the regulation and function of nucleic acid binding proteins are important to understand antiviral immunity and to exploit this knowledge for therapeutic interventions.

Acknowledgments

We want to thank the members of the innate immunity laboratory, particularly Philipp Hubel and Pietro Scaturro, for input and critical discussions. The work in the authors' laboratory is funded by a Max-Planck free floater program, ERC starting grant (StG 311339, iVIP), the German Research Foundation (PI1084/2-1, PI1084/3-1, and TRR179), and the Federal Ministry for Education and Research (ERA-Net grant ERASE).

Author Disclosure Statement

No competing financial interests exist.

References

- Abbas YM, Pichlmair A, Gorna MW, Superti-Furga G, Nagar B. 2013. Structural basis for viral 5'-PPP-RNA recognition by human IFIT proteins. *Nature* 494(7435):60–64.

- Abdullah Z, Schlee M, Roth S, Mraheil MA, Barchet W, Bottcher J, Hain T, Geiger S, Hayakawa Y, Fritz JH, Civril F, Hopfner KP, Kurts C, Ruland J, Hartmann G, Chakraborty T, Knolle PA. 2012. RIG-I detects infection with live *Listeria* by sensing secreted bacterial nucleic acids. *EMBO J* 31(21):4153–4164.
- Ahmad S, Hur S. 2015. Helicases in antiviral immunity: dual properties as sensors and effectors. *Trends Biochem Sci* 40(10):576–585.
- Akhrymuk I, Frolov I, Frolova EI. 2016. Both RIG-I and MDA5 detect alphavirus replication in concentration-dependent mode. *Virology* 487:230–241.
- Anchisi S, Guerra J, Mottet-Osman G, Garcin D. 2016. Mismatches in the influenza A virus RNA panhandle prevent retinoic acid-inducible gene I (RIG-I) sensing by impairing RNA/RIG-I complex formation. *J Virol* 90(1):586–590.
- Athanasiadis A, Rich A, Maas S. 2004. Widespread A-to-I RNA editing of Alu-containing mRNAs in the human transcriptome. *PLoS Biol* 2(12):e391.
- Ayllon J, Garcia-Sastre A. 2015. The NS1 protein: a multi-tasking virulence factor. *Curr Top Microbiol Immunol* 386:73–107.
- Banerjee S, Chakrabarti A, Jha BK, Weiss SR, Silverman RH. 2014. Cell-type-specific effects of RNase L on viral induction of beta interferon. *MBio* 5(2):e00856-14.
- Baum A, Sachidanandam R, Garcia-Sastre A. 2010. Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. *Proc Natl Acad Sci U S A* 107(37):16303–16308.
- Berchtold S, Manncke B, Klenk J, Geisel J, Autenrieth IB, Bohn E. 2008. Forced IFIT-2 expression represses LPS induced TNF-alpha expression at posttranscriptional levels. *BMC Immunol* 9:75.
- Berke IC, Yu X, Modis Y, Egelman EH. 2012. MDA5 assembles into a polar helical filament on dsRNA. *Proc Natl Acad Sci U S A* 109(45):18437–18441.
- Bruns AM, Leser GP, Lamb RA, Horvath CM. 2014. The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly. *Mol Cell* 55(5):771–781.
- Byszewska M, Smietanski M, Purta E, Bujnicki JM. 2014. RNA methyltransferases involved in 5' cap biosynthesis. *RNA Biol* 11(12):1597–1607.
- Cai X, Chiu YH, Chen ZJ. 2014. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Mol Cell* 54(2):289–296.
- Chakrabarti A, Jha BK, Silverman RH. 2011. New insights into the role of RNase L in innate immunity. *J Interferon Cytokine Res* 31(1):49–57.
- Crotta S, Davidson S, Mählakoi T, Desmet CJ, Buckwalter MR, Albert ML, Staeheli P, Wack A. 2013. Type I and type III interferons drive redundant amplification loops to induce a transcriptional signature in influenza-infected airway epithelia. *PLoS Pathog* 9(11):e1003773.
- Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, Lin TY, Schneller S, Züst R, Dong H, Thiel V, Sen GC, Fensterl V, Klimstra WB, Pierson TC, Buller RM, Gale M, Jr, Shi PY, Diamond MS. 2010. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature* 468(7322):452–456.
- Decroly E, Ferron F, Lescar J, Canard B. 2012. Conventional and unconventional mechanisms for capping viral mRNA. *Nat Rev Microbiol* 10(1):51–65.
- Deddouche S, Goubau D, Rehwinkel J, Chakravarty P, Begum S, Maillard PV, Borg A, Matthews N, Feng Q, van Kuppe-

