Cellular origins of dsRNA, their recognition and consequences

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Abstract | Double-stranded RNA (dsRNA) is associated with most viral infections — it either constitutes the viral genome (in the case of dsRNA viruses) or is generated in host cells during viral replication. Hence, nearly all organisms have the capability of recognizing dsRNA and mounting a response, the primary aim of which is to mitigate the potential infection. In vertebrates, a set of innate immune receptors for dsRNA induce a multitude of cell-intrinsic and cell-extrinsic immune responses upon dsRNA recognition. Notably, recent studies showed that vertebrate cells can accumulate self-derived dsRNAs or dsRNA-like species upon dysregulation of several cellular processes, activating the very same immune pathways as in infected cells. On the one hand, such aberrant immune activation in the absence of infection can lead to pathogenesis of immune disorders, such as Aicardi–Goutières syndrome. On the other hand, the same innate immune reaction can be induced in a controlled setting for a therapeutic benefit, as occurs in immunotherapies. In this Review, we describe mechanisms by which immunostimulatory dsRNAs are generated in mammalian cells, either by viruses or by the host cells, and how cells respond to them, with the focus on recent developments regarding the role of cellular dsRNAs in immune modulation.

Since the original proposal of the RNA world more than 50 years ago, which postulates RNA as the sole type of biopolymer for sustaining primitive forms of life, our understanding of functions of RNA has greatly expanded. These functions range from a simple message bearing a linear array of genetic codes to an active player in transcription regulation, protein synthesis, nutrient sensing and many other biological processes[1]. These non-coding functions arise from the abilities of RNA molecules to form secondary, tertiary or quaternary structures, and to engage with DNA, proteins, metabolites or other RNA molecules in temporally and spatially controlled manners. The rapid expansion of the RNA world accompanied the increased appreciation of double-stranded RNAs (dsRNAs) as prevalent molecules with diverse functions.

It has long been thought that the presence of dsRNAs is associated solely with viral infection (including both RNA and DNA viruses). One of the most conserved mechanisms by which cells sense viral infection is through detection of dsRNAs by a set of receptors in the innate immune system that trigger antiviral and inflammatory immune responses[2] (BOX 1). However, accumulating evidence suggests that dsRNAs are not limited to virally infected cells, but can be produced from endogenous sources, such as retroelements and mitochondrial DNA, in various pathophysiological states. These endogenous dsRNAs activate the same receptors that have evolved to detect viral dsRNAs, often serving as a 'danger' signal that alerts misregulated cellular processes. Accordingly, innate immune and inflammatory responses to dsRNAs underlie diverse pathophysiologies from immune disorders to neurodegeneration.

In this Review, we summarize recent reports showing endogenous sources of dsRNA (self dsRNA) and cellular responses that result from activation of dsRNA sensors by these cell-derived dsRNA species. We first discuss the dsRNA sensors in the vertebrate innate immune system, with the focus on their signalling pathways, mechanisms and RNA specificity. We then outline the origins and identities of the immunostimulatory endogenous dsRNAs and describe the consequences of self dsRNA-mediated immune activation.

dsRNA sensors

The immune response to viral infection often begins when viral dsRNA is detected by dsRNA-binding proteins in the host (FIG. 1). These sensors include RIG-I-like receptors (RLRs), protein kinase R (PKR), oligoadenylate synthases (OASes), Toll-like receptors (TLRs) and NOD-, LRR- and pyrin domain-containing 1 (NLRP1). These sensors are broadly expressed in a wide array of tissues, including airway, gut and reproductive tract epithelial cells, which are primary entry sites for many

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Box 1 | **Activation of dsRNA sensors by viruses**

Most viral infections are associated with the generation of double-stranded RNA (dsRNA), but the source of the dsRNA and its recognition by dsRNA sensors in the cell differ depending on the virus (see the figure).

dsRNA viruses

dsRNA viruses often sequester their duplex genome during the entire replication cycle and use additional mechanisms to prevent innate immune activation. For example, both replication and transcription by reovirus occur entirely within the viral core without exposing the dsRNA genome to the cytosolic compartment and to the host innate immune system[184,185]. However, many dsRNA viruses, including reovirus, have been shown to activate cellular dsRNA sensors[186,187], likely by erroneous or host-mediated leakage of the viral dsRNA genome into the cytoplasm[188,189].

Positive-strand RNA viruses

Positive-strand RNA viruses are also known to produce dsRNAs during replication and activate the innate immune system[190,191]. Upon entry, the positive-strand RNA genome is released into the cytoplasm and is translated by the host ribosomes. Newly synthesized RNA-dependent RNA polymerase then copies the genome (positive strand) to produce the antigenome (negative strand), during which long dsRNA of the genome–antigenome hybrid (that is, the replicative form) is generated. For picornaviruses, the replicative form is ~7–8-kb dsRNA, and has been shown to be the major RNA species activating MDA5, protein kinase R (PKR) and oligoadenylate synthases[16,192]. The picornavirus replicative form does not activate RIG-I because both the positive strand and the negative strand of viral RNAs lack 5′-triphosphate.

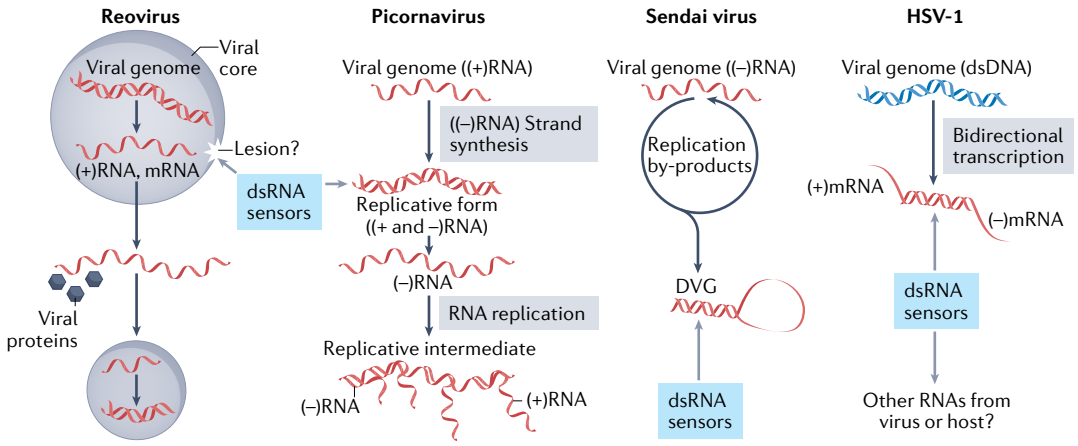
Negative-strand RNA viruses

Negative-strand RNA viruses also replicate through a dsRNA intermediate, but they do not accumulate a significant level of this intermediate[193,194]. Instead, immunostimulatory dsRNA from negative-strand viruses, such as Sendai virus, appears to stem from erroneous replication by-products, namely defective viral genomes (DVGs)[20,195–198]. While there are many types of DVGs, the most immunostimulatory RNAs are copy-back DVGs, which contain a small portion of the genome with two perfectly complementary ends[196]. Copy-back DVGs contain 5′-triphosphate and activate RIG-I, not MDA5. The longer the complementary ends are, the more potent they are in activating RIG-I, suggesting that it is the DVGs, not their replication intermediates, that activate RIG-I[20,197]. However, it is worth noting that copy-back DVGs in the virion particles should also be encapsidated for replication, which would protect them from RIG-I recognition. This suggests that a copy-back DVG is either more prone to escape encapsidation, possibly due to the intrinsic stability of the dsRNA hairpin or other, unknown mechanisms[199].

For most negative-strand RNA viruses, however, the identity of the RIG-I-stimulatory RNA remains less clear. For influenza virus, for example, RIG-I was proposed to be activated by multiple forms of viral RNAs, including the nucleocapsid[200], the full-length genome[201], the 3′ untranslated region[202] and DVGs[203–205]. These conflicting reports highlight the challenge of identifying RIG-I ligands.

DNA viruses

DNA viruses, such as vaccinia virus, herpes simplex virus type 1 (HSV-1) and adenovirus, are also known to accumulate significant levels of dsRNA that activate the innate immune system[193,194]. It has been thought that many DNA viruses generate long sense–antisense hybrids formed by converging bidirectional transcription[206,207]. However, other types of immunostimulatory dsRNAs were also proposed to accumulate during DNA viral infection. For example, EBER1 and EBER2, non-coding RNAs encoded by Epstein–Barr virus and transcribed by the host RNA polymerase III, were proposed to activate RIG-I[208,209]. More recently, aberrant endogenous RNAs were also proposed to activate RIG-I during infection by HSV-1, Kaposi sarcoma-associated herpesvirus or Epstein–Barr virus[126,128]. As with most viruses, however, the precise identity of the dsRNAs activating each of these sensors and the mechanism of activation need further investigation.



viruses. Upon binding to dsRNA, these sensors activate a multitude of immune responses, including activation of antiviral and inflammatory signalling pathways (FIG. 1a), cell growth inhibition (FIG. 1b) and in some cases cell death (FIG. 1c), which altogether inhibit viral replication.

RIG-I-like receptors. RLRs encompass three proteins, RIG-I, MDA5 and LGP2, with all three sharing similar RNA-binding domains, including the conserved DExD/H helicase domain[3,4]. Upon viral dsRNA recognition, RIG-I and MDA5 activate their shared downstream

Type I interferons
A large subgroup of secreted proteins that bind to interferon receptors and stimulate the expression of antiviral genes.

