Review

Slicing and dicing viruses: antiviral RNA interference in mammals

Pierre V Maillard[1],[*] , Annemarthe G van der Veen[2] , Enzo Z Poirier[2] & Caetano Reis e Sousa[2],[**]

Abstract

To protect against the harmful consequences of viral infections, organisms are equipped with sophisticated antiviral mechanisms, including cell-intrinsic means to restrict viral replication and propagation. Plant and invertebrate cells utilise mostly RNA interference (RNAi), an RNA-based mechanism, for cell-intrinsic immunity to viruses while vertebrates rely on the protein-based interferon (IFN)-driven innate immune system for the same purpose. The RNAi machinery is conserved in vertebrate cells, yet whether antiviral RNAi is still active in mammals and functionally relevant to mammalian antiviral defence is intensely debated. Here, we discuss cellular and viral factors that impact on antiviral RNAi and the contexts in which this system might be at play in mammalian resistance to viral infection.

Introduction

Metazoan organisms are constantly exposed to viruses and have evolved diverse mechanisms to combat the invaders. One group of mechanisms operates in a cell-intrinsic fashion, targeting viral nucleic acids and viral proteins for destruction and/or causing the premature shutdown or demise of infected cells to prevent them from serving as virus producers. Cell-intrinsic antiviral mechanisms are part of the innate immune system and include RNA interference (RNAi) and the interferon (IFN) system. The two systems operate very differently even though they can both be triggered by virally derived long double-stranded RNA (dsRNA) or highly base-paired single-stranded RNA (ssRNA). DsRNA can derive from the viral genome (in the case of a dsRNA virus) or from annealing of two strands of complementary RNAs, which are generated as RNA virus replication intermediates or DNA virus convergent transcripts. Highly based-paired ssRNAs are found in hairpins within viral genomes or viral transcripts and are generically referred to as

dsRNA, a nomenclature that we retain here even if technically incorrect. Both types of dsRNA are largely absent from uninfected cells and act as hallmarks of viral infection to trigger innate antiviral immune responses.

In RNAi, long dsRNA is cleaved by the type III endoribonuclease Dicer into small interfering RNA (siRNAs) (Bernstein *et al*, 2001), RNA duplexes of 21–24 nucleotides (nts) in length, with 3′ 2-nt overhangs and a 5′ mono-phosphate and a 3′ hydroxyl group on both strands (Fig 1) (Hamilton & Baulcombe, 1999; Zamore *et al*, 2000; Elbashir *et al*, 2001b,a). One strand of each siRNA duplex is bound by an Argonaute (Ago) protein, which, together with accessory proteins, forms the RNA-induced silencing complex (RISC) and mediates the endonucleolytic cleavage ("slicing") of complementary target RNAs (Hammond *et al*, 2000; MacRae *et al*, 2008). Of the four Ago proteins encoded by the mammalian genome, only Ago2 has catalytic activity and is essential for target slicing and RNAi (Liu *et al*, 2004; Meister *et al*, 2004; Swarts *et al*, 2014; Sheu-Gruttadauria & MacRae, 2017). However, all four Ago proteins are involved in an RNAi-related process, the microRNA (miRNA)-mediated gene silencing pathway, which does not involve slicing but translation inhibition and/or mRNA degradation (Bartel, 2009; Jonas & Izaurralde, 2015). Notably, Dicer is also involved in miRNA biogenesis. Vertebrates and nematodes possess a single Dicer that generates both siRNA and miRNAs while most invertebrates express two Dicer proteins. For example, in *Drosophila melanogaster*, dmDcr-1 is dedicated to the miRNA pathway while dmDcr-2 performs antiviral RNAi (Lee *et al*, 2004).

Three observations indicate that RNAi acts as the major antiviral mechanism of plants and invertebrates (Ding & Voinnet, 2007; Kemp & Imler, 2009; Ding, 2010; Sarkies & Miska, 2013; tenOever, 2016). First, viral infections in these organisms lead to the accumulation of Dicer-dependent virus-derived siRNAs (viRNAs) that originate from dsRNA viral replication intermediates and/or RNA hairpins and are homologous to viral RNA sequences (Yoo *et al*, 2004; Molnar *et al*, 2005; Galiana-Arnoux *et al*, 2006; Ho *et al*, 2006; van Rij *et al*, 2006; Wang *et al*, 2006a; Aliyari *et al*, 2008; Félix *et al*, 2011). Second, inactivation of key components of the RNAi pathway results in an increase in viral load in infected cells (Mourrain *et al*, 2000; Dalmay *et al*, 2001; Li *et al*, 2002; Lu *et al*, 2005; Schott *et al*, 2005; Wilkins *et al*, 2005; Deleris *et al*, 2006; Galiana-Arnoux *et al*, 2006; Wang *et al*, 2006a; Félix *et al*, 2011).

1 Division of Infection and Immunity, University College London, London, UK
2 Immunobiology Laboratory, The Francis Crick Institute, London, UK
*Corresponding author. Tel: +44 2031 082137; E-mail: p.maillard@ucl.ac.uk
**Corresponding author. Tel: +44 2037 961310; E-mail: caetano@crick.ac.uk
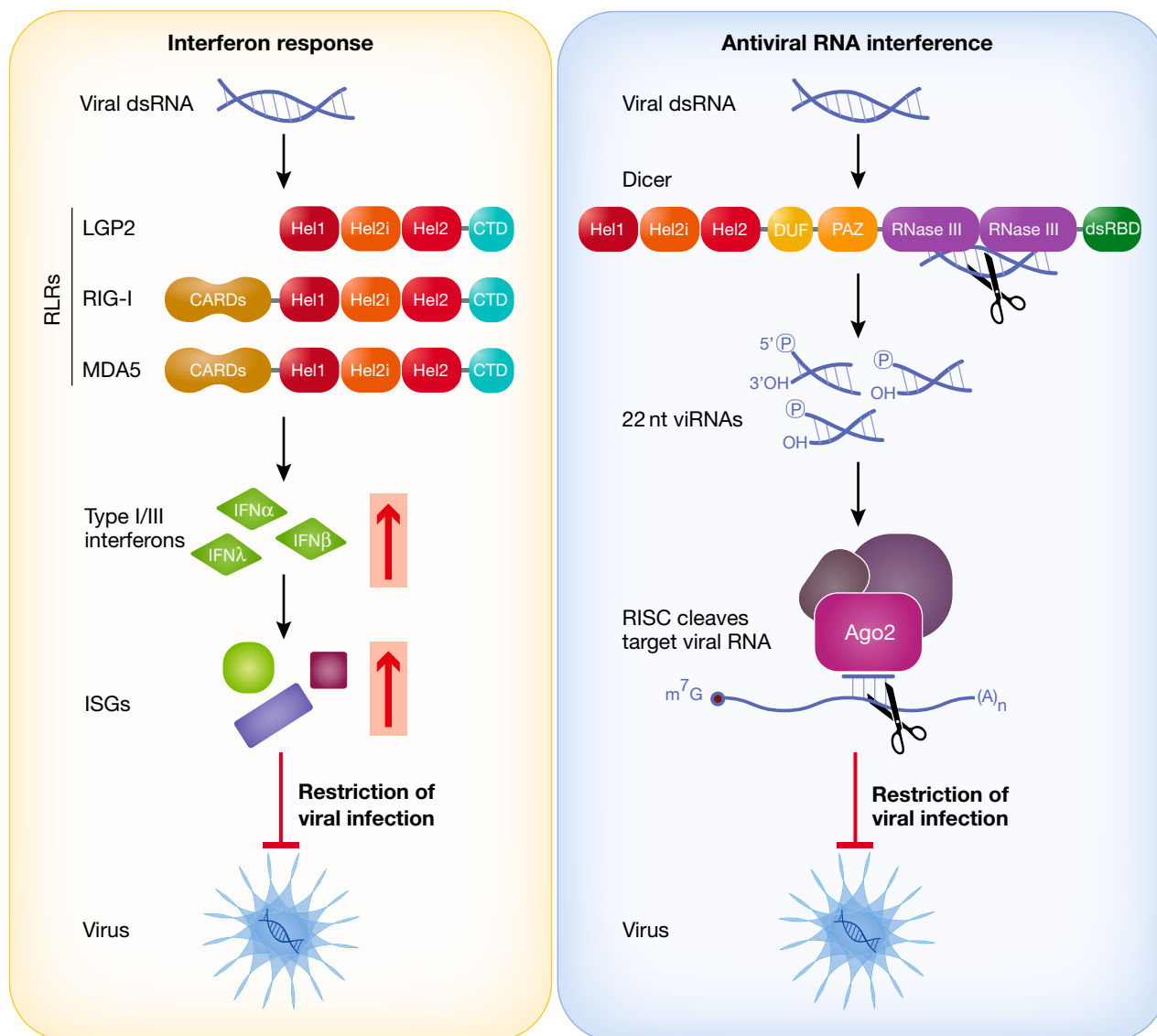
Figure 1. IFN response and antiviral RNAi triggered by viral dsRNA.
In the cytoplasm of mammalian cells, the RIG-I-like receptors (RLRs) RIG-I and MDA5 detect viral dsRNA and trigger the production of type I interferons, which results in the induction of interferon-stimulated genes (ISGs) that encode proteins capable of inhibiting viral replication and virus spread. In antiviral RNAi, Dicer cleaves viral dsRNA into viRNAs that are loaded into a RISC complex. As a protein component of this complex, Ago2 degrades viral RNAs with homology to the viRNAs, thereby inhibiting viral replication. RLRs and Dicer share a common DExD/H domain, composed of three helicases (Hel1, Hel2i and Hel2). RIG-I and MDA5 additionally carry two CARD domains responsible for downstream signalling to MAVS. Dicer possesses two RNase III domains involved in dsRNA dicing.