- veld FJ, Reis e Sousa C. 2014. Identification of an LGP2-associated MDA5 agonist in picornavirus-infected cells. *Elife* 3:e01535.
- Devarkar SC, Wang C, Miller MT, Ramanathan A, Jiang F, Khan AG, Patel SS, Marcotrigiano J. 2016. Structural basis for m7G recognition and 2'-O-methyl discrimination in capped RNAs by the innate immune receptor RIG-I. *Proc Natl Acad Sci U S A* 113(3):596–601.
- Dias A, Bouvier D, Crepin T, McCarthy AA, Hart DJ, Baudin F, Cusack S, Ruigrok RW. 2009. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature* 458(7240):914–918.
- Diebold SS, Montoya M, Unger H, Alexopoulou L, Roy P, Haswell LE, Al-Shamkhani A, Flavell R, Borrow P, Reis e Sousa C. 2003. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. *Nature* 424(6946):324–328.
- Drygin D, Rice WG, Grummt I. 2010. The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. *Annu Rev Pharmacol Toxicol* 50:131–156.
- Eckard SC, Rice GI, Fabre A, Badens C, Gray EE, Hartley JL, Crow YJ, Stetson DB. 2014. The SKIV2L RNA exosome limits activation of the RIG-I-like receptors. *Nat Immunol* 15(9):839–845.
- Errett JS, Suthar MS, McMillan A, Diamond MS, Gale M, Jr. 2013. The essential, nonredundant roles of RIG-I and MDA5 in detecting and controlling West Nile virus infection. *J Virol* 87(21):11416–11425.
- Feng Q, Hato SV, Langereis MA, Zoll J, Virgen-Slane R, Peisley A, Hur S, Semler BL, van Rij RP, van Kuppeveld FJ. 2012. MDA5 detects the double-stranded RNA replicative form in picornavirus-infected cells. *Cell Rep* 2(5):1187–1196.
- Fensterl V, Sen GC. 2015. Interferon-induced Ifit proteins: their role in viral pathogenesis. *J Virol* 89(5):2462–2468.
- Flanagan JB, Petterson RF, Ambros V, Hewlett NJ, Baltimore D. 1977. Covalent linkage of a protein to a defined nucleotide sequence at the 5'-terminus of virion and replicative intermediate RNAs of poliovirus. *Proc Natl Acad Sci U S A* 74(3):961–965.
- Flick R, Neumann G, Hoffmann E, Neumeier E, Hobom G. 1996. Promoter elements in the influenza vRNA terminal structure. *RNA* 2(10):1046–1057.
- Fodor E, Pritlove DC, Brownlee GG. 1994. The influenza virus panhandle is involved in the initiation of transcription. *J Virol* 68(6):4092–4096.
- Fodor E, Pritlove DC, Brownlee GG. 1995. Characterization of the RNA-fork model of virion RNA in the initiation of transcription in influenza A virus. *J Virol* 69(7):4012–4019.
- Fredericksen BL, Keller BC, Fornek J, Katze MG, Gale M, Jr. 2008. Establishment and maintenance of the innate antiviral response to West Nile Virus involves both RIG-I and MDA5 signaling through IPS-1. *J Virol* 82(2):609–616.
- Furuichi Y, Shatkin AJ. 2000. Viral and cellular mRNA capping: past and prospects. *Adv Virus Res* 55:135–184.
- Gack MU, Shin YC, Joo CH, Urano T, Liang C, Sun L, Takeuchi O, Akira S, Chen Z, Inoue S, Jung JU. 2007. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature* 446(7138):916–920.
- Gehrig S, Eberle ME, Botschen F, Rimbach K, Eberle F, Eigenbrod T, Kaiser S, Holmes WM, Erdmann VA, Sprinzl M, Bec G, Keith G, Dalpke AH, Helm M. 2012. Identification of modifications in microbial, native tRNA that suppress immunostimulatory activity. *J Exp Med* 209(2):225–233.
- George CX, John L, Samuel CE. 2014. An RNA editor, adenosine deaminase acting on double-stranded RNA (ADAR1). *J Interferon Cytokine Res* 34(6):437–446.
- George CX, Ramaswami G, Li JB, Samuel CE. 2016. Editing of cellular self-RNAs by adenosine deaminase ADAR1 suppresses innate immune stress responses. *J Biol Chem* 291(12):6158–6168.
- Gerlach P, Malet H, Cusack S, Reguera J. 2015. Structural insights into bunyavirus replication and its regulation by the vRNA promoter. *Cell* 161(6):1267–1279.
- Gilfoy FD, Mason PW. 2007. West Nile virus-induced interferon production is mediated by the double-stranded RNA-dependent protein kinase PKR. *J Virol* 81(20):11148–11158.
- Gitlin L, Barchet W, Gilfillan S, Cella M, Beutler B, Flavell RA, Diamond MS, Colonna M. 2006. Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. *Proc Natl Acad Sci U S A* 103(22):8459–8464.
- Goubau D, Deddouche S, Reis e Sousa C. 2013. Cytosolic sensing of viruses. *Immunity* 38(5):855–869.
- Goubau D, Schlee M, Deddouche S, Pruijssers AJ, Zillinger T, Goldeck M, Schuberth C, Van der Veen AG, Fujimura T, Rehwinkel J, Iskarpatyoti JA, Barchet W, Ludwig J, Dermody TS, Hartmann G, Reis e Sousa C. 2014. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature* 514(7522):372–375.
- Goubau D, van der Veen AG, Chakravarty P, Lin R, Rogers N, Rehwinkel J, Deddouche S, Rosewell I, Hiscott J, Reis ESC. 2015. Mouse superkiller-2-like helicase DDX60 is dispensable for type I IFN induction and immunity to multiple viruses. *Eur J Immunol* 45(12):3386–3403.
- Grunvogel O, Esser-Nobis K, Reustle A, Schult P, Muller B, Metz P, Trippler M, Windisch MP, Frese M, Binder M, Fackler O, Bartenschlager R, Ruggieri A, Lohmann V. 2015. DDX60L is an interferon-stimulated gene product restricting hepatitis C virus replication in cell culture. *J Virol* 89(20):10548–10568.
- Gu L, Fullam A, Brennan R, Schroder M. 2013. Human DEAD box helicase 3 couples IkappaB kinase epsilon to interferon regulatory factor 3 activation. *Mol Cell Biol* 33(10):2004–2015.
- Habjan M, Andersson I, Klingstrom J, Schumann M, Martin A, Zimmermann P, Wagner V, Pichlmair A, Schneider U, Muhlberger E, Mirazimi A, Weber F. 2008. Processing of genome 5' termini as a strategy of negative-strand RNA viruses to avoid RIG-I-dependent interferon induction. *PLoS One* 3(4):e2032.
- Habjan M, Hubel P, Lacerda L, Benda C, Holze C, Eberl CH, Mann A, Kindler E, Gil-Cruz C, Ziebuhr J, Thiel V, Pichlmair A. 2013. Sequestration by IFIT1 impairs translation of 2'-O-unmethylated capped RNA. *PLoS Pathog* 9(10):e1003663.
- Habjan M, Pichlmair A. 2015. Cytoplasmic sensing of viral nucleic acids. *Curr Opin Virol* 11:31–37.
- Hartner JC, Walkley CR, Lu J, Orkin SH. 2009. ADAR1 is essential for the maintenance of hematopoiesis and suppression of interferon signaling. *Nat Immunol* 10(1):109–115.
- Hopper AK. 2013. Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast *Saccharomyces cerevisiae*. *Genetics* 194(1):43–67.
- Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G. 2006. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 314(5801):994–997.
- Hornung V, Hartmann R, Ablasser A, Hopfner KP. 2014. OAS proteins and cGAS: unifying concepts in sensing and re-