Caspase activation and recruitment domains
(CARDs). Interaction motifs found in a wide array of proteins, typically those involved in processes relating to inflammation and apoptosis.

adaptor molecule, mitochondrial antiviral-signalling protein (MAVS) (FIG. 1a). Activated MAVS then recruits multiple signalling molecules, including TNF receptor-associated factors (TRAFs), TANK-binding kinase 1 (TBK1) and interferon-regulatory factor 3/7 (IRF3/7), which in turn induce transcription of type I interferons and other proinflamamtory cytokines[3,4]. Unlike RIG-I and MDA5, LGP2 lacks the ability to directly control the antiviral signalling pathway, but is thought to positively and negatively regulate MDA5 and RIG-I, respectively[5–7].

MDA5 and RIG-I both use RNA duplex length as a key criterion to distinguish viral RNA from cellular RNA that may have local secondary structure, and they do so by forming filaments along the length of dsRNA[4] (FIG. 1a; TABLE 1). For both receptors, filament formation is important for robust antiviral signalling as it increases signalling capacity, which relies on the interaction between the filaments and their activating E3 ligases (RIPLET for RIG-I and TRIM65 for MDA5)[8,9]. These E3 ligases conjugate K63-linked ubiquitin chains, which act as 'molecular glues' to help oligomerize their receptor signalling domains — caspase activation and recruitment domains (CARDs)[10,11] — which occurs more readily when the receptors are preoligomerized on RNA (FIG. 1a).

Despite these similarities, RIG-I and MDA5 use different mechanisms to assemble filaments and regulate filament stability, resulting in their divergence in RNA and viral specificities. For MDA5, filaments form directly within the RNA duplex stem in a highly cooperative manner with little dependence on dsRNA sequence[12–15]. Concurrent to filament formation is ATP hydrolysis within the helicase domain, which in turn stimulates filament disassembly from filament termini[12,13,15]. Therefore, MDA5 filament continuously undergoes cycles of end disassembly and elongation, where the dynamic equilibrium between the two processes determines the stability

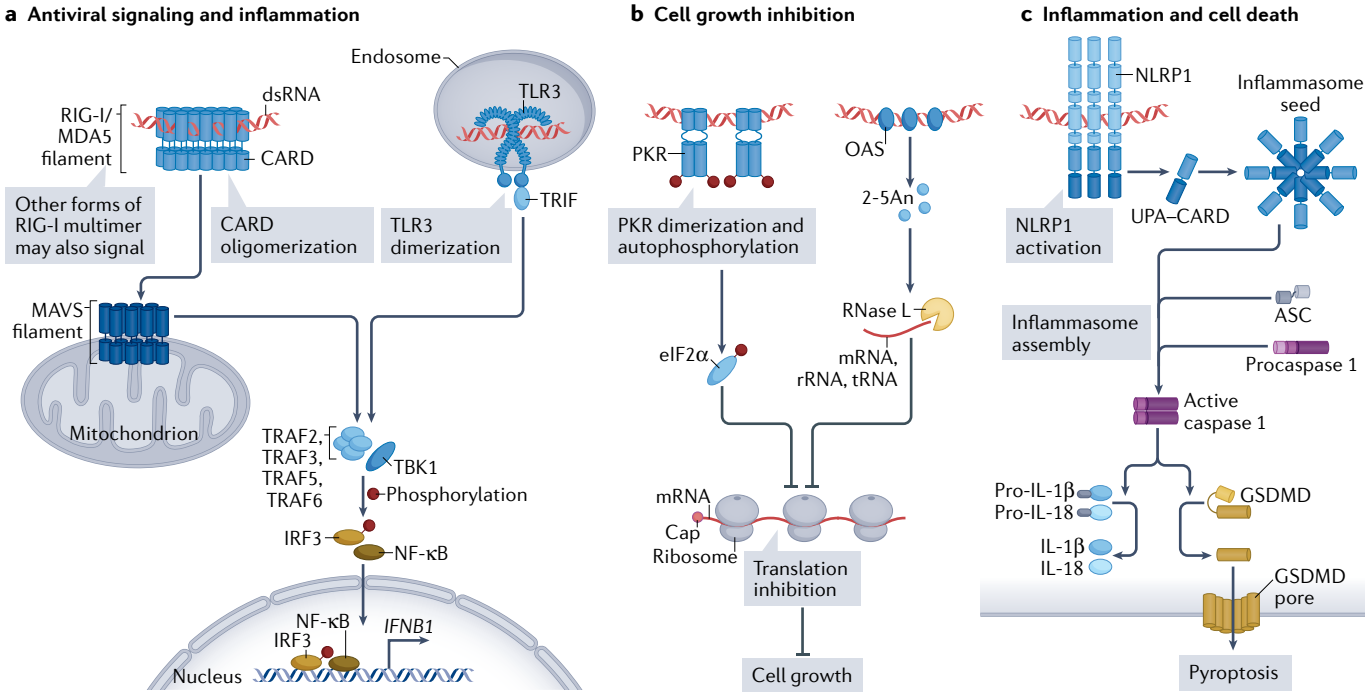


Fig. 1 | **dsRNA sensors and their signalling.** Double stranded RNAs (dsRNAs) from either viral or cellular origins induce three types of cellular responses. They are not mutually exclusive and may occur within the same cell. **a** | The first type of response is classical antiviral innate immune responses mediated by RIG-I or MDA5 (RIG-I-like receptors (RLRs)) and Toll-like receptor 3 (TLR3). RLRs in the cytoplasm and TLRs in the endosome detect dsRNAs and form signalling-competent oligomers to activate their respective downstream adaptors, mitochondrial antiviral-signalling protein (MAVS) and TRIF. Upon dsRNA binding, RLRs form filaments along the length of dsRNA, which promotes oligomerization of their caspase activation and recruitment domains (CARDs) and triggers MAVS filament formation for downstream signal activation. RIG-I may also be stimulated by RNAs besides long dsRNA by forming non-filamentous multimers, but the structural features of such RNAs and RIG-I multimers are unclear. These pathways then converge by activating the common downstream signalling molecules, such as TNF receptor-associated factors (TRAFs) and TANK-binding kinase 1 (TBK1), culminating in the activation of the transcription factors interferon-regulatory factor 3 (IRF3) and NF-κB for producing type I interferons and other proinflammatory cytokines. **b** | The second type of response induces global inhibition of protein synthesis and thus cell growth. This response is mediated by protein kinase R (PKR) and oligoadenylate synthases (OASes), which become active upon binding to dsRNA. PKR activation via dimerization and autophosphorylation results in phosphorylation of a key translation initiation factor (eIF2α) and subsequent inhibition of most protein synthesis. Activated OASes synthesize 2′-5′-linked oligoadenylate (2-5An), which serves as a soluble second messenger to activate ribonuclease L (RNase L). RNase L in turn degrades the bulk of cytosolic RNAs, including mRNA, ribosomal RNA (rRNA) and tRNA, resulting in translation inhibition. **c** | The third type of response to dsRNA is mediated by the NOD-, LRR- and pyrin domain-containing 1 (NLRP1) inflammasome, a macromolecular complex containing the receptor NLRP1, the adaptor ASC and the effector caspase 1. Upon dsRNA binding, NLRP1 triggers release of its UPA and CARD domains, which then assembles the inflammasome seed, inducing inflammasome formation and activating caspase 1. Activated caspase 1 then cleaves precursors of inflammatory cytokines (such as IL-1β and IL-18) and a pore-forming protein gasdermin D (GSDMD). The GSDMD pore forms in the plasma membrane and induces pyroptosis, the inflammatory form of cell death.

Table 1 | **dsRNA length sensitivity of dsRNA sensors**

dsRNA sensors		Preferred duplex length	Mechanism of dsRNA length discrimination	
			At the receptor level	By downstream pathway
RLRs	RIG-I	~22–500 bp with peak activity for ~50–200-bp dsRNA	Filament formation (through ATP-driven translocation of the receptor)	RIPLET binding
	MDA5	~500–1,000 bp; activity progressively increases with dsRNA length	Filament formation (through ATP-independent cooperative binding)	TRIM65 binding
TLR3		40–50 bp; activity progressively increases with dsRNA length	Dimerization or multimerization Activity progressively increases with dsRNA length	ND
PKR		>~33 bp; activity progressively increases with dsRNA length	Proximity of at least two PKR dimers (interdimeric phosphorylation)	ND
OASes	OAS1	<20 bp	ND	ND
	OAS2	Unclear	ND	ND
	OAS3	>50 bp	Binding in an extended conformation (?)	ND
NLRP1		>500 bp	ND	ND

dsRNA, double-stranded RNA; ND, not determined; NLRP1, NOD-, LRR- and pyrin domain-containing 1; OAS, oligoadenylate synthase; PKR, protein kinase R; RIPLET, E3 ubiquitin-protein ligase RNF135; RLR, RIG-I-like receptor; TLR3, Toll-like receptor 3; TRIM65, tripartite motif-containing protein 65.

of the MDA5 filament[13,14]. Since filament disassembly occurs from the filament end, the MDA5 filament stability increases with its increasing length, which is in turn dictated by dsRNA length. As a result, MDA5 signalling increases with dsRNA length and requires an ~0.5–1-kb duplex region[13,16]. This RNA-length dependency also enables detection of duplex structural irregularities introduced by mismatches and bulges, which are common among cellular dsRNAs and interfere with MDA5 filament assembly, allowing it to discriminate between viral and cellular RNAs[17]. Additionally, RNA modifications (FIG. 2), also present in many cellular RNAs, can regulate MDA5 filament formation in a manner dependent on the physicochemical property of the specific modifications. For example, adenosine-to-inosine (A-to-I) modification, which affects Watson–Crick base pairing (discussed further in the section entitled Endogenous sources of RNA), strongly interferes with MDA5 filament assembly[17], while pseudouridylation and N^6-methylation of adenosine have little impact[18] (FIG. 2).