Third, many plant and insect viruses encode viral suppressors of RNAi (VSRs) that interfere with distinct steps of the RNAi pathway, demonstrating the selection pressure imposed by this antiviral system (Pumplin & Voinnet, 2013; Bronkhorst & van Rij, 2014; Csorba *et al*, 2015).

In contrast, in chordate cells, including mouse and human cells, dsRNA and other nucleic acids associated with viral infection trigger cytosolic innate immune pathways that induce the production of type I IFNs (mainly IFNα and IFNβ) and type III IFNs (IFNλ) (Goubau *et al,* 2013; Schneider *et al,* 2014; Wu & Chen, 2014; Schlee & Hartmann, 2016) (Fig 1). These key antiviral cytokines are then secreted and act in an autocrine and paracrine manner by binding to their cognate receptors, i.e. the ubiquitously expressed IFNα/β receptor (IFNAR) and the epithelial cell type-restricted type III IFN receptor (IL-28R), which signal to induce hundreds of interferon-stimulated genes (ISGs) (Schneider *et al,* 2014). The proteins encoded by these ISGs limit viral replication directly (Schoggins *et al,* 2011) and serve to enhance adaptive immune responses to the virus (de Veer *et al,* 2001; Iwasaki & Medzhitov, 2010). For example, the dsRNA-dependent protein kinase R (PKR) is activated by cytosolic dsRNA and phosphorylates and inactivates the eukaryotic translation initiation factor 2 α (eIF2α), resulting in translational arrest and thwarting the production of both viral and host cell proteins (Pindel & Sadler, 2011). This can ultimately lead to the

demise of the infected cell, further undermining the ability of the virus to propagate.

Virally derived long dsRNA or highly based-paired RNA is detected in the cytosol of mammalian cells by RIG-I like receptors (RLRs), which include RIG-I (retinoic acid-inducible gene I), MDA5 (melanoma differentiation factor 5) and LGP2 (laboratory of genetics and physiology 2) (Fig 1). RIG-I recognises based-paired ds or ssRNA with a di- or triphosphate (5′PP/5′PPP) at its 5′ extremity (Hornung *et al*, 2006; Pichlmair *et al*, 2006; Schlee *et al*, 2009; Schmidt *et al*, 2009; Goubau *et al*, 2014), such as found in the genomes of influenza virus, Sendai virus and reovirus (Baum *et al*, 2010; Rehwinkel *et al*, 2010; Weber *et al*, 2013; Goubau *et al*, 2014). MDA5 triggers comprise long dsRNAs that accumulate during infection with certain viruses such as picornaviruses and reovirus (Gitlin *et al*, 2006; Kato *et al*, 2006; Weber *et al*, 2006; Pichlmair *et al*, 2009; Feng *et al*, 2012). RIG-I and MDA5 contain two tandem N-terminal CARDs (caspase activation and recruitment domains) that mediate downstream signalling via the adaptor protein MAVS (mitochondrial antiviral signalling protein), leading to activation of the transcription factors IRF3, IRF7 (interferon regulatory factors 3 and 7) and NFκ-B (nuclear factor kappa-light-chain enhancer of activated B cells). These transcription factors drive the expression of type I and type III IFNs and can directly induce some ISGs. LGP2 lacks CARDs and is unable to induce signalling via MAVS. It is thought to act by modulating responses by the other RLRs (Bruns & Horvath, 2014; Bruns *et al*, 2014; Parisien *et al*, 2018).

Thus, plants and invertebrates lack an IFN system and rely on antiviral RNAi to defend against viruses. In contrast, vertebrates have adopted the IFN system for cell-intrinsic antiviral defence and are thought to have abandoned antiviral RNAi even though they have retained the RNAi machinery and utilise it for miRNA generation and function. Recently, a number of studies have started to question whether the primordial antiviral function of RNAi has truly been abandoned by mammalian cells or whether it can constitute a physiologically relevant antiviral system that complements the IFN pathway. This has become an area of controversy, with some investigators suggesting that RNAi can be a relevant means of cell-intrinsic restriction to virus infection in mammals while others argue that it is an epiphenomenon with no role in antiviral resistance (Cullen *et al*, 2013; Cullen, 2014; Ding & Voinnet, 2014; tenOever, 2014, 2017; Jeffrey *et al*, 2017). In this review, we address this controversy and summarise current understanding of antiviral RNAi pathways in mammals and reflect on the possible contexts in which it might play a role.

Cellular determinants of antiviral RNAi

Detection of dsRNAi in mammalian cells with attenuated IFN responses
To evaluate the possible existence of antiviral RNAi in mammals, it is useful to consider studies that are exempt from virus-dependent variables such as expression of VSRs. Therefore, we first discuss studies that use synthetic long dsRNA, composed of two perfectly complementary strands, to trigger RNAi, termed here long dsRNA-mediated RNAi (dsRNAi). This process depends on the successive processing of long dsRNA into a pool of siRNAs and is distinct from RNAi induced experimentally by the introduction of siRNAs (which

bypasses the Dicer machinery) (Caplen *et al*, 2001; Elbashir *et al*, 2001a) or of short hairpin RNAs (shRNAs, which resemble the structure of pre-miRNAs) (Brummelkamp *et al*, 2002; Paddison *et al*, 2002b; Bartel, 2004).

Long dsRNA-mediated RNAi was first described in *C. elegans* (Fire *et al*, 1998) followed by *Drosophila*, *Trypanosoma brucei*, planarians and plants (Kennerdell & Carthew, 1998; Ngô *et al*, 1998; Waterhouse *et al*, 1998; Sánchez Alvarado & Newmark, 1999). In mammalian cell lines, long dsRNA had either no effect or displayed a non-sequence-specific effect, consistent with activation of the IFN system (Caplen *et al*, 2000; Elbashir *et al*, 2001a). Yet, in preimplantation embryos, as well as in oocytes, embryonic stem cells (ESCs) and embryonal carcinoma (EC) cell lines, the introduction of long dsRNA targeting endogenous genes caused a specific reduction in gene expression and induction of phenotypes comparable to those of null mutants, without causing cell death or translational arrest (Svoboda *et al*, 2000; Wianny & Zernicka-Goetz, 2000; Billy *et al*, 2001; Yang *et al*, 2001; Paddison *et al*, 2002a). The sequence-specific silencing induced by long dsRNA in oocytes and ESCs/ECs correlated with a relative inability of these cells to produce and/or respond to IFN (Burke *et al*, 1978; Francis & Lehman, 1989; Stein *et al*, 2005; D'Angelo *et al*, 2016; Wu *et al*, 2018), which suggested that dsRNAi might be active in mammalian undifferentiated cells but masked or inhibited by the IFN system in differentiated cells.

Antagonism between the IFN response and dsRNAi
Antagonism between the IFN system and dsRNAi was formally tested in somatic cells genetically deficient in MAVS or IFNAR (Maillard *et al*, 2016). In such cells, introduction of dsRNA resulted in Dicer-dependent accumulation of siRNAs and Ago2-dependent sequence-specific gene silencing (Maillard *et al*, 2016). A subsequent study showed that the IFN system actively inhibits dsRNAi at least in part through induction of LGP2, which binds Dicer and inhibits processing of long dsRNA into siRNAs (Van der Veen *et al*, 2018) (Fig 2). In that study (Van der Veen *et al*, 2018), LGP binding to Dicer did not impact the biogenesis of two household miRNAs although LGP2 has also been reported to interact with the Dicer co-factor TRBP (HIV TAR RNA-binding protein) and inhibit the processing of a subset of TRBP-bound miRNAs (Komuro *et al*, 2016; Takahashi *et al*, 2018). Whether LGP2 additionally inhibits dsRNAi via TRBP remains to be addressed.