- sponding to cytosolic nucleic acids. *Nat Rev Immunol* 14(8):521–528.
- Hsu MT, Parvin JD, Gupta S, Krystal M, Palese P. 1987. Genomic RNAs of influenza viruses are held in a circular conformation in virions and in infected cells by a terminal panhandle. *Proc Natl Acad Sci U S A* 84(22):8140–8144.
- Hyde JL, Gardner CL, Kimura T, White JP, Liu G, Trobaugh DW, Huang C, Tonelli M, Paessler S, Takeda K, Klimstra WB, Amarasinghe GK, Diamond MS. 2014. A viral RNA structural element alters host recognition of nonself RNA. *Science* 343(6172):783–787.
- Imaizumi T, Yoshida H, Hayakari R, Xing F, Wang L, Matsumiya T, Tanji K, Kawaguchi S, Murakami M, Tanaka H. 2016. Interferon-stimulated gene (ISG) 60, as well as ISG56 and ISG54, positively regulates TLR3/IFN-beta/STAT1 axis in U373MG human astrocytoma cells. *Neurosci Res* 105: 35–41.
- Isaacs A, Lindenmann J. 1957. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci* 147(927):258–267.
- Jockel S, Nees G, Sommer R, Zhao Y, Cherkasov D, Hori H, Ehm G, Schnare M, Nain M, Kaufmann A, Bauer S. 2012. The 2'-O-methylation status of a single guanosine controls transfer RNA-mediated Toll-like receptor 7 activation or inhibition. *J Exp Med* 209(2):235–241.
- Kaiser S, Rimbach K, Eigenbrod T, Dalpke AH, Helm M. 2014. A modified dinucleotide motif specifies tRNA recognition by TLR7. *RNA* 20(9):1351–1355.
- Kang R, Tang D. 2012. PKR-dependent inflammatory signals. *Sci Signal* 5(247):pe47.
- Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S. 2008. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med* 205(7): 1601–1610.
- Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S. 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 441(7089):101–105.
- Killip MJ, Smith M, Jackson D, Randall RE. 2014. Activation of the interferon induction cascade by influenza A viruses requires viral RNA synthesis and nuclear export. *J Virol* 88(8):3942–3952.
- Killip MJ, Young DF, Precious BL, Goodbourn S, Randall RE. 2012. Activation of the beta interferon promoter by paramyxoviruses in the absence of virus protein synthesis. *J Gen Virol* 93(Pt 2):299–307.
- Kim HJ, Fodor E, Brownlee GG, Seong BL. 1997. Mutational analysis of the RNA-fork model of the influenza A virus vRNA promoter in vivo. *J Gen Virol* 78(Pt 2):353–357.
- Kirchner S, Ignatova Z. 2015. Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. *Nat Rev Genet* 16(2):98–112.
- Ko C, Lee S, Windisch MP, Ryu WS. 2014. DDX3 DEAD-box RNA helicase is a host factor that restricts hepatitis B virus replication at the transcriptional level. *J Virol* 88(23):13689–13698.
- Kohler A, Hurt E. 2007. Exporting RNA from the nucleus to the cytoplasm. *Nat Rev Mol Cell Biol* 8(10):761–773.
- Kohlway A, Luo D, Rawling DC, Ding SC, Pyle AM. 2013. Defining the functional determinants for RNA surveillance by RIG-I. *EMBO Rep* 14(9):772–779.
- Koppstein D, Ashour J, Bartel DP. 2015. Sequencing the cap-snatching repertoire of H1N1 influenza provides insight into the mechanism of viral transcription initiation. *Nucleic Acids Res* 43(10):5052–5064.
- Kumar P, Sweeney TR, Skabkina OV, Hellen CU, Pestova TV. 2014. Inhibition of translation by IFIT family members is determined by their ability to interact selectively with the 5'-terminal regions of cap0-, cap1- and 5'ppp-mRNAs. *Nucleic Acids Res* 42(5):3228–3245.
- Lässig C, Matheisl S, Sparrer KM, de Oliveira Mann CC, Moldt M, Patel JR, Goldeck M, Hartmann G, Garcia-Sastre A, Hornung V, Conzelmann KK, Beckmann R, Hopfner KP. 2015. ATP hydrolysis by the viral RNA sensor RIG-I prevents unintentional recognition of self-RNA. *Elife* 4:e10859.
- Lee YF, Nomoto A, Detjen BM, Wimmer E. 1977. A protein covalently linked to poliovirus genome RNA. *Proc Natl Acad Sci U S A* 74(1):59–63.
- Leung DW, Amarasinghe GK. 2016. When your cap matters: structural insights into self vs non-self recognition of 5' RNA by immunomodulatory host proteins. *Curr Opin Struct Biol* 36:133–141.
- Levanon EY, Eisenberg E, Yelin R, Nemzer S, Hallegger M, Shemesh R, Fligelman ZY, Shoshan A, Pollock SR, Sztybel D, Olshansky M, Rechavi G, Jantsch MF. 2004. Systematic identification of abundant A-to-I editing sites in the human transcriptome. *Nat Biotechnol* 22(8):1001–1005.
- Liddicoat BJ, Piskol R, Chalk AM, Ramaswami G, Higuchi M, Hartner JC, Li JB, Seeburg PH, Walkley CR. 2015. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. *Science* 349(6252):1115–1120.
- Liedmann S, Hrincius ER, Guy C, Anhlén D, Dierkes R, Carter R, Wu G, Staeheli P, Green DR, Wolff T, McCullers JA, Ludwig S, Ehrhardt C. 2014. Viral suppressors of the RIG-I-mediated interferon response are pre-packaged in influenza virions. *Nat Commun* 5:5645.
- Lingel A, Simon B, Izaurralde E, Sattler M. 2005. The structure of the flock house virus B2 protein, a viral suppressor of RNA interference, shows a novel mode of double-stranded RNA recognition. *EMBO Rep* 6(12):1149–1155.
- Loo YM, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, Akira S, Gill MA, Garcia-Sastre A, Katze MG, Gale M, Jr. 2008. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J Virol* 82(1):335–345.
- Mackenzie J. 2005. Wrapping things up about virus RNA replication. *Traffic* 6(11):967–977.
- Maharaj NP, Wies E, Stoll A, Gack MU. 2012. Conventional protein kinase C- α (PKC- α) and PKC- β negatively regulate RIG-I antiviral signal transduction. *J Virol* 86(3):1358–1371.
- Malathi K, Dong B, Gale M, Jr., Silverman RH. 2007. Small self-RNA generated by RNase L amplifies antiviral innate immunity. *Nature* 448(7155):816–819.
- Malathi K, Saito T, Crochet N, Barton DJ, Gale M, Jr, Silverman RH. 2010. RNase L releases a small RNA from HCV RNA that refolds into a potent PAMP. *RNA* 16(11):2108–2119.
- Marq JB, Hausmann S, Veillard N, Kolakofsky D, Garcin D. 2011. Short double-stranded RNAs with an overhanging 5' ppp-nucleotide, as found in arenavirus genomes, act as RIG-I decoys. *J Biol Chem* 286(8):6108–6116.
- McCartney SA, Thackray LB, Gitlin L, Gilfillan S, Virgin HW, Colonna M. 2008. MDA-5 recognition of a murine norovirus. *PLoS Pathog* 4(7):e1000108.