Unlike MDA5, RIG-I filament assembles from a dsRNA end with a 5′-triphosphate group (5′ppp) or a 5′-diphosphate group[19–21]. 5′ppp is present in all nascent transcripts, but is removed from host RNAs during normal 5′ processing[1]. Viral RNAs, especially those generated in the cytoplasm of the host cell, often do not undergo such 5′ processing and thus retain 5′ppp. Binding of an individual RIG-I molecule to a 5′ppp-containing dsRNA end stimulates the ATPase activity in the RIG-I helicase domain. This then drives RIG-I to translocate from the dsRNA end to the interior[22] (the activity not shared with MDA5), re-exposing the 5′ppp-containing dsRNA end and allowing recruitment of another RIG-I molecule. Iterations of end recruitment and translocation lead to the formation of a filamentous oligomer of RIG-I, albeit not as long as MDA5 filaments[19,20]. Even though filamentous oligomerization is also important for RIG-I, the importance of the dsRNA end limits the ability of RIG-I to form filaments on very long dsRNA (longer than ~500-bp dsRNA)[8,19] (TABLE 1). Thus, the preference of RIG-I for dsRNA displays a bell-shaped curve with respect to dsRNA length[8]. This exemplifies how RIG-I, unlike MDA5, utilizes two distinct pathogen-associated molecular patterns (PAMPs) – the presence of 5′ppp and RNA duplex length – to recognize viral dsRNAs that differ from those stimulating MDA5. Some reports suggest that monomeric RIG-I stimulated by a short (~14-bp) hairpin RNA is sufficient for activation[23]. However, RIG-I signalling requires the E3 ligase RIPLET, which in turn needs at least dimeric RIG-I for bivalent binding and ubiquitin-mediated tetramerization of the RIG-I CARD. It is possible that the short hairpin multimerizes to form longer duplex RNA (as shown before)[24], which may then support RIG-I multimerization and signalling (FIG. 1a). It should also be noted that while RIG-I filament is the only RNA-bound multimer characterized to date, other forms of RIG-I multimer may exist on unknown RNA and activate signalling. In general, there is a limited understanding of the diversity of RNA structure (besides the simple duplex stem structure) for RIG-I stimulation, which is an intriguing area for future studies.

TLR3. TLR3 is another dsRNA receptor that activates the antiviral signalling pathway that transcriptionally induces interferon-β (IFNβ) and proinflammatory cytokines. Unlike RLRs, which are cytosolic, TLR3 is anchored to the endosomal membrane, surveying the presence of dsRNA in the lumen of the endosome (FIG. 1a). In some cell types, TLR3 is also present on the cell surface[25], but dsRNA binding requires an acidic environment[26], suggesting that recognition occurs within the endosome. For this reason, it is generally believed that TLR3 detects dsRNAs that are released from infected and dying cells through endocytosis (cell-extrinsic sensing). This differs from cytosolic dsRNA sensors (such as RLRs), which sense dsRNAs originating within the cell (cell-intrinsic sensing). TLR3

Pathogen-associated molecular patterns (PAMPs). Conserved motifs that are associated with pathogen infection that serve as ligands for host pattern recognition receptors.

Stress granules
Molecular condensates that form in response to various cellular stresses. They often result from accumulation of stalled translation initiation complexes that expose mRNAs.

2′-5′-linked oligomers of adenosines
The polymerized form of ATP with the general formula pppA(2′p5′A)$_n$ that is catalysed by 2′-5′-oligoadenylate synthase. The oligomers activate ribonuclease L (RNase L), which mediates RNA degradation.

deficiency results in increased susceptibility to a broad range of viruses, including poliovirus[27] and herpes simplex virus type 1 (HSV-1)[28]. In humans, TLR3 deficiency causes HSV-1 encephalitis in children due to failure to restrict the virus in the central nervous system (CNS)[29]. Interestingly, such patients display normal immunity against HSV-1 in non-CNS tissues and against most other microorganisms in general. This suggests that TLR3 has a unique role in host defence against HSV-1 in the CNS, while its function is redundant with those of other receptors (such as RLRs) in most other tissues.

Like for RLRs, dsRNA recognition by TLR3 also depends on the RNA duplex length, with a minimal requirement of 40–50 bp (REFS[26,30]) (TABLE 1). Structural and biochemical studies showed that this length sensitivity stems from the requirement of TLR3 to form a dimer[26] or a multimer[29]. Ectodomains of two TLR3 molecules cooperatively bind one dsRNA molecule in a way that the cytoplasmic carboxy-terminal signalling domains are juxtaposed to allow recruitment and activation of the downstream adaptor molecule TRIF[30]. TLR3 interacts with the ribose phosphate backbone of dsRNA and thus has no RNA sequence specificity. Nucleoside modification can suppress recognition by TLR3 (REF.[31]).

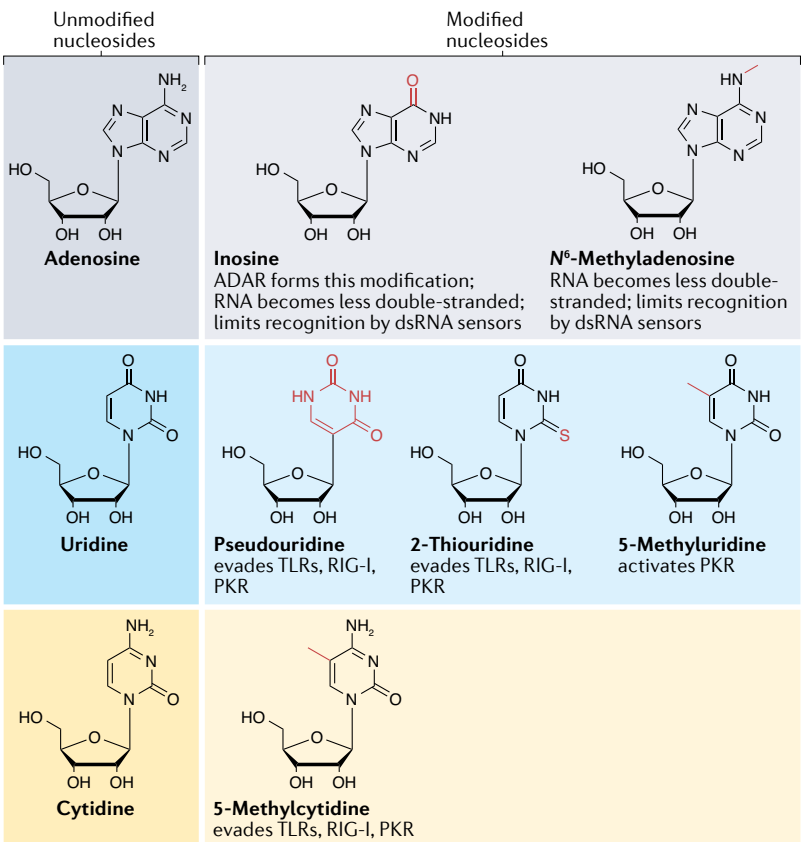


Fig. 2 | **RNA modifications affect the RNA's secondary structures and interaction with immune sensors.** The canonical nucleosides adenosine, uridine and cytidine can be modified by enzymes that install new chemical groups (shown in red). The RNA modifications can change base-pairing interactions, protein binding and secondary structures, which can prevent the modified RNAs from forming immunogenic structures, such as double-stranded RNAs (dsRNAs), and evading detection by immune sensors, including Toll-like receptors (TLRs), RIG-I and protein kinase R (PKR). ADAR, double-stranded RNA-specific adenosine deaminase.

Some studies, however, reported that incomplete duplex structures within single-stranded RNA (ssRNA) molecules can also activate TLR3 (REF.[32]), although the mechanistic basis for this observation is unclear.

PKR. PKR is a dsRNA-dependent protein kinase. Upon dsRNA binding, PKR dimerizes, autophosphorylates and becomes an active kinase that can phosphorylate the α-subunit of the translation initiation factor eIF2 (REF.[2]) (FIG. 1b). Phosphorylation of eIF2α prevents functions of eIF2, leading to the global shutdown of protein synthesis and cell growth inhibition. Since all viruses depend on the host machineries for viral protein synthesis, PKR-mediated eIF2 restriction has a broad antiviral function, although some viruses evade this restriction mechanism through eIF2-independent translation[33].

PKR was also found to modulate RLR signalling, although the detailed mechanism remains controversial. First, PKR activation and the consequent inhibition of translation initiation induce stress granules. Stress granule formation has been proposed to amplify RLR signalling by serving as a signalling hub[34,35], although the opposite effect was also reported in a recent preprint[36]. PKR was also reported to promote production of IFNα and IFNβ by stabilizing their mRNAs, rather than by upregulating their transcription[37]. Finally, PKR was proposed to modulate RLR signalling simply by suppressing global protein synthesis, as some inhibitors of the RLR pathway, such as IκB, have a short half-life, requiring continuous protein synthesis to maintain its level and inhibitory function[38,39].

PKR contains two tandem repeats of dsRNA-binding domains and a kinase domain[40,41]. Data to date suggest that PKR activation requires stable dimerization, which is induced by dsRNA binding and stabilized by autophosphorylation[41,42] (FIG. 1b). The PKR dimeric structure has a back-to-back dimeric configuration where the two active sites face away from each other[41,42]. Therefore, autophosphorylation likely occurs through interdimeric phosphorylation rather than intradimeric or intramolecular phosphorylation[43], although intradimeric phosphorylation has also been proposed[44]. It has been reported that the minimal length of dsRNA required for PKR activation is ~33 bp (REF.[45]) (TABLE 1), likely reflecting the requirement for clustering of at least two PKR dimers on a single RNA molecule to facilitate interdimeric phosphorylation. While other RNA features, such as 5′ppp and single-stranded regions, were also proposed to activate PKR[46], the molecular details remain unclear. It is also worth noting that certain RNAs, such as adenovirus-associated RNA 1 (REF.[47]), were shown to inhibit PKR through competition with other activating dsRNAs and by preventing PKR dimerization or higher-order clustering.