It is unclear why somatic cells should inhibit dsRNAi during an IFN response. A clue may come from the observation that mammalian cells stably expressing *Drosophila* dcr-2 to artificially boost dsRNAi have impaired induction of IFN upon treatment with poly(I:C), a dsRNA analog (Girardi *et al*, 2015). Viral infection or treatment with poly(I:C) also induces poly-ADP-ribosylation of Ago2 and other RISC components, which inhibits RISC activity and causes a relief in miRNA-mediated repression of some ISGs (Seo *et al*, 2013). Perhaps, inhibition of Dicer and RISC is essential for effective stimulation of the IFN pathway, in part by preventing loss of dsRNA substrates for RLR activation. Preservation of dsRNA in infected cells may also ensure that the activity of antiviral proteins encoded by ISGs is not compromised. For example, PKR requires dsRNA of > 30 nts to dimerise and become active for translational repression (Husain *et al*, 2012). Dicer-mediated cleavage of long dsRNA could starve the cell of substrates for PKR activation or, more likely, lead
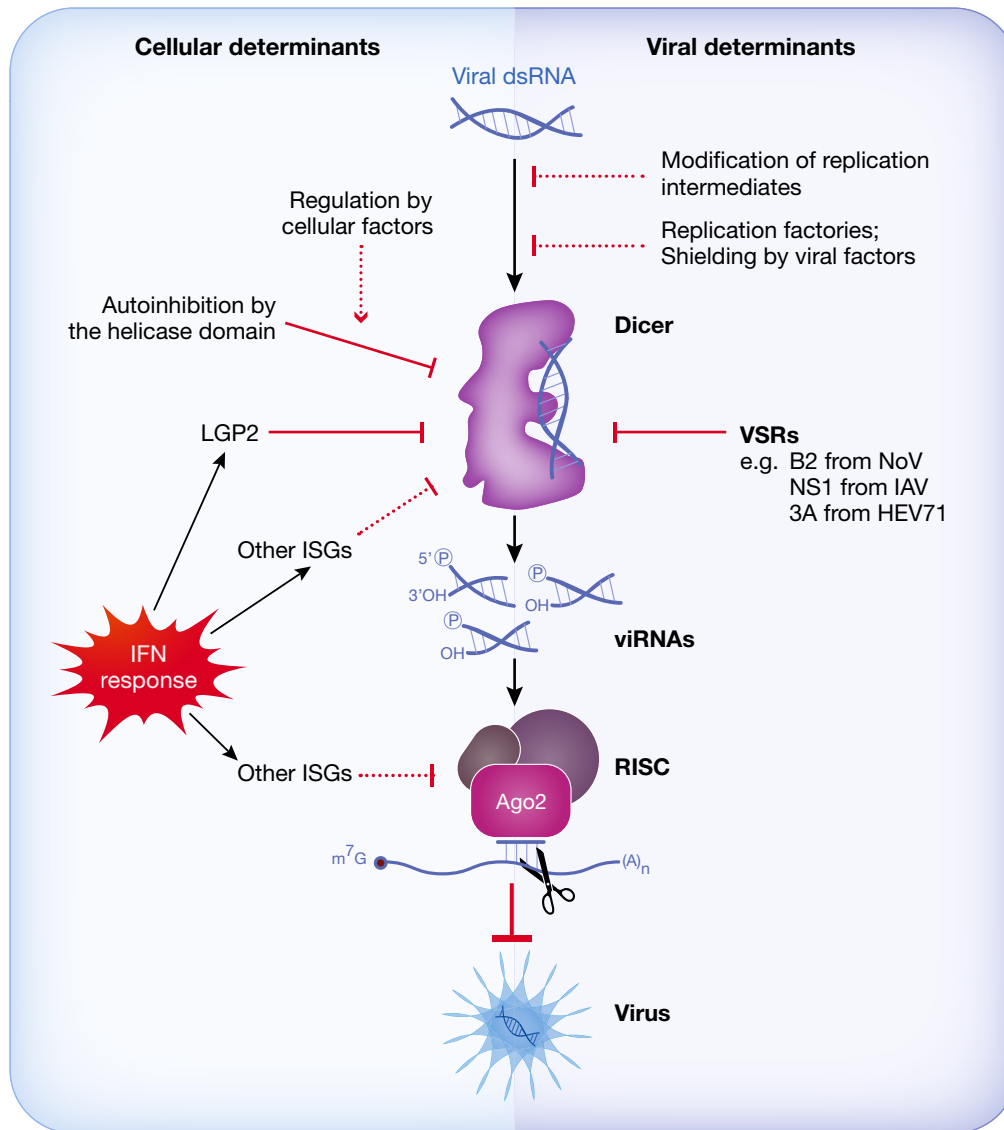
Figure 2. Impact of viral and cellular determinants on antiviral RNAi.
Recognition and dicing of viral dsRNA by Dicer can be influenced by various viral determinants or cellular factors, as described in main text. Known or putative mechanisms that counteract antiviral RNAi are represented with a plain or dashed red line, respectively. Structure of Dicer is based on Liu *et al* (2018).

to accumulation of 21–22 nt siRNA duplexes that would "quench" PKR monomers, blocking substrate-dependent dimerisation.

Intrinsic inefficiency of mammalian Dicer in processing long dsRNA
DsRNAi in mammalian cells is further influenced by the molecular properties of its central component: Dicer. This large multi-domain enzyme comprises an N-terminal DExD/H helicase domain (containing an ATPase site) followed by a small domain of unknown function (DUF283), a Piwi Argonaute Zwille (PAZ) domain, two tandem RNAse III domains and a C-terminal dsRNA-binding domain (dsRBD —Fig 1). The PAZ domain binds the 3′ 2nt-overhangs found at the extremity of dsRNA substrates, while the RNAse III domains each mediate the cleavage of one strand of the RNA duplex. *In vitro* studies revealed that human Dicer (hDcr) processes long dsRNA into

siRNAs less efficiently than pre-miRNA into miRNAs (Ma *et al*, 2008; Chakravarthy *et al*, 2010). Deletion or partial proteolysis of the helicase domain increases rate of dsRNA cleavage, while only modestly affecting the cleavage of pre-miRNAs (Provost *et al*, 2002; Zhang *et al*, 2002; Ma *et al*, 2008). Similarly, a deletion mutant of hDcr lacking nearly the entire helicase domain displayed an enhanced ability to process endogenously transcribed long dsRNA and long hairpin RNAs into siRNAs and conferred dsRNAi activity to engineered cells (Kennedy *et al*, 2015). Finally, mouse oocytes, in which dsRNAi is active, express a shortened isoform of Dicer (Dicer^O) that lacks the N-terminal helicase domain and processes endogenous or ectopically expressed long hairpin RNAs more efficiently (Flemr *et al*, 2013). Together, these data suggest that the helicase domain of Dicer inhibits its catalytic activity for long

dsRNA and that its incorporation into the mature enzyme might be regulated by alternative transcription. However, expression of Dicer[O] has not been detected outside mouse germ cells and expression of truncated Dicer isoforms in humans has been reported only in certain cancer cell lines (Potenza *et al*, 2010; Hinkal *et al*, 2011; Cantini *et al*, 2014). An alternative possibility is that modulation of inhibition by the helicase domain could come about not through alternative transcription but through the activity of Dicer-associated proteins. Structural studies show that the co-factors TRBP and PACT (Protein Activator of PKR) induce a conformational change in the Dicer helicase domain (Taylor *et al*, 2013) that could mimic the effect of deletion (Lee *et al*, 2006; Ma *et al*, 2008; Chakravarthy *et al*, 2010; Ota *et al*, 2013). The exact role and cellular context in which TRBP and PACT might modulate the ability of Dicer to process long dsRNA *in vivo* remains to be explored.

Viral determinants of antiviral RNAi

Antiviral RNAi is distinct from dsRNAi in (i) the origin of the substrate dsRNA (viral RNA vs exogenous sources) and (ii) the RNAs targeted by the RISC (viral RNA vs host cell mRNA). Antiviral RNAi therefore depends on the efficient production of viRNAs from viral dsRNA and the efficient targeting of viral RNA by the RISC machinery.

Targeting of viral RNA by exogenous small RNAs

Cells transfected with siRNAs or expressing an shRNA targeting viral genomes display sequence-specific reductions in viral RNA accumulation and virus replication upon challenge with homologous viruses, including human immunodeficiency virus (HIV), hepatitis C virus (HCV), influenza A virus (IAV), West Nile virus (WNV), SARS coronavirus, human papilloma virus and various picornaviruses (Gitlin *et al*, 2002; Jacque *et al*, 2002; Lee *et al*, 2002; Ge *et al*, 2003; Kapadia *et al*, 2003; Konishi *et al*, 2003; Randall *et al*, 2003; Lu *et al*, 2004; Phipps *et al*, 2004; Takigawa *et al*, 2004; Bitko *et al*, 2005; Shi *et al*, 2005; Sim *et al*, 2005; Werk *et al*, 2005; Yuan *et al*, 2005; Kumar *et al*, 2006; Bousarghin *et al*, 2009; Qureshi *et al*, 2018). Similarly, delivery of siRNAs to mice, prior to or concomitant with viral challenge, provides protection against infection with various viruses (Ge *et al*, 2003; Giladi *et al*, 2003; McCaffrey *et al*, 2003; Tompkins *et al*, 2004; Bitko *et al*, 2005; Tan *et al*, 2007; Shah & Schaffer, 2011). RNA viruses engineered to contain perfectly complementary target sites for cellular miRNAs are restricted in cells or tissues expressing the cognate miRNAs (Cawood *et al*, 2009; Perez *et al*, 2009; Kelly *et al*, 2010; Langlois *et al*, 2013). Finally, in IFN-defective somatic cells, introduction of long dsRNA conferred sequence-specific protection from viral challenge dependent on the "slicing" activity of Ago2 (Maillard *et al*, 2016). Together, these studies show that, if siRNAs are provided directly (bypassing Dicer processing) or are generated from substrates in the absence of an IFN response (shRNAs or dsRNAs in IFN-defective cells), RISC can access and target viral RNAs to limit viral accumulation. As such, much of the current debate on the role of antiviral RNAi in mammalian cells ultimately centres on the question of whether, during a viral infection, viRNAs are ever produced in sufficient amounts to engage the latent antiviral activity of RISC and exert an antiviral effect.

viRNA production and antiviral RNAi in mammalian cells

viRNAs have key features: (i) a discrete length of ~22 nt, (ii) extremities with 3′ 2nt overhangs and (iii) a strand derived from the positive (+)-sense viral RNA and a complementary strand commonly derived from the negative (−)-sense viral RNA or, less frequently, from intramolecular base pairing of viral ssRNA. Initial attempts failed to detect viRNAs in mammalian cells infected with viruses (Pfeffer *et al*, 2005; Lin & Cullen, 2007), yet the recent emergence of high-throughput sequencing has allowed the question to be re-explored more thoroughly. Deep sequencing of differentiated mammalian cells infected with five mammalian viruses [HCV, dengue virus (DENV), WNV, poliovirus and vesicular stomatitis virus (VSV)] revealed the presence of small RNAs derived from the viral genome (viral small RNAs or vsRNAs) (Parameswaran *et al*, 2010). These vsRNAs, however, did not display size uniformity except in cells carrying a HCV replicon or infected with HCV virions (Parameswaran *et al*, 2010). Additional deep sequencing experiments of human cells infected with a range of viruses [WNV, DENV, Borna disease virus, IAV, Sindbis virus (SINV)] also reported the detection of vsRNAs but not specifically 22 nt long ones (Girardi *et al*, 2013; Backes *et al*, 2014; Bogerd *et al*, 2014). In addition, deep sequencing of RIG-I- and MDA5-deficient cells infected with SINV, YFV and the picornavirus coxsackie virus B3 did not reveal viRNA accumulation (Schuster *et al*, 2017) [although the inhibition of Dicer activity by LGP2 might have dampened the response (Van der Veen *et al*, 2018)]. Altogether, these experiments argue for limited Dicer-mediated generation of viRNAs in IFN-competent differentiated somatic cells.