- McCracken S, Fong N, Rosonina E, Yankulov K, Brothers G, Siderovski D, Hessel A, Foster S, Shuman S, Bentley DL. 1997. 5'-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. *Genes Dev* 11(24):3306–3318.
- Miyashita M, Oshiumi H, Matsumoto M, Seya T. 2011. DDX60, a DEXD/H box helicase, is a novel antiviral factor promoting RIG-I-like receptor-mediated signaling. *Mol Cell Biol* 31(18):3802–3819.
- Molleston JM, Sabin LR, Moy RH, Menghani SV, Rausch K, Gordesky-Gold B, Hopkins KC, Zhou R, Jensen TH, Wilusz JE, Cherry S. 2016. A conserved virus-induced cytoplasmic TRAMP-like complex recruits the exosome to target viral RNA for degradation. *Genes Dev* 30(14):1658–1670.
- Monroe KM, McWhirter SM, Vance RE. 2009. Identification of host cytosolic sensors and bacterial factors regulating the type I interferon response to *Legionella pneumophila*. *PLoS Pathog* 5(11):e1000665.
- Moy RH, Cole BS, Yasunaga A, Gold B, Shankarling G, Varble A, Molleston JM, tenOever BR, Lynch KW, Cherry S. 2014. Stem-loop recognition by DDX17 facilitates miRNA processing and antiviral defense. *Cell* 158(4):764–777.
- Muller-McNicoll M, Neugebauer KM. 2013. How cells get the message: dynamic assembly and function of mRNA-protein complexes. *Nat Rev Genet* 14(4):275–287.
- Neumann G, Hobom G. 1995. Mutational analysis of influenza virus promoter elements in vivo. *J Gen Virol* 76(Pt 7):1709–1717.
- Nikonov A, Molder T, Sikut R, Kiiver K, Mannik A, Toots U, Lulla A, Lulla V, Utt A, Merits A, Ustav M. 2013. RIG-I and MDA-5 detection of viral RNA-dependent RNA polymerase activity restricts positive-strand RNA virus replication. *PLoS Pathog* 9(9):e1003610.
- O'Neill LA, Golenbock D, Bowie AG. 2013. The history of Toll-like receptors – redefining innate immunity. *Nat Rev Immunol* 13(6):453–460.
- Onoguchi K, Yoneyama M, Fujita T. 2011. Retinoic acid-inducible gene-I-like receptors. *J Interferon Cytokine Res* 31(1):27–31.
- Oshiumi H, Miyashita M, Matsumoto M, Seya T. 2013. A distinct role of Riplet-mediated K63-Linked polyubiquitination of the RIG-I repressor domain in human antiviral innate immune responses. *PLoS Pathog* 9(8):e1003533.
- Oshiumi H, Miyashita M, Okamoto M, Morioka Y, Okabe M, Matsumoto M, Seya T. 2015. DDX60 is involved in RIG-I-dependent and independent antiviral responses, and its function is attenuated by virus-induced EGFR activation. *Cell Rep* 11(8):1193–1207.
- Oshiumi H, Sakai K, Matsumoto M, Seya T. 2010. DEAD/H BOX 3 (DDX3) helicase binds the RIG-I adaptor IPS-1 to up-regulate IFN-beta-inducing potential. *Eur J Immunol* 40(4):940–948.
- Painter MM, Morrison JH, Zoecklein LJ, Rinkoski TA, Watzlawik JO, Papke LM, Warrington AE, Bieber AJ, Matchett WE, Turkowski KL, Poeschla EM, Rodriguez M. 2015. Antiviral protection via RdRP-mediated stable activation of innate immunity. *PLoS Pathog* 11(12):e1005311.
- Paul D, Bartenschlager R. 2015. Flaviviridae replication organelles: oh, what a tangled web we weave. *Annu Rev Virol* 2(1):289–310.
- Peisley A, Lin C, Wu B, Orme-Johnson M, Liu M, Walz T, Hur S. 2011. Cooperative assembly and dynamic disassembly of MDA5 filaments for viral dsRNA recognition. *Proc Natl Acad Sci U S A* 108(52):21010–21015.