Oligoadenylate synthases. As with PKR, OASes are enzymes whose catalytic activities are regulated by dsRNA. Upon dsRNA binding, OASes undergo a conformational change in the active site, allowing joining of two ATP molecules through the 2′-5′ linkage. Iteration of ATP joining leads to production of 2′-5′-linked oligomers of adenosines[48,49], which then serve as second

Ribonuclease L
(RNase L). An interferon-induced endoribonuclease that degrades RNA when activated.

Cyclic GMP–AMP synthase
(cGAS). A cytosolic DNA sensor that activates a type I interferon response, part of the cGAS–STING DNA sensing pathway.

Inflammasome
A multiprotein complex that forms in response to a variety of inflammatory triggers (both pathogen derived and host derived). Assembly of the inflammasome often leads to activation of caspase 1, which then processes inflammatory cytokines into their mature forms and induces gasdermin pore formation.

Gasdermin D
A member of the gasdermin family, which form pores in response to inflammasome activation. Gasdermin D pore formation leads to release of inflammatory cytokines and causes a highly inflammatory form of programmed cell death known as pyroptosis

Transposable elements
(TEs). DNA sequences that can change their position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size.

Endogenous retroviruses
(ERVs). Endogenous viral elements in the genome that closely resemble and can be derived from retroviruses. These elements constitute up to 8% of the human genome.

Long interspersed nuclear elements
(LINEs). A group of retrotransposons that are not long terminal repeats. LINEs constitute ~21% of the human genome. They are transcribed into mRNA and translated into a protein that acts as a reverse transcriptase, which makes a DNA copy of the LINE RNA, which then can be integrated into the genome at a new site.

messengers to activate the downstream effector ribonuclease L (RNase L)[50] (FIG. 1b). Activated RNase L degrades both cellular and viral ssRNA with limited sequence specificity, which culminates in global suppression of protein synthesis, cellular proliferation and viral replication[50,51].

The use of non-canonical oligonucleotide as a signalling molecule during antiviral response is analogous to that of cyclic GMP–AMP synthase (cGAS), an innate immune receptor that synthesizes cyclic GMP–AMP (cGAMP) upon binding foreign dsDNA. OASes and cGAS share close structural homology and belong to a family of template-independent nucleotidyltransferases (NTase). They also use a similar activation mechanism where the binding of the substrate nucleic acid induces an active site conformational change to stimulate the catalytic activity[52].

In humans, there are four isoforms of OASes; OAS1, OAS2 and OAS3 are enzymatically active, while OASL is not. Accordingly, OAS1, OAS2 and OAS3 have the ability to activate RNase L, while OASL does not (FIG. 1b). Instead, OASL is thought to regulate other antiviral immune pathways through diverse mechanisms, including direct binding to and activation of RIG-I[53,54]. OAS1, OAS2 and OAS3 respectively harbour one, two and three tandem repeats of the NTase domain. For OAS2 and OAS3, only one NTase domain is catalytically active and the other NTase domains (pseudo-NTase domains) lack the catalytic triad in the active site[55,56]. The pseudo-NTase domains, however, appear to play an important function as a dsRNA-binding domain, explaining the higher dsRNA affinity of OAS3 than the other two isoforms[57,58]. Consistent with this observation, OAS3 is the primary receptor for activating RNase L during viral infection[59]. OAS3 was also found to preferentially recognize long dsRNAs (more than 50 bp)[55] (TABLE 1), but the mechanism of dsRNA length recognition is unclear. It also remains to be investigated what functions OAS1 and OAS2 have and whether they recognize different types of dsRNA for non-redundant antiviral functions.

NLRP1. NLRP1 is an inflammasome-forming sensor that detects microorganisms and activates the cytokines IL-1β and IL-18 or promotes gasdermin D-mediated pore formation in the plasma membrane[60] (FIG. 1c). In humans, NLRP1 relies on both RNA secondary structure and RNA length for sensing[61]. The carboxy-terminal leucine-rich repeat (LRR) domain and the amino-terminal deathfold domain that engages caspase 1 (NACHT) region on NLRP1 directly sense dsRNAs longer than 500 bp to activate the inflammasome pathway[61] (TABLE 1). The strength of NLRP1 activation positively correlates with dsRNA length. Duplex RNA structure is required for the activity, as even long (2,500-bp) ssRNAs do not induce NLRP1 inflammasome activity[61]. Collectively, dsRNA length dependence appears to be a shared property among many dsRNA sensors, suggesting its importance in self versus non-self discrimination (TABLE 1).

Upon dsRNA binding, NLRP1 hydrolyses ATP using the NACHT domain, which was shown to be crucial for inflammasome activation[62]. Strikingly, a 15-bp dsRNA was sufficient for ATP hydrolysis by NLRP1 (REF.[61]). The difference in dsRNA length dependence for NLRP1 activation (500 bp) and for ATPase activity (15 bp) is of interest. How the extra length beyond 15 bp contributes to NLRP1 inflammasome activity and antiviral response, as well as the tolerance for imperfect base pairing within the dsRNAs, is worthy of further investigation. There is still an incomplete understanding of the impact of Watson–Crick base mismatches (see the section entitled Endogenous sources of RNA) or bulges or other duplex RNA structural irregularities on NLRP1 activation. In addition, it is not clear whether multiple NLRP1 molecules oligomerize on a single, long dsRNA to ensure activation. Finally, investigation of the potential contributions of other cellular factors would be useful to illuminate the complete mechanisms of NLRP1 activation.

Endogenous sources of dsRNA

dsRNAs were originally considered as PAMPs and their generation was associated solely with infection by pathogens (BOX 1). However, studies in the past two decades point to diverse cellular sources for dsRNAs and their frequent occurrence either during normal physiological process or upon various types of physiological perturbations. Just like viral dsRNA, self-derived dsRNAs activate cellular dsRNA sensors and activate antiviral innate immune pathways and cell stress responses (FIG. 3). The presence of endogenous dsRNA has been understood largely on the basis of the observation that dsRNA sensors can be activated in the absence of viral infection. Since the precise identities of the endogenous dsRNAs responsible for activation of dsRNA sensors are only beginning to be understood, we discuss here circumstances where endogenous dsRNA-mediated activation of these sensors were reported, the current understanding of the identity of these immunostimulatory endogenous dsRNAs and how they are recognized to mount the immunostimulatory response.

Dysregulated epigenetic control. Cancer chemotherapy has long been known to trigger antiviral signalling pathways, but its mechanism of immune activation had been unclear. Multiple studies with 5-aza-2′-deoxycytidine (5-aza-CdR), an inhibitor of DNA methyltransferases (DNMTs) and a common chemotherapeutic agent, showed that its therapeutic efficacy is in part mediated by activation of a broad range of dsRNA sensors (including MDA5, TLR3 and OASes), indicating that derepression of transcription achieved by suppressing DNA methylation can lead to dsRNA formation[63–66] (FIG. 3a). Indeed, 5-aza-CdR strongly stimulates transcriptional upregulation of various types of transposable elements (TEs) that are otherwise epigenetically silenced. These include endogenous retroviruses (ERVs), long interspersed nuclear elements (LINEs) and short interspersed nuclear elements. Some studies proposed that dysregulated, bidirectional transcription of these genomic regions leads to production of sense and antisense transcripts that can then hybridize to form dsRNA[63,64]. However, the concomitant presence of sense and antisense transcripts does not necessarily result in RNA duplex formation. In a more recent study[17], MDA5 activation was found to be driven largely