In contrast, deep sequencing of mESCs infected with the picornavirus encephalomyocarditis virus (EMCV) revealed the accumulation of viral reads with a specific peak at 21-23 nt and reads that mapped within the first 200-nt of the EMCV genome and, to a lesser extent, to the 3′end and that, importantly, derived in equal parts from the (+) strand and (−) strand (Maillard *et al*, 2013). The sequences formed perfectly paired duplexes with 3′ 2-nt overhangs and were produced in a phase pattern indicative of successive cleavage by Dicer. Finally, the duplexes could be shown to associate with Ago2 and require Dicer for their generation, thereby fulfilling all criteria for *bona fide* viRNAs (Maillard *et al*, 2013). Interestingly, production of these viRNAs by mESCs was greatly reduced upon cell differentiation (Maillard *et al*, 2013), in line with the aforementioned studies reporting little viRNA generation in differentiated somatic cells.

Thus, in some IFN-deficient cells, including ESCs, infection with viruses allows for viRNA production. Can it elicit a protective RNAi-dependent response? Early work suggested that knockdown of Dicer in Vero cells, an African green monkey cell line that lacks IFN-α and IFN-β genes (Diaz *et al*, 1988), causes a modest increase in virus production upon IAV infection (Matskevich & Moelling, 2007). In contrast, later reports found that absence of Dicer in HEK 293T cells and/or in mouse embryonic fibroblasts did not impact the accumulation of flaviviruses (DENV, WNV, YFV), alphaviruses (SINV, Venezuelan equine encephalitis virus [VEEV]), IAV, measles virus, HIV and reovirus (Shapiro *et al*, 2010; Bogerd *et al*, 2014). Similarly, in engineered cells overexpressing an artificial Dicer that lacks the helicase domain, infection with IAV or poliovirus led to low-level accumulation of viRNAs, which were loaded onto RISC but had little impact on replication (Kennedy *et al*, 2015). Finally,

expression of a slicing-deficient Ago2 mutant in *Ifnar1$^{-/-}$* mouse embryonic fibroblasts did not impact infection with Semliki Forest virus (SFV), reovirus or IAV (Maillard *et al*, 2016). The overall message from those studies is that, even in a context permissive for dsRNAi, viRNA production from viral replication intermediates is too weak to inhibit viral infection. Replication of (+)-sense RNA viruses often occurs within membranous structures (replication factories), whereas RNAs generated during replication of (−)-sense RNA viruses rapidly associate with nucleocapsid proteins (Conzelmann, 1998; Romero-Brey & Bartenschlager, 2014). In addition, the 5′ extremities of certain viral genomes (and replication intermediates) can display various modifications, including a 7-methylguanosine (Cap) structure, a covalently linked protein (e.g. Vpg, viral protein genome-linked), highly structured regions or 2–3 phosphates (Fig 2). Whether these features prevent efficient access of Dicer to viral RNA is unknown although it is worth remembering they do not prevent antiviral RNAi in insect cells. A key issue might therefore be antagonism of RNAi by VSRs. This will be discussed in the next section.

VSRs in mammalian viruses

Several proteins encoded by mammalian viruses display VSR activity (1; 2). Expression of influenza virus NS1, vaccinia virus E3L, reovirus σ3 and Nodamura virus (NoV) B2 proteins inhibits RNAi in plants and/or insect cells (Lichner *et al*, 2003; Bucher *et al*, 2004; Delgadillo *et al*, 2004; Li *et al*, 2004). In mammalian cells, a plethora of mammalian virus-encoded VSRs, including primate foamy virus type 1 (PFV-1) Tas, NoV B2, HCV core, IAV NS1, HIV Tat, Ebola VP35, VP30 and VP40, CoV N, SARS-CoV 7a, YFV capsid, DENV NS4B, human enterovirus 71 (HEV 71) 3A and adenovirus virus-associated RNA I (VA1), can reduce shRNA/siRNA-mediated knockdown of reporter genes (Table 1) (Lu & Cullen, 2004; Andersson *et al*, 2005; Lecellier *et al*, 2005; Sullivan & Ganem, 2005; Wang *et al*, 2006b; Haasnoot *et al*, 2007; Chen *et al*, 2008; de Vries *et al*, 2009; Karjee *et al*, 2010; Fabozzi *et al*, 2011; Kakumani *et al*, 2013; Cui *et al*, 2015; Samuel *et al*, 2016; Qiu *et al*, 2017). Most viral proteins identified thus far that display VSR activity share the ability to bind dsRNA and mutations that affect their dsRNA-binding domain block VSR activity, arguing that their principal mode of action is sequestration of dsRNA from Dicer (Table 1). As dsRNA is a potent inducer of the IFN pathway, most of these VSRs also act as IFN antagonists (García-Sastre, 2017). It is therefore unclear whether these viral proteins specifically evolved to block RNAi or whether their VSR activity is a byproduct of their role as IFN antagonists (Cullen, 2006). However, some VSRs may function through mechanisms other than dsRNA sequestration (Kakumani *et al*, 2013), including binding to components of the RNAi pathway: e.g. Ebola virus VP35 and VP30 proteins interact with Dicer co-factors TRBP and PACT, while HCV core associates with Dicer (Table 1) (Wang *et al*, 2006b; Chen *et al*, 2008; Fabozzi *et al*, 2011). Whether these interactions contribute to VSR activity is unclear. Finally, adenovirus VA1s are small, highly structured RNAs that inhibit shRNA-mediated RNAi by acting as decoy substrates for Dicer, RISC and exportin 5 (required for nuclear export of pre-miRNAs and shRNAs) (Lu & Cullen, 2004; Andersson *et al*, 2005).

Despite the evidence that many viral proteins from mammalian viruses can act as VSRs in overexpression (i.e. gain-of-function) studies, there are relatively few loss-of-function studies that show

that they actively suppress mammalian antiviral RNAi defence. Persuasive experiments have been done with NoV, a member of the *Nodavirus* family. Nodaviruses express B2 proteins, which bind long dsRNA and siRNAs *in vitro* and associate with replication intermediates and viRNAs in infected cells (Chao *et al*, 2005; Lu *et al*, 2005; Sullivan & Ganem, 2005; Aliyari *et al*, 2008). B2 proteins act as potent VSRs in insect cells (Wang *et al*, 2006a; Aliyari *et al*, 2008). Notably, B2-deficient NoV (NoV ΔB2) also replicates less efficiently than parental NoV in mESCs but its accumulation is rescued in mESCs lacking all Ago genes (Maillard *et al*, 2013). In suckling mice, NoV ΔB2 is highly attenuated and induces accumulation of viRNAs (Li *et al*, 2013). viRNAs are also detected, although to a lesser degree, upon infection of somatic cells (BHK-21) with NoV ΔB2, but not with NoV WT (Li *et al*, 2013). Altogether, these data suggest that the ability of Dicer to process NoV replication intermediates is actively antagonised by the B2 protein.

The NS1 protein from IAV also displays VSR activity when expressed in plants and insect cells (Bucher *et al*, 2004; Delgadillo *et al*, 2004; Li *et al*, 2004). In mammalian cells, NS1 is ineffective against RISC-loaded siRNAs (Kok & Jin, 2006; Haasnoot *et al*, 2007; de Vries *et al*, 2009; Kennedy *et al*, 2015) but infection of human and African green monkey cells with IAV ΔNS1 but not IAV WT yields readily detectable levels of canonical viRNAs derived from the termini of both strands of the eight viral RNA segments (Li *et al*, 2016; Tsai *et al*, 2018). It has been reported that IAV ΔNS1, and to a lesser extent parental wild-type IAV, VSV and EMCV, replicates more extensively in mouse embryonic fibroblasts expressing a slicing-deficient Ago2 mutant (Li *et al*, 2016). However, in other studies, loss of RNAi components did not cause an increase in replication of IAV ΔNS1 (Maillard *et al*, 2016; Tsai *et al*, 2018). Interestingly, IAV engineered to express an shRNA or a miRNA targeting a viral gene or a reporter gene integrated in the viral genome, respectively, was attenuated compared to non-targeting controls (Benitez *et al*, 2015). This restriction was Dicer-dependent but independent of NS1 suggesting that, in the context of an infection, NS1′s VSR activity inhibits viRNA production from genome segments but not from short dsRNA hairpins (Benitez *et al*, 2015; Li *et al*, 2016; Tsai *et al*, 2018).

Finally, the HEV71-encoded protein 3A inhibits shRNA-mediated silencing in mammalian cells, as well as antiviral RNAi in insect cells, and suppresses Dicer-mediated biogenesis of siRNAs by binding and sequestering long dsRNA *in vitro* (Qiu *et al*, 2017). A point mutation that inactivates 3A's VSR activity reduces viral replication in somatic cells and in suckling mice. Concomitantly, canonical viRNAs derived from both strands of the 5′terminal region of the HEV71 genome are produced, loaded into RISC and able to silence a reporter bearing complementary sites (Qiu *et al*, 2017). Interestingly, the absence of Dicer increases HEV71 accumulation in infected cells despite the presence of an intact IFN pathway, suggesting that, in this case, antiviral RNAi could function irrespective of the IFN system (Qiu *et al*, 2017).

Niches for antiviral RNAi?

The generally observed antagonism between the IFN response and dsRNAi suggests that antiviral RNAi may be especially important in cellular niches in which the induction of or the response to IFN is limited. One of those niches might be stem cells. Pluripotent stem cells do not produce IFN upon viral infection or exposure to

Table 1.   List of mammalian virus-encoded proteins with VSR activity.