- Peisley A, Wu B, Yao H, Walz T, Hur S. 2013. RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner. *Mol Cell* 51(5):573–583.
- Pelka K, Shibata T, Miyake K, Latz E. 2016. Nucleic acid-sensing TLRs and autoimmunity: novel insights from structural and cell biology. *Immunol Rev* 269(1):60–75.
- Pestal K, Funk CC, Snyder JM, Price ND, Treuting PM, Stetson DB. 2015. Isoforms of RNA-editing enzyme ADAR1 independently control nucleic acid sensor MDA5-driven autoimmunity and multi-organ development. *Immunity* 43(5):933–944.
- Pflug A, Guilligay D, Reich S, Cusack S. 2014. Structure of influenza A polymerase bound to the viral RNA promoter. *Nature* 516(7531):355–360.
- Pham AM, Santa Maria FG, Lahiri T, Friedman E, Marie IJ, Levy DE. 2016. PKR transduces MDA5-dependent signals for type I IFN induction. *PLoS Pathog* 12(3):e1005489.
- Pichlmair A, Lassnig C, Eberle CA, Gorna MW, Baumann CL, Burkard TR, Burckstummer T, Stefanovic A, Krieger S, Bennett KL, Rulicke T, Weber F, Colinge J, Muller M, Superti-Furga G. 2011. IFIT1 is an antiviral protein that recognizes 5'-triphosphate RNA. *Nat Immunol* 12(7):624–630.
- Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F, Reis e Sousa C. 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science* 314(5801):997–1001.
- Pichlmair A, Schulz O, Tan CP, Rehwinkel J, Kato H, Takeuchi O, Akira S, Way M, Schiavo G, Reis e Sousa C. 2009. Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J Virol* 83(20):10761–10769.
- Pinto AK, Williams GD, Szretter KJ, White JP, Proenca-Modena JL, Liu G, Olejnik J, Brien JD, Ebihara H, Muhlberger E, Amarasinghe G, Diamond MS, Boon AC. 2015. Human and murine IFIT1 proteins do not restrict infection of negative-sense RNA viruses of the orthomyxoviridae, bunyaviridae, and filoviridae families. *J Virol* 89(18):9465–9476.
- Portal MM, Pavet V, Erb C, Gronemeyer H. 2015. Human cells contain natural double-stranded RNAs with potential regulatory functions. *Nat Struct Mol Biol* 22(1):89–97.
- Rassa JC, Ross SR. 2003. Viruses and toll-like receptors. *Microbes Infect* 5(11):961–968.
- Rehwinkel J, Tan CP, Goubau D, Schulz O, Pichlmair A, Bier K, Robb N, Vreede F, Barclay W, Fodor E, Reis e Sousa C. 2010. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. *Cell* 140(3):397–408.
- Reich S, Guilligay D, Pflug A, Malet H, Berger I, Crepin T, Hart D, Lunardi T, Nanao M, Ruigrok RW, Cusack S. 2014. Structural insight into cap-snatching and RNA synthesis by influenza polymerase. *Nature* 516(7531):361–366.
- Resa-Infante P, Jorba N, Coloma R, Ortin J. 2011. The influenza virus RNA synthesis machine: advances in its structure and function. *RNA Biol* 8(2):207–215.
- Rice GI, Kashner PR, Forte GM, Mannion NM, Greenwood SM, Szykiewicz M, Dickerson JE, Bhaskar SS, Zampini M, Briggs TA, Jenkinson EM, Bacino CA, Battini R, Bertini E, Brogan PA, Brueton LA, Carpanelli M, De Laet C, de Lonlay P, del Toro M, Desguerre I, Fazzi E, Garcia-Cazorla A, Heiberg A, Kawaguchi M, Kumar R, Lin JP, Lourenco CM, Male AM, Marques W, Jr., Mignot C, Olivieri I, Orcesi S, Prabhakar P, Rasmussen M, Robinson RA, Rozenberg F, Schmidt JL, Steindl K, Tan TY, van der Merwe WG, Vanderver A, Vassallo G, Wakeling EL, Wassmer E, Whittaker