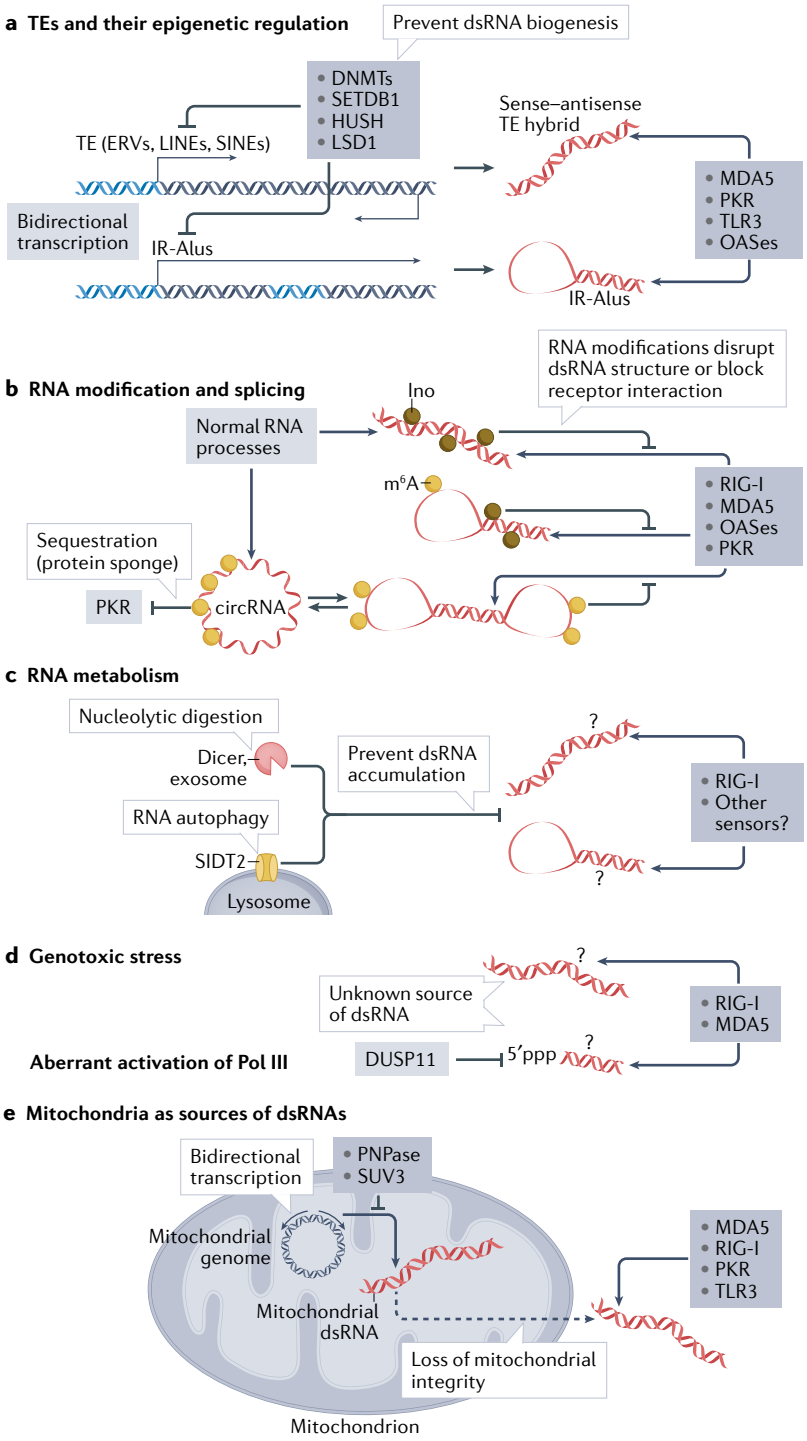


Fig. 3 | **Endogenous sources of dsRNA and cellular regulatory processes.** Cells have diverse endogenous sources of double-stranded RNA (dsRNA) and utilize multiple mechanisms to suppress its biogenesis and accumulation. **a** | Epigenetic derepression of transposable elements (TEs), such as endogenous retroviruses (ERVs), long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs), can lead to dsRNA generation. These elements can be transcribed in a bidirectional manner or as an inverted repeat, forming sense–antisense hybrid or fold-back hairpin dsRNA. Biogenesis of TE-based dsRNAs is normally suppressed by epigenetic silencing mechanisms involving DNA methyltransferases (DNMTs), the histone H3 K9 methyltransferase SETDB1, its partner the human silencing hub (HUSH) and the histone demethylase LSD1. The only exception is inverted repeat Alus (IR-Alus), some of which are constitutively produced within the 3′ untranslated region of many mRNAs. **b** | Once transcribed, cellular RNAs are regulated by post-transcriptional modifications, such as adenosine deamination to inosine (Ino) and N^6-adenosine methylation (to produce N^6-methyladenosine (m^6A)), both of which disrupt the structure of dsRNA and lower its immunogenicity (shown by inhibitory arrows). Deregulation of these (and potentially other) RNA modifications can result in the recognition of normal cellular transcripts as foreign, owing to the formation of local duplex structures. Splicing inhibition can lead to an increase in the levels of dsRNAs, as a result of the increase in the levels of transcripts with retained introns, which may form double-stranded structures. Splicing is also associated with the generation of circular RNAs (circRNAs), which can form dsRNA structures more easily than their linear counterparts. On the one hand, these dsRNA structures can be recognized by RIG-I, but this is negatively regulated by m^6A modification, normally present in circRNAs. On the other hand, circRNAs can also act as protein sponges and sequester protein kinase R (PKR) and prevent its activation in sterile conditions. **c** | RNA degradation mechanisms, such as those involving Dicer, RNA exosome complex and the lysosomal RNA transporter SIDT2, may prevent excessive accumulation of dsRNA through poorly understood mechanisms (question marks). **d** | Genotoxic stress (for example, resulting from exposure to ionizing radiation) and aberrant activation of RNA polymerase III (Pol III; for example when MYC is activated in cancer) can promote biogenesis of RLR-stimulatory dsRNAs, but the precise nature of these dsRNAs remains unclear (question marks). These may be aberrantly processed RNA components of the spliceosome (U1 and U2 small nuclear RNAs) or products of Pol III which contain 5′-triphosphate (5′ppp). The recognition of the latter could be regulated by the phosphatase DUSP11, which can remove 5′ppp. **e** | Mitochondria are a rich source of dsRNA as mitochondrial RNAs are produced by bidirectional transcription of the circular DNA. Normally, the level of mitochondrial dsRNA is regulated by the mitochondrial RNA degradosome, which includes the nuclease polynucleotide phosphorylase (PNPase) and the helicase SUV3. During mitochondrial dysregulation, mitochondrial dsRNA can gain access to cytosolic dsRNA sensors and activate them through a poorly understood mechanism. OAS, oligoadenylate synthase; TLR3, Toll-like receptor 3.

by Alu repeats that are juxtaposed in the inverted configuration (inverted repeat Alu elements (IR-Alus)), which fold back and form long (~300-bp) hairpins[65]. While ~300 bp is below the usual length threshold for MDA5 stimulation, it is likely that the abundance of Alu dsRNA in cells compensates for the suboptimal length for MDA5 activation.

Alu is a primate-specific TE, constituting ~10% of the genome. While most Alus are transcriptionally silenced through various epigenetic means, including DNA methylation, a significant number of Alus are normally transcribed within untranslated regions (UTRs) of many common mRNAs. Earlier investigations of MDA5 with gain-of-function mutations causing inflammatory diseases showed that IR-Alus in 3′ UTRs of endogenous mRNAs are the main source of dsRNA for stimulating MDA5 in the absence of infection[17]. In the case of

Short interspersed nuclear elements
Non-autonomous, non-coding retrotransposons that are capable of copying and pasting themselves into another region of the genome via an RNA intermediate and the action of reverse transcriptase. They constitute ~15% of the human genome.

Alu repeats
A clustered arrangement of Alu (a kind of short interspersed nuclear element), which can often be transcribed within a single transcript.

5-aza-CdR treatment, IR-Alus that stimulate MDA5 originate from intergenic and intronic regions downstream of CpG islands, rather than those embedded within mRNA UTRs[65]. Regardless of the specific origin of IR-Alus, these studies together suggest that duplex formation associated with IR-Alus is mediated largely by *in cis* hairpin formation between adjacent Alus, rather than *in trans* hybridization between separate Alus or sense/antisense transcripts containing these elements (FIG. 3a). Similarly, IR-Alus have been linked to PKR activity in the absence of infection[67], suggesting that IR-Alus may be the major source of dsRNA molecules that activate a broad range of dsRNA sensors.

Similarly to the inhibition of DNMTs, perturbation of the activity of other epigenetic modifiers has been shown to lead to the activation of dsRNA sensors. For example, trimethylation of histone H3 K9 (H3K9) is an epigenetic mark typically associated with transcriptional suppression of TEs and heterochromatin regions. Genetic depletion of an H3K9 methyltransferase (SETDB1)[68] or its partner (the human silencing hub (HUSH))[69] causes induction of a broad range of TEs and concomitant activation of RLRs signalling (FIG. 3a). Similarly, inhibition of the histone demethylase LSD1 (also known as KDM1A) activates TLR3 and RLRs[70] (FIG. 3a). LSD1 can act as a transcription co-repressor of ERVs through removal of methyl groups on H3K4 (REF.[71]), but can also function as a transcription activator by demethylating H3K9 (the suppressive mark)[72]. This raises the question of whether the effect of the LSD1 inhibition on the dsRNA sensors is through increased methylation of H3K4, increased methylation of H3K9 or both. Regardless, these studies collectively suggest that epigenetic regulation is a key mechanism for suppressing biogenesis of endogenous dsRNAs, in particular those formed by TEs (FIG. 3a).

Changes to RNA modification. RNA modifications have been shown to regulate RNA metabolism, secondary structure and protein association. Defects in the installation or recognition of RNA modifications cause self RNAs to appear foreign, which induces immune signalling (FIG. 3b). Here, we discuss two main types of RNA modifications — adenosine deamination and methylation — both of which affect local dsRNA structure and decrease the recognition of endogenous RNAs by dsRNA sensors.

ADAR1 is a dsRNA-specific adenosine deaminase and one of the three adenosine deaminases in vertebrates (ADAR1, ADAR2 and ADAR3)[73–75]. Unlike ADAR2 and ADAR3, ADAR1 is ubiquitously expressed and has an important role in suppressing basal innate immune activity. Upon dsRNA binding, ADAR1 deaminates adenosines within the duplex structure with minimal sequence dependence. Deaminated adenosine (inosine) can impact RNA structure and function in at least two major ways (FIG. 3b). First, inosine is recognized as guanosine by reverse transcriptase and by the ribosome, and is therefore mutagenic. By introducing mutations, ADAR1 can thus antagonize viruses in the case of infection[76]. However, the role of ADAR1 in host RNA biology seems largely independent of this mutagenic effect as most ADAR1 modifications in host RNAs occur in non-coding regions[77]. One of the major functions of ADAR1-mediated A-to-I editing seems to be through its effect on dsRNA structure[78,79]. A-to-I editing replaces the A•U pairs by less stable I•U pairs, destabilizing the dsRNA structure and thereby limiting recognition by dsRNA sensors. Therefore, ADAR1 deficiency leads to constitutive activation of MDA5, PKR and OASes[67,80–83] (discussed further in the section entitled Consequences of endogenous dsRNA).