Viral genome	Virus name	Virus family	VSR	Properties	Proposed mode of action	References
(+)-ssRNA	Coronavirus (CoV)	Coronaviridae	N	dsRNA binding	dsRNA sequestration	Cui et al (2015)
	Severe acute respiratory syndrome coronavirus (SARS-CoV)	Coronaviridae	7a	–	–	Karjee et al (2010)
	Dengue virus (DENV)	Flaviviridae	NS4B	lack of dsRNA binding	inhibition of Dicer activity	Kakumani et al (2013)
	Hepatitis C virus (HCV)	Flaviviridae	capsid	Dicer binding	inhibition of Dicer activity	Wang et al (2006b), Chen et al (2008)
	Yellow Fever virus (YFV)	Flaviviridae	capsid[a]	dsRNA binding	dsRNA sequestration	Samuel et al (2016)
	Human enterovirus 71 (HEV71)	Picornaviridae	3A	dsRNA binding	dsRNA sequestration	Qiu et al (2017)
	Human immunodeficiency virus 1 (HIV-1)	Retroviridae	Tat	dsRNA binding	–	Bennasser et al (2005), Triboulet et al (2007), Lin and Cullen (2007)[b], Sanghvi & Steel (2011)[b]
	Primate foamy virus type 1 (PFV-1)	Retroviridae	Tas[a]	–	–	Lecellier et al (2005)
	Nodamura virus (NoV)	Nodaviridae	B2	dsRNA binding	dsRNA sequestration	Sullivan and Ganem (2005), Aliyari et al (2008), Li et al (2013), Maillard et al (2013)
(−)-ssRNA	Ebolavirus	Filoviridae	VP30	Dicer and TRBP binding	inhibition of Dicer activity	Fabozzi et al (2011)
			VP35	PACT, TRBP, dsRNA binding	inhibition of Dicer activity	Haasnoot et al (2007), Fabozzi et al (2011)
			VP40	–	–	Fabozzi et al (2011)
	Marburg virus	Filoviridae	VP35	dsRNA binding	–	Li et al (2016)
	Influenza virus	Orthomyxoviridae	NS1	dsRNA binding	dsRNA sequestration	Li et al (2004), Bucher et al (2004), Delgadillo et al (2004), Kok and Jin (2006)[b], de Vries et al (2009), Kennedy et al (2015), Benitez et al (2015)[b], Li et al (2016), Tsai et al (2018)
			NP	–	–	Kennedy et al (2015)
	La Crosse virus	Peribunyaviridae	NSs	–	–	Soldan et al (2004)
dsRNA	Reovirus	Reoviridae	σ3[a]	dsRNA binding	dsRNA sequestration	Lichner et al (2003)
dsDNA	Adenovirus	Adenoviridae	VA I, VA II	Dicer binding	Dicer sequestration by acting as decoy RNAs	Lu and Cullen (2004), Andersson et al (2005)
	Vaccinia virus	Poxviridae	E3L	dsRNA binding	dsRNA sequestration	Li et al (2004), Haasnoot et al (2007)

[a]VSR activity shown only in non-mammalian hosts.
[b]Studies questioning VSR activity.

poly(I:C) and respond poorly to IFN treatment (Chen *et al*, 2010; Hong & Carmichael, 2013; Wang *et al*, 2013; Guo *et al*, 2015; D'Angelo *et al*, 2016). It is unclear why pluripotent stem cells are refractory to IFN but it may have to do with the fact that self-renewal is incompatible with the anti-proliferative effects and pro-apoptotic effects of the cytokines (Hertzog *et al*, 1994; de Veer *et al*, 2001). Furthermore, artificial induction of an IFN response in engineered

pluripotent cells compromises differentiation potential (Eggenberger *et al*, 2019). Thus, pluripotent stem cells may be forced to rely on IFN-independent mechanisms to combat virus infections. These may include the ability to constitutively express some ISGs that confer an efficient and permanent antiviral state (Wu *et al*, 2018). In this scenario, antiviral RNAi would constitute an additional mechanism to protect the integrity and function of tissue stem cells in the face of

virus infection and thereby contribute to tissue maintenance, repair and regeneration (Xia *et al*, 2018). Notably, the ability of a virus to infect stem cells might not be needed for its propagation and, therefore, stem cell-intrinsic antiviral RNAi would benefit the host but not impact on virus transmission.

Future directions in antiviral RNAi

In line with other facets of immunity, it is likely that antiviral RNAi is highly tuneable and that it operates in conjunction with multiple other mechanisms of defence. Further studies are clearly needed to disentangle the complex web that regulates dsRNAi in mammals and to understand its ability to act as a cell-intrinsic mechanism of antiviral defence. Open questions include what are the cellular factors regulating the activity of Dicer on long dsRNA? Apart from murine germ cells, are there similar truncated Dicer isoforms expressed in other cell types and/or other species? Which viral proteins act as *bona fide* VSRs in the context of an infection? What are the cell types in which antiviral RNAi is active? Does antiviral RNAi directly impact on viral accumulation upon infection *in vivo*? These and other questions are likely to enliven the debate on the role of RNAi in mammalian defence from virus attack for years to come.

Note added in proof

While this review was in production, a study reported that Zika virus (ZIKV) infection resulted in the production of canonical viRNAs in human neural progenitor cells (hNPCs), the major target cells of ZIKV, but not in neurons differentiated from hNPCs (Xu et al, 2019). This study supports the notion that RNAi can play an antiviral role in certain progenitor cells, including those involved in brain development.

References

Aliyari R, Wu Q, Li HW, Wang XH, Li F, Green LD, Han CS, Li WX, Ding SW (2008) Mechanism of induction and suppression of antiviral immunity directed by virus-derived small RNAs in *Drosophila*. *Cell Host Microbe* 4: 387–397

Andersson MG, Haasnoot PCJ, Xu N, Berenjian S, Berkhout B, Akusjarvi G (2005) Suppression of RNA interference by adenovirus virus-associated RNA. *J Virol* 79: 9556–9565

Backes S, Langlois RA, Schmid S, Varble A, Shim JV, Sachs D, tenOever BR (2014) The Mammalian response to virus infection is independent of small RNA silencing. *Cell Rep* 8: 114–125

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116: 281–297

Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. *Cell* 136: 215–233

Baum A, Sachidanandam R, García-Sastre A (2010) Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. *Proc Natl Acad Sci USA* 107: 16303–16308

Benitez AA, Spanko LA, Bouhaddou M, Sachs D, tenOever BR (2015) Engineered Mammalian RNAi can elicit antiviral protection that negates the requirement for the interferon response. *Cell Rep* 13: 1456–1466

Bennasser Y, Le S-Y, Benkirane M, Jeang K-T (2005) Evidence that HIV-1 Encodes an siRNA and a Suppressor of RNA Silencing. *Immunity* 22: 607–619

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409: 363–366

Billy E, Brondani V, Zhang H, Muller U, Filipowicz W (2001) Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. *Proc Natl Acad Sci USA* 98: 14428–14433

Bitko V, Musiyenko A, Shulyayeva O, Barik S (2005) Inhibition of respiratory viruses by nasally administered siRNA. *Nat Med* 11: 50–55

Bogerd HP, Skalsky RL, Kennedy EM, Furuse Y, Whisnant AW, Flores O, Schultz KLW, Putnam N, Barrows NJ, Sherry B, Scholle F, Garcia-Blanco MA, Griffin DE, Cullen BR (2014) Replication of many human viruses is refractory to inhibition by endogenous cellular microRNAs. *J Virol* 88: 8065–8076

Bousarghin L, Touze A, Gaud G, Iochmann S, Alvarez E, Reverdiau P, Gaitan J, Jourdan ML, Sizaret PY, Coursaget PL (2009) Inhibition of cervical cancer cell growth by human papillomavirus virus-like particles packaged with human papillomavirus oncoprotein short hairpin RNAs. *Mol Cancer Ther* 8: 357–365

Bronkhorst AW, van Rij RP (2014) The long and short of antiviral defense: small RNA-based immunity in insects. *Curr Opin Virol* 7: 19–28

Brummelkamp TR, Bernards R, Agami R (2002) A system for stable expression of short interfering RNAs in mammalian cells. *Science* 296: 550–553

Bruns AM, Horvath CM (2014) Antiviral RNA recognition and assembly by RLR family innate immune sensors. *Cytokine Growth Factor Rev* 25: 507–512

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: 771–781

Bucher E, Hemmes H, de Haan P, Goldbach R, Prins M (2004) The influenza A virus NS1 protein binds small interfering RNAs and suppresses RNA silencing in plants. *J Gen Virol* 85: 983–991

Burke DC, Graham CF, Lehman JM (1978) Appearance of interferon inducibility and sensitivity during differentiation of murine teratocarcinoma cells in vitro. *Cell* 13: 243–248

Cantini LP, Andino LM, Attaway CC, Butler B, Dumitriu A, Blackshaw A, Jakymiw A (2014) Identification and characterization of Dicer1e, a Dicer1 protein variant, in oral cancer cells. *Mol Cancer* 13: 190

Caplen NJ, Fleenor J, Fire A, Morgan RA (2000) dsRNA-mediated gene silencing in cultured *Drosophila* cells: a tissue culture model for the analysis of RNA interference. *Gene* 252: 95–105

Caplen NJ, Parrish S, Imani F, Fire A, Morgan RA (2001) Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. *Proc Natl Acad Sci USA* 98: 9742–9747

Cawood R, Chen HH, Carroll F, Bazan-Peregrino M, Van Rooijen N, Seymour LW (2009) Use of tissue-specific MicroRNA to control pathology of wild-type adenovirus without attenuation of its ability to kill cancer cells. *PLoS Pathog* 5: e1000440–10

Chakravarthy S, Sternberg SH, Kellenberger CA, Doudna JA (2010) Substrate-specific kinetics of Dicer-catalyzed RNA processing. *J Mol Biol* 404: 392–402

Chao JA, Lee JH, Chapados BR, Debler EW, Schneemann A, Williamson JR (2005) Dual modes of RNA-silencing suppression by Flock House virus protein B2. *Nat Struct Mol Biol* 12: 952–957