- E, Livingston JH, Lebon P, Suzuki T, McLaughlin PJ, Keegan LP, O'Connell MA, Lovell SC, Crow YJ. 2012. Mutations in ADAR1 cause Aicardi-Goutieres syndrome associated with a type I interferon signature. *Nat Genet* 44(11):1243–1248.
- Roers A, Hiller B, Hornung V. 2016. Recognition of endogenous nucleic acids by the innate immune system. *Immunity* 44(4):739–754.
- Rosenthal JJ. 2015. The emerging role of RNA editing in plasticity. *J Exp Biol* 218(Pt 12):1812–1821.
- Runge S, Sparrer KM, Lassig C, Hembach K, Baum A, Garcia-Sastre A, Soding J, Conzelmann KK, Hopfner KP. 2014. In vivo ligands of MDA5 and RIG-I in measles virus-infected cells. *PLoS Pathog* 10(4):e1004081.
- Saito T, Hirai R, Loo YM, Owen D, Johnson CL, Sinha SC, Akira S, Fujita T, Gale M, Jr. 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc Natl Acad Sci U S A* 104(2):582–587.
- Sanchez David RY, Combredet C, Sismeiro O, Dillies MA, Jagla B, Coppee JY, Mura M, Guerbois Galla M, Despres P, Tangy F, Komarova AV. 2016. Comparative analysis of viral RNA signatures on different RIG-I-like receptors. *Elife* 5:e11275.
- Sancho D, Reis e Sousa C. 2013. Sensing of cell death by myeloid C-type lectin receptors. *Curr Opin Immunol* 25(1):46–52.
- Sarin LP, Leidel SA. 2014. Modify or die?—RNA modification defects in metazoans. *RNA Biol* 11(12):1555–1567.
- Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, Tsujimura T, Fujita T, Akira S, Takeuchi O. 2010. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci U S A* 107(4):1512–1517.
- Schlee M. 2013. Master sensors of pathogenic RNA – RIG-I like receptors. *Immunobiology* 218(11):1322–1335.
- Schlee M, Roth A, Hornung V, Hagmann CA, Wimmenauer V, Barchet W, Coch C, Janke M, Mihailovic A, Wardle G, Juranek S, Kato H, Kawai T, Poeck H, Fitzgerald KA, Takeuchi O, Akira S, Tuschl T, Latz E, Ludwig J, Hartmann G. 2009. Recognition of 5' triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity* 31(1):25–34.
- Schmidt A, Schwerdt T, Hamm W, Hellmuth JC, Cui S, Wenzel M, Hoffmann FS, Michallet MC, Besch R, Hopfner KP, Endres S, Rothenfusser S. 2009. 5'-Triphosphate RNA requires base-paired structures to activate antiviral signaling via RIG-I. *Proc Natl Acad Sci U S A* 106(29):12067–12072.
- Schneider U, Martin A, Schwemmle M, Staeheli P. 2007. Genome trimming by Borna disease viruses: viral replication control or escape from cellular surveillance? *Cell Mol Life Sci* 64(9):1038–1042.
- Schuberth-Wagner C, Ludwig J, Bruder AK, Herzner AM, Zillinger T, Goldeck M, Schmidt T, Schmid-Burgk JL, Kerber R, Wolter S, Stumpel JP, Roth A, Bartok E, Drosten C, Coch C, Hornung V, Barchet W, Kummerer BM, Hartmann G, Schlee M. 2015. A conserved histidine in the RNA sensor RIG-I controls immune tolerance to N1-2'O-methylated self RNA. *Immunity* 43(1):41–51.
- Schulz O, Pichlmair A, Rehwinkel J, Rogers NC, Scheuner D, Kato H, Takeuchi O, Akira S, Kaufman RJ, Reis e Sousa C. 2010. Protein kinase R contributes to immunity against specific viruses by regulating interferon mRNA integrity. *Cell Host Microbe* 7(5):354–361.
- Shi Y, Yuan B, Qi N, Zhu W, Su J, Li X, Qi P, Zhang D, Hou F. 2015. An autoinhibitory mechanism modulates MAVS activity in antiviral innate immune response. *Nat Commun* 6:7811.