Studies showed that IR-Alus in 3′ UTRs are the major substrates for ADAR1 in human cells[77,84,85]. Some IR-Alus are highly edited, with more than ~50% of the adenosines capable of being edited[85]. The correlation between the editing efficiency and proximity between adjacent Alus in the inverted orientation is consistent with the notion that IR-Alus form a duplex through intrastrand Alu–Alu interaction as discussed earlier herein[84]. Similarly to the response of MDA5 with gain-of-function mutations, IR-Alus within the mRNA 3′ UTR were found to be responsible for activation of wild-type MDA5 under ADAR1 deficiency[17]. IR-Alus were also proposed to be primarily responsible for constitutive activation of PKR and OASes upon ADAR1 deletion[67,82]. Collectively, these results suggest that IR-Alus are an important source of dsRNA that can breach the immunological threshold, and that ADAR1 is one of the major regulators that suppresses immunostimulatory activities of IR-Alus and guards immune homeostasis.

N^6-Methyladenosine (m^6A) is the most abundant internal modification on mRNAs and long non-coding RNAs in eukaryotes[86,87] (FIG. 2). m^6A can regulate RNA splicing, stability, cellular localization, translation, immune stimulation and secondary structure[86,88–91] (FIG. 3b). The diverse effects of m^6A are caused by the modification being recognized by a plethora of 'reader' proteins. Other m^6A-specific machinery includes 'writers' that install and 'erasers' that remove the modification. Dysregulation of m^6A modification levels has been correlated with various diseases, including autoimmune disorders, cancer, metabolic disorders and neurological disorders[88,92].

The m^6A RNA modification is essential to prevent haematopoietic failure and perinatal lethality during murine fetal development[93]. The lack of m^6A induces the formation of dsRNAs and activation of the dsRNA sensors (FIG. 3b). The dsRNAs are predominantly protein coding and have extensive m^6A modifications in their native state. How the regulation of m^6A modification contributes to the secondary structure for the dsRNAs remains to be investigated. Potentially, m^6A could act as a structural switch on these RNAs where the modification inhibits base pairing; in the absence of m^6A, the transcript becomes double-stranded[91].

m^6A could also regulate TE expression, but the effects of m^6A on TE levels and function differ depending on the species and cell type. In mouse embryonic stem cells, the m^6A modification on ERVs associates with the m^6A-reader protein YTHDF2, which leads to the destabilization of ERVs and prevention of dsRNA accumulation in the cell. However, TEs in murine fetal liver had similar expression in both low-m^6A-level and wild-type conditions[93]. Another study in murine

embryonic stem cells found that m^{6}A on TEs interacts with YTHDC1 (REF.[94]) and the modified RNAs associate with chromatin to affect downstream gene expression and the transcription rate. Differences between these studies could be attributed to cell-specific m^{6}A modification patterns or the different approaches to deplete cellular m^{6}A levels.

In addition to modifying host transcripts, m^{6}A also modifies viral RNAs. Positive-strand RNA viruses (for example, HIV, hepatitis C virus, Zika virus and enterovirus 71), negative-strand segmented RNA viruses (for example, influenza virus) and negative-strand non-segmented RNA viruses (for example, human metapneumovirus, Sendai virus and vesicular stomatitis virus) have all been characterized to contain the m^{6}A modification on their RNAs[95–98]. The m^{6}A modification on viral RNAs enables them to evade host immune surveillance by weakening the association between the immune sensor and the modified RNA, which leads to lowered immune signalling and interferon production. Typically, RIG-I is the main cytosolic receptor that senses viral dsRNA, in a manner dependent on m^{6}A, while MDA5 also seems to be involved, albeit to a lesser extent[95]. Similarly to viral dsRNAs, m^{6}A also prevents recognition of endogenous circular RNAs (circRNAs) by RIG-I (see the section entitled Splicing inhibition).

In addition to inosine and m^{6}A, more than 170 other RNA modifications have been identified thus far, but their roles are still mostly unknown[99]. Some of these RNA modifications have the potential to affect the formation of dsRNAs and/or their recognition by immune receptors. The incorporation of pseudouridine, 2-thiouridine or 5-methylcytidine (FIG. 2) onto exogenously introduced RNAs (such as therapeutic mRNA) allows them to evade detection by TLRs, RIG-I and PKR[100]. By contrast, 5-methyluridine activates PKR, which reveals that there are differences in how immune receptors respond to the various RNA modifications[100].

Splicing inhibition. Similarly to the use of DNMT inhibitors that induce the expression of ERVs, use of small molecules that block proper spliceosome activity in conjunction with MYC hyperactivation leads to dsRNA accumulation in the cytoplasm and promotes antiviral-like responses[101]. Cells treated with spliceosome inhibitors increase the levels of transcripts with retained introns, and those transcripts form double-stranded structures. Both RIG-I and MDA5 detect the induced dsRNAs[101]. Anti-J2 enrichment, which captures dsRNAs greater than 40 bp in length[102,103], revealed that both transcripts with introns that lack retrotransposons and those that have Alus form double-stranded secondary structures. A major question is how do the transcripts with retained introns bypass cellular quality control, including nuclear retention and decay as well as cytoplasmic nonsense-mediated decay? One possibility could be that the stress of inhibiting an essential cellular process, splicing, on top of MYC amplification significantly impacts the cell's ability to monitor and degrade mis-spliced transcripts. An alternative and mutually inclusive possibility could be that the transcripts that are inappropriately spliced may encode proteins that are involved in the quality control of cytoplasmic RNAs. Dysregulation of splicing could affect both potential pathways to enable the transcripts with retained introns to escape and accumulate in the cytoplasm.

Deregulation of circRNAs. circRNAs are a class of endogenous transcripts found throughout the eukaryotic kingdom. The spliceosome produces circRNAs from pre-mRNAs by back-splicing the end of one exon to the beginning of a previous exon to form a covalent loop[104]. Even though circRNAs are identical in primary sequence to their linear RNA counterparts, except for the back-splice junction, emerging studies show that endogenous circRNAs and linear RNAs have different secondary structures and RNA modification patterns, which lead to differential recognition by nucleic acid sensors and immune receptors[105–107].

Endogenous circRNAs are more likely to form imperfect short RNA duplexes (16–26 bp) than their corresponding linear RNAs[107]. circRNAs have been shown to function as targets of RIG-I in the presence of K63-linked polyubiquitin chains to initiate immune signalling. However, the m^{6}A modification — normally present on endogenous circRNAs — interacts with the reader protein YTHDF2, which prevents the recognition by RIG-I[105] (FIG. 3b). Therefore, even though mammalian cells contain up to thousands of endogenous circRNAs[108], they are not under a state of constant inflammation and immune stress because RIG-I does not associate with m^{6}A-modified circRNAs (FIG. 3b). Indeed, the addition of the m^{6}A modification to exogenous circRNAs masks their 'non-selfness' to significantly reduce their binding to RIG-I and subsequent immune signalling[105].

The increase in dsRNA structure as compared with the linear transcripts enables the circRNAs to associate with PKR. Remarkably, this interaction does not lead to PKR activation, presumably due to the limited size of the duplexed secondary structure. Instead, the circRNA–PKR interaction was found to sequester PKR and prevent its activation in sterile conditions[107]. Upon stimulation of cells with exogenous dsRNA or viral infection, RNase L degrades circRNAs globally, leading to the release and activation of PKR for innate immune response. In vitro binding assays between immune-sensing receptors and linear RNA or circRNA revealed that the dsRNA-binding domain of PKR is required for interaction and recognition of circRNA. Since these binding assays were conducted solely with the protein and the RNA, the presence of other cellular factors, including proteins that associate with endogenous circRNAs, may provide further insight into the recognition of circRNAs by PKR in physiological conditions and in disease. For example, sequestration of PKR by circRNAs has been suggested to prevent the initiation of autoimmune diseases such as systemic lupus erythematosus (SLE)[107]. Accordingly, overexpression of circRNAs with double-stranded secondary structure in peripheral blood mononuclear cells from patients with SLE reduced PKR autoactivation and expression of type I interferon. Whether other autoimmune diseases have a high aberrant activation of PKR due to lack of circRNA binding is also of interest.

Circular RNAs
(circRNAs). Single-stranded RNAs where the 5′ and 3′ ends are joined through a phosphodiester bond.

MYC
A family of regulatory genes and proto-oncogenes that code for transcription factors. Many types of cancers have dysregulated MYC levels and activity.

Anti-J2 enrichment
Use of the anti-double-stranded RNA (dsRNA) J2 antibody to capture dsRNAs that are longer than 40 bp.

Nonsense-mediated decay
An evolutionarily conserved mechanism of degradation of mRNA species with a premature termination codon.

Systemic lupus erythematosus
(SLE). An autoimmune disease that causes widespread inflammation and tissue damage in the affected organs. It can affect the joints, skin, brain, lungs, kidneys and blood vessels.

Peripheral blood mononuclear cells
Any peripheral blood cell having a round nucleus, which includes lymphocytes and monocytes.

Defects in RNA processing and degradation. Multiple lines of evidence suggest that cells may accumulate immunostimulatory dsRNAs when there is a defect in RNA processing and degradation (FIG. 3c). While most ribonucleases in cells are inefficient at degrading dsRNAs, Dicer contains an RNase III domain, which specializes in cleaving dsRNAs. Dicer is best known for its role in processing microRNA precursors to generate mature microRNAs, but it can also process long dsRNAs from both endogenous and exogenous sources and generate small interfering RNAs[109,110]. Investigation of the sources of endogenous small interfering RNAs revealed that many of them are from sense–antisense hybrids of TEs or pseudogenes, or transcripts containing inverted repeats of retroelements. For example, LINE-1 has been shown to be subject to Dicer-mediated processing and to be the source of endogenous small interfering RNAs, which in turn mediate RNA-directed DNA methylation and silencing[111,112]. Therefore, defects in Dicer function can lead to accumulation of LINE-1 transcripts, presumably in the form of long dsRNAs[112,113].