Chen W, Zhang Z, Chen J, Zhang J, Zhang J, Wu Y, Huang Y, Cai X, Huang A (2008) HCV core protein interacts with Dicer to antagonize RNA silencing. *Virus Res* 133: 250–258

Chen L-L, Yang L, Carmichael GG (2010) Molecular basis for an attenuated cytoplasmic dsRNA response in human embryonic stem cells. *Cell Cycle* 9: 3552–3564

Conzelmann KK (1998) Nonsegmented negative-strand RNA viruses: genetics and manipulation of viral genomes. *Annu Rev Genet* 32: 123–162

Csorba T, Kontra L, Burgyán J (2015) viral silencing suppressors: Tools forged to. *Virology* 479–480: 85–103

Cui L, Wang H, Ji Y, Yang J, Xu S, Huang X, Wang Z, Qin L, Tien P, Zhou X, Guo D, Chen Y (2015) the nucleocapsid protein of coronaviruses acts as a viral suppressor of RNA silencing in mammalian cells. *J Virol* 89: 9029–9043

Cullen BR (2006) Is RNA interference involved in intrinsic antiviral immunity in mammals? *Nat Immunol* 7: 563–567

Cullen BR, Cherry S, tenOever BR (2013) Is RNA interference a physiologically relevant innate antiviral immune response in mammals? *Cell Host Microbe* 14: 374–378

Cullen BR (2014) Viruses and RNA interference: issues and controversies. *J Virol* 88: 12934–12936

D'Angelo W, Acharya D, Wang R, Wang J, Gurung C, Chen B, Bai F, Guo Y-L (2016) Development of antiviral innate immunity during in vitro differentiation of mouse embryonic stem cells. *Stem Cells Dev* 25: 648–659

Dalmay T, Horsefield R, Braunstein TH, Baulcombe DC (2001) SDE3 encodes an RNA helicase required for post-transcriptional gene silencing in *Arabidopsis*. *EMBO J* 20: 2069–2078

Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, Voinnet O (2006) Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. *Science* 313: 68–71

Delgadillo MO, Sáenz P, Salvador B, García JA, Simón-Mateo C (2004) Human influenza virus NS1 protein enhances viral pathogenicity and acts as an RNA silencing suppressor in plants. *J Gen Virol* 85: 993–999

Diaz MO, Ziemin S, Le Beau MM, Pitha P, Smith SD, Chilcote RR, Rowley JD (1988) Homozygous deletion of the alpha- and beta 1-interferon genes in human leukemia and derived cell lines. *Proc Natl Acad Sci USA* 85: 5259–5263

Ding SW, Voinnet O (2007) Antiviral immunity directed by small RNAs. *Cell* 130: 413–426

Ding SW (2010) RNA-based antiviral immunity. *Nat Rev Immunol* 10: 632–644

Ding SW, Voinnet O (2014) Antiviral RNA silencing in mammals: no news is not good news. *Cell Rep* 9: 795–797

Eggenberger J, Blanco-Melo D, Panis M, Brennand KJ, tenOever BR (2019) Type I interferon response impairs differentiation potential of pluripotent stem cells. *Proc Natl Acad Sci USA* 172: 201812449–10

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001a) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411: 494–498

Elbashir SM, Martinez J, Patkaniowska A, Lendeckel W, Tuschl T (2001b) Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J* 20: 6877–6888

Fabozzi G, Nabel CS, Dolan MA, Sullivan NJ (2011) Ebolavirus proteins suppress the effects of small interfering RNA by direct interaction with the mammalian RNA interference pathway. *J Virol* 85: 2512–2523

Félix M-A, Ashe A, Piffaretti J, Wu G, Nuez I, Bélicard T, Jiang Y, Zhao G, Franz CJ, Goldstein LD, Sanroman M, Miska EA, Wang D (2011) Natural and experimental infection of *Caenorhabditis* nematodes by novel viruses related to nodaviruses. *PLoS Biol* 9: e1000586

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: 1187–1196

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806–811

Flemr M, Malik R, Franke V, Nejepinska J, Sedlacek R, Vlahovicek K, Svoboda P (2013) A retrotransposon-driven dicer isoform directs endogenous small interfering RNA production in mouse oocytes. *Cell* 155: 807–816

Francis MK, Lehman JM (1989) Control of beta-interferon expression in murine embryonal carcinoma F9 cells. *Mol Cell Biol* 9: 3553–3556

Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL (2006) Essential function in vivo for Dicer-2 in host defense against RNA viruses in *Drosophila*. *Nat Immunol* 7: 590–597

García-Sastre A (2017) Ten strategies of interferon evasion by viruses. *Cell Host Microbe* 22: 176–184

Ge Q, McManus MT, Nguyen T, Shen C-H, Sharp PA, Eisen HN, Chen J (2003) RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. *Proc Natl Acad Sci USA* 100: 2718–2723

Giladi H, Ketzinel-Gilad M, Rivkin L, Felig Y, Nussbaum O, Galun E (2003) Small interfering RNA inhibits hepatitis B virus replication in mice. *Mol Ther* 8: 769–776

Girardi E, Chane-Woon-Ming B, Messmer M, Kaukinen P, Pfeffer S (2013) Identification of RNase L-dependent, 3′-end-modified, viral small RNAs in sindbis virus-infected mammalian cells. *MBio* 4: e00698–13.

Girardi E, Lefèvre M, Chane-Woon-Ming B, Paro S, Claydon B, Imler J-L, Meignin C, Pfeffer S (2015) Cross-species comparative analysis of Dicer proteins during Sindbis virus infection. *Sci Rep* 5: 10693–12

Gitlin L, Karelsky S, Andino R (2002) Short interfering RNA confers intracellular antiviral immunity in human cells. *Nature* 418: 430–434

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 USA* 103: 8459–8464

Goubau D, Deddouche S, Reis e Sousa C (2013) Cytosolic sensing of viruses. *Immunity* 38: 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 59-diphosphates. *Nature* 514: 372–375

Guo Y-L, Carmichael GG, Wang R, Hong X, Acharya D, Huang F, Bai F (2015) Attenuated innate immunity in embryonic stem cells and its implications in developmental biology and regenerative medicine. *Stem Cells* 33: 3165–3173

Haasnoot J, de Vries W, Geutjes E-J, Prins M, de Haan P, Berkhout B (2007) The Ebola virus VP35 protein is a suppressor of RNA silencing. *PLoS Pathog* 3: e86

Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286: 950–952

Hammond SM, Bernstein E, Beach D, Hannon GJ (2000) An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404: 293–296

Hertzog PJ, Hwang SY, Kola I (1994) Role of interferons in the regulation of cell proliferation, differentiation, and development. *Mol Reprod Dev* 39: 226–232

Hinkal GW, Grelier G, Puisieux A, Moyret-Lalle C (2011) Complexity in the regulation of Dicer expression: Dicer variant proteins are differentially expressed in epithelial and mesenchymal breast cancer cells and decreased during EMT. *Br J Cancer* 104: 387–388

Ho T, Pallett D, Rusholme R, Dalmay T, Wang H (2006) A simplified method for cloning of short interfering RNAs from *Brassica juncea* infected with *Turnip mosaic* potyvirus and *Turnip crinkle* carmovirus. *J Virol Methods* 136: 217–223

Hong XX, Carmichael GG (2013) Innate immunity in pluripotent human cells: attenuated response to interferon-beta. *J Biol Chem* 288: 16196–16205

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: 994–997

Husain B, Mukerji I, Cole JL (2012) Analysis of high-affinity binding of protein kinase R to double-stranded RNA. *Biochemistry* 51: 8764–8770

Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. *Science* 327: 291–295

Jacque J-M, Triques K, Stevenson M (2002) Modulation of HIV-1 replication by RNA interference. *Nature* 418: 435–438

Jeffrey KL, Li Y, Ding SW (2017) Reply to "questioning antiviral RNAi in mammals". *Nat Microbiol* 2: 17053

Jonas S, Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. *Nat Rev Genet* 16: 421–433

Kakumani PK, Ponia SS, Rajgokul KS, Sood V, Chinnappan M, Banerjea AC, Medigeshi GR, Malhotra P, Mukherjee SK, Bhatnagar RK (2013) Role of RNA interference (RNAi) in dengue virus replication and identification of NS4B as an RNAi suppressor. *J Virol* 87: 8870–8883

Kapadia SB, Brideau-Andersen A, Chisari FV (2003) Interference of hepatitis C virus RNA replication by short interfering RNAs. *Proc Natl Acad Sci USA* 100: 2014–2018

Karjee S, Minhas A, Sood V, Ponia SS, Banerjea AC, Chow VTK, Mukherjee SK, Lal SK (2010) The 7a accessory protein of severe acute respiratory syndrome coronavirus acts as an RNA silencing suppressor. *J Virol* 84: 10395–10401

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: 101–105

Kelly EJ, Nace R, Barber GN, Russell SJ (2010) Attenuation of vesicular stomatitis virus encephalitis through microRNA targeting. *J Virol* 84: 1550–1562

Kemp C, Imler J-L (2009) Antiviral immunity in *Drosophila*. *Curr Opin Immunol* 21: 3–9

Kennedy EM, Whisnant AW, Kornepati AVR, Marshall JB, Bogerd HP, Cullen BR (2015) Production of functional small interfering RNAs by an amino-terminal deletion mutant of human Dicer. *Proc Natl Acad Sci USA* 112: E6945–E6954

Kennerdell JR, Carthew RW (1998) Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* 95: 1017–1026

Kok K-H, Jin D-Y (2006) Influenza A virus NS1 protein does not suppress RNA interference in mammalian cells. *J Gen Virol* 87: 2639–2644

Komuro A, Homma Y, Negoro T, Barber GN, Horvath CM (2016) The TAR-RNA binding protein is required for immunoresponses triggered by Cardiovirus infection. *Biochem Biophys Res Commun* 480: 187–193