- Singh R, Reddy R. 1989. Gamma-monomethyl phosphate: a cap structure in spliceosomal U6 small nuclear RNA. *Proc Natl Acad Sci U S A* 86(21):8280–8283.
- Strahle L, Garcin D, Kolakofsky D. 2006. Sendai virus defective-interfering genomes and the activation of interferon-beta. *Virology* 351(1):101–111.
- Sun Z, Ren H, Liu Y, Teeling JL, Gu J. 2011. Phosphorylation of RIG-I by casein kinase II inhibits its antiviral response. *J Virol* 85(2):1036–1047.
- Takahashi K, Yoneyama M, Nishihori T, Hirai R, Kumeta H, Narita R, Gale M, Jr, Inagaki F, Fujita T. 2008. Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. *Mol Cell* 29(4):428–440.
- Thulasi Raman SN, Liu G, Pyo HM, Cui YC, Xu F, Ayalew LE, Tikoo SK, Zhou Y. 2016. DDX3 interacts with influenza A virus NS1 and NP proteins and exerts antiviral function through regulation of stress granule formation. *J Virol* 90(7):3661–3675.
- Tiley LS, Hagen M, Matthews JT, Krystal M. 1994. Sequence-specific binding of the influenza virus RNA polymerase to sequences located at the 5' ends of the viral RNAs. *J Virol* 68(8):5108–5116.
- Topisirovic I, Svitkin YV, Sonenberg N, Shatkin AJ. 2011. Cap and cap-binding proteins in the control of gene expression. *Wiley Interdiscip Rev RNA* 2(2):277–298.
- Triantafyllou K, Vakakis E, Kar S, Richer E, Evans GL, Triantafyllou M. 2012. Visualisation of direct interaction of MDA5 and the dsRNA replicative intermediate form of positive strand RNA viruses. *J Cell Sci* 125(Pt 20):4761–4769.
- Uchikawa E, Lethier M, Malet H, Brunel J, Gerlier D, Cusack S. 2016. Structural analysis of dsRNA binding to anti-viral pattern recognition receptors LGP2 and MDA5. *Mol Cell* 62(4):586–602.
- Venkataraman T, Valdes M, Elsby R, Kakuta S, Caceres G, Saijo S, Iwakura Y, Barber GN. 2007. Loss of DEXD/H box RNA helicase LGP2 manifests disparate antiviral responses. *J Immunol* 178(10):6444–6455.
- Versteeg GA, Garcia-Sastre A. 2010. Viral tricks to grid-lock the type I interferon system. *Curr Opin Microbiol* 13(4):508–516.
- Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR. 2006. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J Virol* 80(10):5059–5064.
- Weber M, Gawanbacht A, Habjan M, Rang A, Borner C, Schmidt AM, Veitinger S, Jacob R, Devignot S, Kochs G, Garcia-Sastre A, Weber F. 2013. Incoming RNA virus nucleocapsids containing a 5'-triphosphorylated genome activate RIG-I and antiviral signaling. *Cell Host Microbe* 13(3):336–346.
- Wolferstatter M, Schweneker M, Spath M, Lukassen S, Klingenberg M, Brinkmann K, Wielert U, Lauterbach H, Hochrein H, Chaplin P, Suter M, Hausmann J. 2014. Recombinant modified vaccinia virus Ankara generating excess early double-stranded RNA transiently activates protein kinase R and triggers enhanced innate immune responses. *J Virol* 88(24):14396–14411.
- Wu B, Peisley A, Richards C, Yao H, Zeng X, Lin C, Chu F, Walz T, Hur S. 2013. Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5. *Cell* 152(1–2):276–289.
- Wu B, Peisley A, Tetrault D, Li Z, Egelman EH, Magor KE, Walz T, Penczek PA, Hur S. 2014. Molecular imprinting as a signal-activation mechanism of the viral RNA sensor RIG-I. *Mol Cell* 55(4):511–523.