Dicer deficiency was also shown to cause accumulation of Alu RNAs, and was proposed to cause age-related macular degeneration, a disease characterized by inflammation in the retinal pigment epithelium[114,115]. Accumulation of Alu RNAs in Dicer-deficient cells was reported to activate the NLRP3 inflammasome[115] and cGAS[116] through unclear mechanisms. Unlike NLRP1, NLRP3 has not been shown to directly recognize RNA, raising the question of whether NLRP3 is a direct sensor or whether there is another RNA sensor that directly detects Alu accumulation. Intriguingly, Alu upregulation induced by the Dicer deficiency was blocked by RNA polymerase III (Pol III) inhibition. Considering that Alu RNA synthesized by Pol III contains a single Alu (not Alu repeats) within individual transcripts, this observation raises the question as to what features of Pol III-transcribed Alu RNA are recognized by Dicer and cause inflammation.

Dicer was also proposed to regulate the level of RNAs with trinucleotide repeats and possibly contribute to the cause of triplet repeat expansion disorders. Triplet repeat expansion disorders, such as Huntington disease and spinocerebellar ataxia, are neurological and neuromuscular disorders that are associated with expansion of a trinucleotide repeat (in particular CNG) within either coding or non-coding regions of the causal genes. Expanded CNG repeats are partially self-complementary and thus can form a hairpin, a dimer and higher-order oligomers. It was found that Dicer can recognize and cleave RNAs containing a trinucleotide repeat expansion, producing small CNG-repeated RNAs[117]. Therefore, impaired function of Dicer may lead to accumulation of RNAs harbouring trinucleotide repeats, which may cause RNA-mediated toxicity that could potentially involve dsRNA sensor activation[118].

SKIV2L is an RNA helicase and a component of the RNA exosome responsible for turnover of the bulk of cytosolic RNAs. SKIV2L assists the exosome by disrupting RNA secondary structure using the helicase activity, which is necessary for degrading RNA with secondary structures[119]. It was found that a defect in SKIV2L causes hyperactivation of RIG-I and is possibly involved in the pathogenesis of trichohepatoenteric syndrome, a disease associated with mutations in SKIV2L[120]. However, the identity of the RIG-I-stimulatory RNAs and the mechanism of RIG-I activation remain unclear.

In addition to exosome-mediated degradation, cytosolic RNAs can be cleared through a recently described process termed 'RNautophagy', where RNA is directly taken up by lysosomes through the RNA transporter SIDT2 (REF.[121]) (FIG. 3c). SIDT2 is an orthologue of the dsRNA transporter SID-1, and was found to transport dsRNA in a bidirectional manner[122]. Whether deficiency of SIDT2 has a role in accumulation of endogenous dsRNAs would be an interesting area of future investigation.

Dysregulated RNA Pol III. Unlike RNA Pol II transcripts, which are generally processed to contain a 7-methylguanosine cap at the 5′ end, many RNA Pol III transcripts, such as U6 small nuclear RNAs (snRNA), 5S ribosomal RNA, 7SK RNA and 7SL RNA, retain 5′ppp as in nascent transcripts[123], although some acquire a monomethyl group on the γ-phosphate of 5′ppp[124]. Many Pol III transcripts also contain RNA secondary structures, which together with 5′ppp confers them with a potential to stimulate RIG-I (FIG. 3d). Consistent with this notion, several studies suggest that Pol III-transcribed RNAs can activate RIG-I in response to a variety of stimuli. For example, 7SL RNA, a component of the signal recognition particle (SRP) ribonucleoprotein complex, was found to activate RIG-I in breast cancer cells, causing tumorigenic inflammation[125]. Although 7SL RNA is abundant in cells, it does not normally activate RIG-I because it is bound by the protein partner SRP9/14, which shields 5′ppp and secondary structure. In breast cancer cells, 'naked' 7SL RNA accumulates as a result of hyperstimulation of Pol III through MYC activation, without the corresponding increase in the level of SRP9/14. Similarly, other Pol III transcripts were also reported to activate RIG-I in response to infection with several viruses, such as HSV-1, influenza A virus and Kaposi sarcoma-associated herpesvirus[126–128]. More recently, Pol III-mediated RIG-I activation was found to be regulated by the phosphatase DUSP11, which can remove 5′ppp from both endogenous and exogenous RNA[128,129].

Genotoxic stress. Ionizing radiation and other genotoxic stresses can potently activate antiviral signalling pathways, which have recently emerged as important part of cancer therapies. Multiple studies revealed that ionizing radiation-induced antiviral signalling is associated with activation of the cGAS pathway[130–133]. However, more recent studies suggested that both RIG-I and MDA5 also contribute to ionizing radiation-mediated immune activation in a cell type-dependent manner[134,135] (FIG. 3d). Inhibition of the cell cycle checkpoint accelerates immune activation through both the cGAS pathway and the RLR pathway[134,135]. In an independent study, RIG-I, but not MDA5, was found to be activated upon ionizing radiation treatment[136]. Germline deletion of RIG-I, but not MDA5, protected mice from death following total

Dicer
An endoribonuclease specialized in processing double-stranded RNA. It is typically involved in biogenesis of small regulatory RNAs such as microRNAs and small interfering RNAs.

Pseudogenes
DNA sequences that resemble functional genes, but are inactive due to mutations.

RNA exosome
A multisubunit protein complex that catalyses 3′-to-5′ processing or degradation of cellular RNAs

Trichohepatoenteric syndrome
An inherited autosomal recessive condition that affects the hair, liver and intestines. Can be caused by mutations in *SKIV2L* or *TTC37*.

Signal recognition particle (SRP) ribonucleoprotein complex
A ribonucleoprotein complex that recognizes the signal sequence of a nascent peptide and targets it to the endoplasmic reticulum in eukaryotes and the plasma membrane in prokaryotes.

Cell cycle checkpoint
A checkpoint in the eukaryotic cell cycle at which the cell monitors the progression of cell division and decides whether or not to move forward.

body irradiation. LGP2, a third member of the RIG-I-like receptor family, was also found to be important for conferring radioresistance, consistent with its role in inhibiting RIG-I[137].

Exactly which RNAs activate RIG-I or MDA5 upon ionizing radiation exposure remains unclear. The cell-type dependence of the activated receptor suggests that production of immunostimulatory ligands for each receptor differs depending on the cellular context. A study suggests that RNA components of the spliceosome, U1/U2 snRNAs[136], are co-purified with RIG-I upon ionizing radiation treatment. U1/U2 snRNAs are synthesized by Pol II, and thus are co-transcriptionally processed to remove 5′ppp. It is unclear whether RIG-I activation is mediated by fully mature U1/U2 snRNAs or improperly processed RNA still retaining 5′ppp. In another study, it was proposed that RIG-I senses Pol III transcripts of certain cellular DNA fragments generated during ionizing radiation stress[134]. The identity of ionizing radiation-induced RNAs that activate RIG-I and MDA5 and their cell-type dependence need further investigation.

Mitochondrion-derived dsRNAs. Mitochondria have traditionally been thought to be the major organelles for regulating cell metabolism and apoptosis. However, more recent studies have suggested their broader roles in cell stress response and innate immunity[138]. These include the role of mitochondrial DNA in activating cGAS[116,139] and the role of the mitochondrial unfolded protein response in activating the cytosolic integrated stress response[140]. More recently, mitochondrial RNA has emerged as yet another 'danger' signal that indicates cell dysfunction and that can alert the innate immune system.

Mitochondrial RNAs are transcribed from the circular mitochondrial genome in a bidirectional manner[141] (FIG. 3e). Both RNA strands (H and L strands) are synthesized in long polycistronic precursor transcripts, which are processed into mature transcripts by endoribonucleolytic cleavage events. Despite these processing steps being in place, studies found that a detectable level of RNA exists as full-length RNA for both strands, and that sense–antisense hybrids are present[142–145]. The level of mitochondrial dsRNAs increases upon depletion of polynucleotide phosphorylase (PNPase) or SUV3, components of the mitochondrial RNA degradosome[144]. Intriguingly, deficiency in PNPase, but not SUV3, leads to both accumulation of mitochondrial dsRNAs and their leakage into the cytoplasm. This is in line with previous reports implicating PNPase in mitochondrial RNA transport as well as degradation[146,147]. As a result, knockdown of PNPase, but not SUV3, activates MDA5 (REF.[144]). A similar hyperinflammatory phenotype was observed in PNPase-deletion mice and cells derived from patients harbouring biallelic hypomorphic mutations in *PNPT1* (the gene encoding PNPase)[144].

Mitochondrial dsRNA can also activate PKR, but this does not require deficiency of PNPase or SUV3 (REF.[145]). Intriguingly, while the majority of PKR functions in the cytoplasm, a subset of PKR was found in the mitochondrial matrix and intermembrane space, explaining how PKR can be activated by mitochondrial dsRNAs without their erroneous leakage into the cytoplasm[145,148]. However, it remains unclear how PKR enters the mitochondria, and how activated PKR exits mitochondria to phosphorylate eIF2α.