Konishi M, Wu CH, Wu GY (2003) Inhibition of HBV replication by siRNA in a stable HBV-producing cell line. *Hepatology* 38: 842–850

Kumar P, Lee SK, Shankar P, Manjunath N (2006) A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. *PLoS Med* 3: e96–10

Langlois RA, Albrecht RA, Kimble B, Sutton T, Shapiro JS, Finch C, Angel M, Chua MA, Gonzalez-Reiche AS, Xu K, Perez D, Garcia-Sastre A, tenOever BR (2013) MicroRNA-based strategy to mitigate the risk of gain-of-function influenza studies. *Nat Biotechnol* 31: 844–847

Lecellier CH, Dunoyer P, Arar K, Lehmann-Che J, Eyquem S, Himber C, Saib A, Voinnet O (2005) A cellular microRNA mediates antiviral defense in human cells. *Science* 308: 557–560

Lee NS, Dohjima T, Bauer G, Li H, Li M-J, Ehsani A, Salvaterra P, Rossi J (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nat Biotechnol* 20: 500–505

Lee YS, Nakahara K, Pham JW, Kim K, He Z, Sontheimer EJ, Carthew RW (2004) Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* 117: 69–81

Lee Y, Hur I, Park SY, Kim YK, Suh MR, Kim VN (2006) The role of PACT in the RNA silencing pathway. *EMBO J* 25: 522–532

Li H, Li WX, Ding SW (2002) Induction and suppression of RNA silencing by an animal virus. *Science* 296: 1319–1321

Li WX, Li H, Lu R, Li F, Dus M, Atkinson P, Brydon EW, Johnson KL, Garcia-Sastre A, Ball LA, Palese P, Ding SW (2004) Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. *Proc Natl Acad Sci USA* 101: 1350–1355

Li Y, Lu J, Han Y, Fan X, Ding SW (2013) RNA interference functions as an antiviral immunity mechanism in mammals. *Science* 342: 231–234

Li Y, Basavappa M, Lu J, Dong S, Cronkite DA, Prior JT, Reinecker H-C, Hertzog P, Han Y, Li W-X, Cheloufi S, Karginov FV, Ding SW, Jeffrey KL (2016) Induction and suppression of antiviral RNA interference by influenza A virus in mammalian cells. *Nat Microbiol* 2: 16250

Lichner Z, Silhavy D, Burgyan J (2003) Double-stranded RNA-binding proteins could suppress RNA interference-mediated antiviral defences. *J Gen Virol* 84: 975–980

Lin J, Cullen BR (2007) Analysis of the interaction of primate retroviruses with the human RNA interference machinery. *J Virol* 81: 12218–12226

Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song J-J, Hammond SM, Joshua-Tor L, Hannon GJ (2004) Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 305: 1437–1441

Liu Z, Wang J, Cheng H, Ke X, Sun L, Zhang QC, Wang H-W (2018) Cryo-EM structure of human dicer and its complexes with a pre-miRNA substrate. *Cell* 173: 1191–1203.e12

Lu S, Cullen BR (2004) Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and MicroRNA biogenesis. *J Virol* 78: 12868–12876

Lu W-W, Hsu Y-Y, Yang J-Y, Kung S-H (2004) Selective inhibition of enterovirus 71 replication by short hairpin RNAs. *Biochem Biophys Res Commun* 325: 494–499

Lu R, Maduro M, Li F, Li HW, Broitman-Maduro G, Li WX, Ding SW (2005) Animal virus replication and RNAi-mediated antiviral silencing in *Caenorhabditis elegans*. *Nature* 436: 1040–1043

Ma E, MacRae IJ, Kirsch JF, Doudna JA (2008) Autoinhibition of human dicer by its internal helicase domain. *J Mol Biol* 380: 237–243

MacRae IJ, Ma E, Zhou M, Robinson CV, Doudna JA (2008) In vitro reconstitution of the human RISC-loading complex. *Proc Natl Acad Sci USA* 105: 512–517

Maillard PV, Ciaudo C, Marchais A, Li Y, Jay F, Ding SW, Voinnet O (2013) Antiviral RNA interference in mammalian cells. *Science* 342: 235–238

Maillard PV, Van der Veen AG, Deddouche Grass S, Rogers NC, Merits A, Reis e Sousa C (2016) Inactivation of the type I interferon pathway reveals long double-stranded RNA-mediated RNA interference in mammalian cells. *EMBO J* 35: 2505–2518

Matskevich AA, Moelling K (2007) Dicer is involved in protection against influenza A virus infection. *J Gen Virol* 88: 2627–2635

McCaffrey AP, Nakai H, Pandey K, Huang Z, Salazar FH, Xu H, Wieland SF, Marion PL, Kay MA (2003) Inhibition of hepatitis B virus in mice by RNA interference. *Nat Biotechnol* 21: 639–644

Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol Cell* 15: 185–197

Molnar A, Csorba T, Lakatos L, Varallyay E, Lacomme C, Burgyan J (2005) Plant virus-derived small interfering RNAs originate predominantly from highly structured single-stranded viral RNAs. *J Virol* 79: 7812–7818

Mourrain P, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Jouette D, Lacombe AM, Nikic S, Picault N, Rémoué K, Sanial M, Vo TA, Vaucheret H (2000) *Arabidopsis* SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* 101: 533–542

Ngô H, Tschudi C, Gull K, Ullu E (1998) Double-stranded RNA induces mRNA degradation in Trypanosoma brucei. *Proc Natl Acad Sci USA* 95: 14687–14692

Ota H, Sakurai M, Gupta R, Valente L, Wulff BE, Ariyoshi K, Iizasa H, Davuluri RV, Nishikura K (2013) ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. *Cell* 153: 575–589

Paddison PJ, Caudy AA, Hannon GJ (2002a) Stable suppression of gene expression by RNAi in mammalian cells. *Proc Natl Acad Sci USA* 99: 1443–1448

Paddison PJ, Caudy AA, Bernstein E, Hannon GJ, Conklin DS (2002b) Short hairpin RNAs (shRNAs) induce sequence-specific silencing in mammalian cells. *Genes Dev* 16: 948–958

Parameswaran P, Sklan E, Wilkins C, Burgon T, Samuel MA, Lu R, Ansel KM, Heissmeyer V, Einav S, Jackson W, Doukas T, Paranjape S, Polacek C, dos Santos FB, Jalili R, Babrzadeh F, Gharizadeh B, Grimm D, Kay M, Koike S, *et al* (2010) Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems. *PLoS Pathog* 6: e1000764

Parisien JP, Lenoir JJ, Mandhana R, Rodriguez KR, Qian K, Bruns AM, Horvath CM (2018) RNA sensor LGP2 inhibits TRAF ubiquitin ligase to negatively regulate innate immune signaling. *EMBO Rep* 19: e45176–14

Perez JT, Pham AM, Lorini MH, Chua MA, Steel J, tenOever BR (2009) MicroRNA-mediated species-specific attenuation of influenza A virus. *Nat Biotechnol* 27: 572–576

Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien M, Russo JJ, Ju J, Randall G, Lindenbach BD, Rice CM, Simon V, Ho DD, Zavolan M, Tuschl T (2005) Identification of microRNAs of the herpesvirus family. *Nat Methods* 2: 269–276

Phipps KM, Martinez A, Lu J, Heinz BA, Zhao G (2004) Small interfering RNA molecules as potential anti-human rhinovirus agents: in vitro potency, specificity, and mechanism. *Antiviral Res* 61: 49–55

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: 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: 10761–10769

Pindel A, Sadler A (2011) The role of protein kinase R in the interferon response. *J Interferon Cytokine Res* 31: 59–70

Potenza N, Papa U, Scaruffi P, Mosca N, Tonini GP, Russo A (2010) A novel splice variant of the human dicer gene is expressed in neuroblastoma cells. *FEBS Lett* 584: 3452–3457

Provost P, Dishart D, Doucet J, Frendewey D, Samuelsson B, Rådmark O (2002) Ribonuclease activity and RNA binding of recombinant human Dicer. *EMBO J* 21: 5864–5874

Pumplin N, Voinnet O (2013) RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. *Nat Rev Micro* 11: 745–760

Qiu Y, Xu Y, Zhang Y, Zhou H, Deng Y-Q, Li X-F, Miao M, Zhang Q, Zhong B, Hu Y, Zhang F-C, Wu L, Qin C-F, Zhou X (2017) Human virus-derived small RNAs can confer antiviral immunity in mammals. *Immunity* 46: 992–1004.e5

Qureshi A, Tantray VG, Kirmani AR, Ahangar AG (2018) A review on current status of antiviral siRNA. *Rev Med Virol* 9: e1976–11

Randall G, Grakoui A, Rice CM (2003) Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. *Proc Natl Acad Sci USA* 100: 235–240

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: 397–408

van Rij RP, Saleh MC, Berry B, Foo C, Houk A, Antoniewski C, Andino R (2006) The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes Dev* 20: 2985–2995

Romero-Brey I, Bartenschlager R (2014) Membranous replication factories induced by plus-strand RNA viruses. *Viruses* 6: 2826–2857

Samuel GH, Wiley MR, Badawi A, Adelman ZN, Myles KM (2016) Yellow fever virus capsid protein is a potent suppressor of RNA silencing that binds double-stranded RNA. *Proc Natl Acad Sci USA* 113: 13863–13868

Sánchez Alvarado A, Newmark PA (1999) Double-stranded RNA specifically disrupts gene expression during planarian regeneration. *Proc Natl Acad Sci USA* 96: 5049–5054

Sanghvi VR, Steel LF (2011) A re-examination of global suppression of RNA interference by HIV-1. *PLoS One* 6: e17246

Sarkies P, Miska EA (2013) RNAi pathways in the recognition of foreign RNA: antiviral responses and host-parasite interactions in nematodes. *Biochem Soc Trans* 41: 876–880