- Xu J, Mercado-Lopez X, Grier JT, Kim WK, Chun LF, Irvine EB, Del Toro Duany Y, Kell A, Hur S, Gale M, Jr, Raj A, Lopez CB. 2015. Identification of a natural viral RNA motif that optimizes sensing of viral RNA by RIG-I. *MBio* 6(5): e01265-15.
- Yao H, Dittmann M, Peisley A, Hoffmann HH, Gilmore RH, Schmidt T, Schmid-Burgk JL, Hornung V, Rice CM, Hur S. 2015. ATP-dependent effector-like functions of RIG-I-like receptors. *Mol Cell* 58(3):541–548.
- Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo YM, Gale M, Jr., Akira S, Yonehara S, Kato A, Fujita T. 2005. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol* 175(5):2851–2858.
- Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 5(7): 730–737.
- Zamanian-Daryoush M, Mogensen TH, DiDonato JA, Williams BR. 2000. NF-kappaB activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF-kappaB-inducing kinase and IkappaB kinase. *Mol Cell Biol* 20(4):1278–1290.
- Zhang B, Liu X, Chen W, Chen L. 2013. IFIT5 potentiates anti-viral response through enhancing innate immune signaling pathways. *Acta Biochim Biophys Sin (Shanghai)* 45(10): 867–874.
- Zhang Z, Kim T, Bao M, Facchinetti V, Jung SY, Ghaffari AA, Qin J, Cheng G, Liu YJ. 2011a. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. *Immunity* 34(6):866–878.
- Zhang Z, Yuan B, Lu N, Facchinetti V, Liu YJ. 2011b. DHX9 pairs with IPS-1 to sense double-stranded RNA in myeloid dendritic cells. *J Immunol* 187(9):4501–4508.

Address correspondence to:
Dr. Andreas Pichlmair
Innate Immunity Laboratory
Max-Planck Institute of Biochemistry
Am Klopferspitz 18
82152 Martinsried/Munich
Germany

E-mail: apichl@biochem.mpg.de

Received 19 September 2016/Accepted 5 January 2017