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, *et al* (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: 25–34

Schlee M, Hartmann G (2016) Discriminating self from non-self in nucleic acid sensing. *Nat Rev Immunol* 16: 566–580

Schmidt A, Schwerd 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 USA* 106: 12067–12072

Schneider WM, Chevillotte MD, Rice CM (2014) Interferon-stimulated genes: a complex web of host defenses. *Annu Rev Immunol* 32: 513–545

Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM (2011) A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472: 481–485

Schott DH, Cureton DK, Whelan SP, Hunter CP (2005) An antiviral role for the RNA interference machinery in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 102: 18420–18424

Schuster S, Tholen LE, Overheul GJ, van Kuppeveld FJM, van Rij RP (2017) Deletion of cytoplasmic double-stranded rna sensors does not uncover viral small interfering RNA production in human cells. *mSphere* 2: e00333–17

Seo GJ, Kincaid RP, Phanaksri T, Burke JM, Pare JM, Cox JE, Hsiang T-Y, Krug RM, Sullivan CS (2013) Reciprocal inhibition between intracellular antiviral signaling and the RNAi machinery in mammalian cells. *Cell Host Microbe* 14: 435–445

Shah PS, Schaffer DV (2011) Antiviral RNAi: translating science towards therapeutic success. *Pharm Res* 28: 2966–2982

Shapiro JS, Varble A, Pham AM, tenOever BR (2010) Noncanonical cytoplasmic processing of viral microRNAs. *RNA* 16: 2068–2074

Sheu-Gruttadauria J, MacRae IJ (2017) Structural foundations of RNA silencing by argonaute. *J Mol Biol* 429: 2619–2639

Shi Y, Yang DH, Xiong J, Jia J, Huang B, Jin YX (2005) Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs. *Cell Res* 15: 193–200

Sim ACN, Luhur A, Tan TMC, Chow VTK, Poh CL (2005) RNA interference against Enterovirus 71 infection. *Virology* 341: 72–79

Soldan SS, Plassmeyer ML, Matukonis MK, Gonzalez-Scarano F (2004) La Crosse virus nonstructural protein NSs counteracts the effects of short interfering RNA. *J Virol* 79: 234–244

Stein P, Zeng F, Pan H, Schultz RM (2005) Absence of non-specific effects of RNA interference triggered by long double-stranded RNA in mouse oocytes. *Dev Biol* 286: 464–471

Sullivan CS, Ganem D (2005) A virus-encoded inhibitor that blocks RNA interference in mammalian cells. *J Virol* 79: 7371–7379

Svoboda P, Stein P, Hayashi H, Schultz RM (2000) Selective reduction of dormant maternal mRNAs in mouse oocytes by RNA interference. *Development* 127: 4147–4156

Swarts DC, Makarova K, Wang Y, Nakanishi K, Ketting RF, Koonin EV, Patel DJ, van der Oost J (2014) The evolutionary journey of Argonaute proteins. *Nat Struct Mol Biol* 21: 743–753

Takahashi T, Nakano Y, Onomoto K, Murakami F, Komori C, Suzuki Y, Yoneyama M, Ui-Tei K (2018) LGP2 virus sensor regulates gene expression network mediated by TRBP-bound microRNAs. *Nucleic Acids Res* 42: D68–14

Takigawa Y, Nagano-Fujii M, Deng L, Hidajat R, Tanaka M, Mizuta H, Hotta H (2004) Suppression of hepatitis C virus replicon by RNA interference directed against the NS3 and NS5B regions of the viral genome. *Microbiol Immunol* 48: 591–598

Tan EL, Tan TMC, Tak Kwong Chow V, Poh CL (2007) Inhibition of enterovirus 71 in virus-infected mice by RNA interference. *Mol Ther* 15: 1931–1938

Taylor DW, Ma E, Shigematsu H, Cianfrocco MA, Noland CL, Nagayama K, Nogales E, Doudna JA, Wang H-W (2013) Substrate-specific structural rearrangements of human Dicer. *Nat Struct Mol Biol* 20: 662–670

tenOever BR (2014) Response to Voinnet *et al*. *Cell Rep* 9: 798–799

tenOever BR (2016) The evolution of antiviral defense systems. *Cell Host Microbe* 19: 142–149

tenOever BR (2017) Questioning antiviral RNAi in mammals. *Nat Microbiol* 2: 17052

Tompkins SM, Lo C-Y, Tumpey TM, Epstein SL (2004) Protection against lethal influenza virus challenge by RNA interference in vivo. *Proc Natl Acad Sci USA* 101: 8682–8686

Triboulet R, Mari B, Lin YL, Chable-Bessia C, Bennasser Y, Lebrigand K, Cardinaud B, Maurin T, Barbry P, Baillat V, Reynes J, Corbeau P, Jeang KT, Benkirane M (2007) Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science* 315: 1579–1582

Tsai K, Courtney DG, Kennedy EM, Cullen BR (2018) Influenza A virus-derived siRNAs increase in the absence of NS1 yet fail to inhibit virus replication. *RNA* 24: 1172–1182

Van der Veen AG, Maillard PV, Schmidt JM, Lee SA, Deddouche Grass S, Borg A, Kjær S, Snijders AP, Reis e Sousa C (2018) The RIG-I-like receptor LGP2 inhibits Dicer-dependent processing of long double-stranded RNA and blocks RNA interference in mammalian cells. *EMBO J* 37: e97479

de Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, Silverman RH, Williams BR (2001) Functional classification of interferon-stimulated genes identified using microarrays. *J Leukoc Biol* 69: 912–920

de Vries W, Haasnoot J, Fouchier R, de Haan P, Berkhout B (2009) Differential RNA silencing suppression activity of NS1 proteins from different influenza A virus strains. *J Gen Virol* 90: 1916–1922

Wang XH, Aliyari R, Li WX, Li HW, Kim K, Carthew R, Atkinson P, Ding SW (2006a) RNA interference directs innate immunity against viruses in adult *Drosophila*. *Science* 312: 452–454

Wang Y, Kato N, Jazag A, Dharel N, Otsuka M, Taniguchi H, Kawabe T, Omata M (2006b) Hepatitis C virus core protein is a potent inhibitor of RNA silencing-based antiviral response. *Gastroenterology* 130: 883–892

Wang R, Wang J, Paul AM, Acharya D, Bai F, Huang F, Guo YL (2013) Mouse embryonic stem cells are deficient in type I interferon expression in response to viral infections and double-stranded RNA. *J Biol Chem* 288: 15926–15936

Waterhouse PM, Graham MW, Wang MB (1998) Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proc Natl Acad Sci USA* 95: 13959–13964

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: 5059–5064

Weber M, Gawanbacht A, Habjan M, Rang A, Borner C, Schmidt AM, Veitinger S, Jacob R, Devignot S, Kochs G, García-Sastre A, Weber F (2013) Incoming RNA virus nucleocapsids containing a 5′-triphosphorylated genome activate RIG-I and antiviral signaling. *Cell Host Microbe* 13: 336–346

Werk D, Schubert S, Lindig V, Grunert H-P, Zeichhardt H, Erdmann VA, Kurreck J (2005) Developing an effective RNA interference strategy against a plus-strand RNA virus: silencing of coxsackievirus B3 and its cognate coxsackievirus-adenovirus receptor. *Biol Chem* 386: 857–863

Wianny F, Zernicka-Goetz M (2000) Specific interference with gene function by double-stranded RNA in early mouse development. *Nat Cell Biol* 2: 70–75

Wilkins C, Dishongh R, Moore SC, Whitt MA, Chow M, Machaca K (2005) RNA interference is an antiviral defence mechanism in *Caenorhabditis elegans*. *Nature* 436: 1044–1047

Wu J, Chen ZJ (2014) Innate immune sensing and signaling of cytosolic nucleic acids. *Annu Rev Immunol* 32: 461–488

Wu X, Dao Thi VL, Huang Y, Billerbeck E, Saha D, Hoffmann H-H, Wang Y, Silva LAV, Sarbanes S, Sun T, Andrus L, Yu Y, Quirk C, Li M, MacDonald MR,

Schneider WM, An X, Rosenberg BR, Rice CM (2018) Intrinsic immunity shapes viral resistance of stem cells. *Cell* 172: 423–438.e25

Xia H, Li X, Gao W, Fu X, Fang RH, Zhang L, Zhang K (2018) Tissue repair and regeneration with endogenous stem cells. *Nat Rev Mater* 3: 174–193

Xu Y-P, Qiu Y, Zhang B, Chen G, Chen Q, Wang M, Mo F, Xu J, Wu J, Zhang R-R, Cheng M-L, Zhang N-N, Lyu B, Zhu W-L, Wu M-H, Ye Q, Zhang D, Man J-H, Li X-F, Cui J *et al* (2019) Zika virus infection induces RNAi-mediated antiviral immunity in human neural progenitors and brain organoids. *Cell Res.* 46: 509

Yang S, Tutton S, Pierce E, Yoon K (2001) Specific double-stranded RNA interference in undifferentiated mouse embryonic stem cells. *Mol Cell Biol* 21: 7807–7816

Yoo BC, Kragler F, Varkonyi-Gasic E, Haywood V, Archer-Evans S, Lee YM, Lough TJ, Lucas WJ (2004) A systemic small RNA signaling system in plants. *Plant Cell* 16: 1979–2000

Yuan J, Cheung PKM, Zhang HM, Chau D, Yang D (2005) Inhibition of coxsackievirus B3 replication by small interfering RNAs requires perfect sequence match in the central region of the viral positive strand. *J Virol* 79: 2151–2159

Zamore PD, Tuschl T, Sharp PA, Bartel DP (2000) RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* 101: 25–33

Zhang H, Kolb FA, Brondani V, Billy E, Filipowicz W (2002) Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. *EMBO J* 21: 5875–5885