Mitochondrial dsRNA was also proposed to mediate the innate immune response during ionizing radiation exposure[149]. Ionizing radiation was found to damage both nuclear and mitochondrial DNA, and the latter can lead to production of aberrant dsRNAs and their leakage into the cytoplasm through loss of mitochondrial membrane integrity. This observation raises the question as to whether other genotoxic stresses, such as those mediated by DNA-modifying chemotherapeutic agents, can also activate RLRs through similar mechanism involving mitochondria. The specific identity of the RIG-I ligand again requires further studies.

Consequences of endogenous dsRNAs

In the previous section, we described various physiological conditions activating dsRNA sensors where endogenous dsRNA is shown or suspected to mediate the virus-independent, 'sterile' immune activation. As discussed earlier herein, in many cases, the exact identity of the dsRNA remains speculative and requires further research. In this section, we focus on biological consequences of such sterile immune activation involving dsRNA sensors.

There are at least three categories of situations where sterile activation of dsRNA sensors has been observed (FIG. 4). First, sterile activation of dsRNA sensors can occur in a controlled fashion during normal physiological processes and can have important roles in maintaining cellular homeostasis. Second, sterile immune activation, when occurring in an uncontrolled and chronic fashion, can cause a broad range of disorders, including inflammatory disorders. Finally, the immune functions of dsRNA sensors can be leveraged for therapeutic purposes, where therapeutic agents induce endogenous dsRNA production to activate innate immune responses. Here, we describe examples of all three cases.

Normal physiological process. PKR, TLR3 and RLRs were found to be activated in several normal physiological conditions. During mitosis, PKR is activated and regulates the levels of multiple mitotic factors, likely through global suppression of protein synthesis and JNK phosphorylation, to ensure proper progression of mitosis[145,148]. PKR also regulates neuronal excitation during normal brain functions by suppressing the level of IFNγ and promoting GABAergic synaptic transmission[150]. Similarly, sterile activation of TLR3 by dsRNAs released from dead cells and consequent activities of downstream mediators, such as IL-6 and STAT3, are critical for skin regeneration following tissue damage[151]. Finally, activation of RLRs by TE RNA was found to occur during the formation of haematopoietic stem and progenitor cells, and the consequent inflammatory signalling is important for development of these cells[152]. These observations suggest dsRNA can serve as a cellular signalling molecule to coordinate

Unfolded protein response
An evolutionarily conserved adaptive reaction that reduces unfolded protein load to maintain cell viability and function. Depending on the cell type involved and the nature of the stress stimuli, unfolded protein response signalling has different consequences and kinetics.

Integrated stress response
An evolutionarily conserved cellular stress response that downregulates protein synthesis and upregulates specific genes in response to internal or environmental stresses.

JNK
JUN N-terminal kinase (JNK) that plays key roles in many cellular stress and inflammatory signalling pathways.

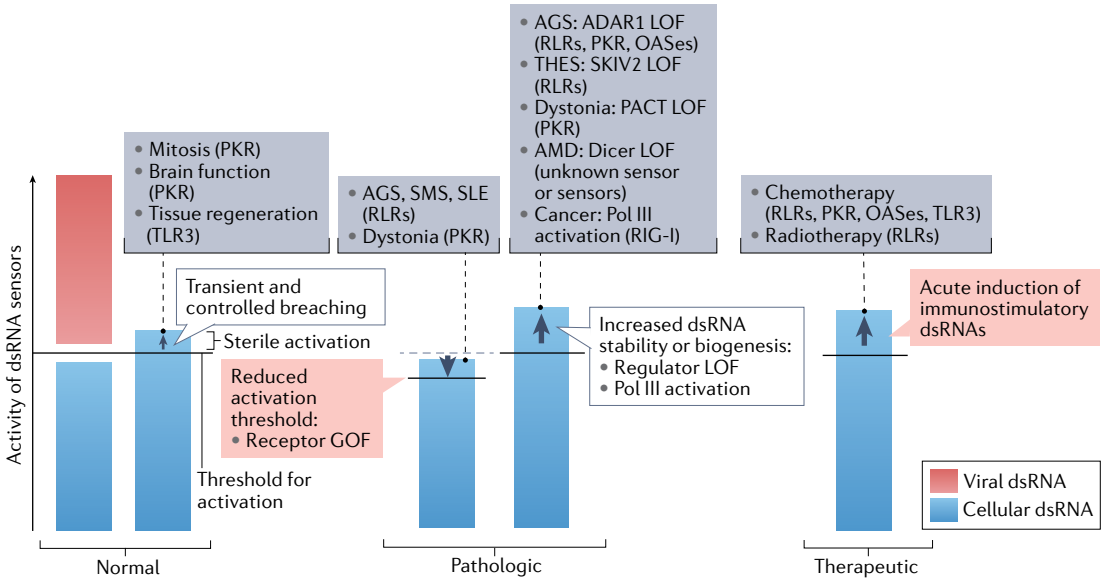


Fig. 4 | **Consequences of dsRNA recognition.** Sterile activation of double-stranded RNA (dsRNA) sensors can occur in normal, pathologic and therapeutic conditions. Here, we use an immunological threshold model to summarize examples in each category. dsRNA sensors have an evolutionarily optimized activation threshold that allows the receptors to tolerate a certain level and certain kinds of dsRNAs (for example, dsRNA with short and imperfect complementarity is normally tolerated by MDA5), while those RNAs beyond the threshold (for example, viral dsRNA) would activate the dsRNA sensors. In normal conditions, the levels of cellular dsRNAs are well below the activation threshold of dsRNA sensors. However, there are cases where a subset of cellular dsRNAs breach the threshold in a transient and controlled fashion, and innate immune functions of the dsRNA sensors are integrated into the normal biological processes. This includes protein kinase R (PKR) activation during mitosis, neuronal excitation in the brain and Toll-like receptor 3 (TLR3) activation during tissue regeneration in the skin. By contrast, constitutive and uncontrolled breaching of the tolerance threshold leads to pathogenesis of immune disorders and other diseases. This can occur through gain-of-function (GOF) mutations of the dsRNA sensor, which lowers the threshold, leading to misrecognition of otherwise inert cellular dsRNA. Such cases include Aicardi–Goutières syndrome (AGS), Singleton–Merten syndrome (SMS) and systemic lupus erythematosus (SLE) caused by GOF RIG-I-like receptors (RLRs) and dystonia caused by GOF PKR. Alternatively, similar diseases can be caused by loss-of-function (LOF) mutations in the regulators. For example, LOF in ADAR1 causes AGS through constitutive activation of RLRs, PKR or oligoadenylate synthases (OASes), LOF in SKIV2 causes trichohepatoenteric syndrome (THES), where constitutive activation of RIG-I is thought to contribute, LOF in PACT causes dystonia through constitutive activation of PKR and LOF in Dicer has been associated with age-related macular degeneration (AMD), although in the last case the exact sensor involved has not been determined. In addition, increased generation of immunostimulatory dsRNAs has been observed in cancer, where aberrant activation of RNA polymerase III (Pol III) downstream of the activity of the oncogenic protein MYC leads to increased generation of RNAs prone to forming secondary structures and RNAs containing 5′-triphosphate, which are recognized by RIG-I. Finally, immune functions of dsRNA sensors can be leveraged in cancer immunotherapy. Chemotherapy and radiotherapy were shown to confer anticancer efficacy partly by inducing biogenesis of immunostimulatory dsRNAs, which activate a broad range of dsRNA sensors.

innate immune response with various steps of normal physiological processes.

Disease process. Earlier studies showed that mice with a single point mutation (G821S) in MDA5 display multi-organ inflammatory symptoms[153]. In humans, gain-of-function mutations in MDA5 and RIG-I were shown to cause a broad spectrum of autoinflammatory diseases, such as Aicardi–Goutières syndrome, Singleton–Merten syndrome, neuroregression and spastic dystonia[154–159]. In many cases, the RNA-binding activity of the receptors is important, suggesting the role of endogenous dsRNAs in driving the pathogenesis[17,154,160–162]. A detailed mechanistic study showed that the mutations increase the ability of MDA5 to form filaments on cellular dsRNAs, which are normally inert due to their structural irregularities, such as mismatches and bulges[17]. For RIG-I, wild-type RIG-I uses a kinetic proofreading mechanism to prevent its oligomerization on self dsRNAs, and the disease mutations impair this regulatory function, allowing activation by self dsRNAs[161,162]. The diseases mentioned above and the mutations involved are rare. More common single-nucleotide polymorphisms in MDA5 have been linked to type 1 diabetes[163,164] and SLE[165,166], where the impact of an individual single-nucleotide polymorphism is probably subtler and context dependent.

Aberrant activation of PKR has also been implicated in several diseases, including dystonia[167,168], SLE[107], Alzheimer disease[169,170] and Huntington disease[171]. However, dystonia is one of the few cases with clear genetic evidence supporting the role of PKR in disease pathogenesis. In a subset of patients with dystonia, mutations were found in the genes encoding PKR and a PKR suppressor, PACT[167,168], both of which lead to the constitutive activation of PKR and the consequent integrated stress response involving translational suppression.

Aicardi–Goutières syndrome
An inherited encephalopathy that affects newborns and usually results in severe mental and physical handicap. It can be caused by gain-of-function mutations in *IFIH1* (the gene encoding MDA5). Loss-of-function mutations in *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1* and *ADAR1* were also shown to cause Aicardi–Goutières syndrome.

Singleton–Merten syndrome
A rare autoimmune disorder characterized by tooth abnormalities, calcifications in the aorta and certain valves of the heart, and osteoporosis in the hands and feet. It can be caused by a gain-of-function mutation in *IFIH1* (the gene encoding MDA5) or *DDX58* (the gene encoding RIG-I).

Single-nucleotide polymorphisms
Germline substitutions of a single nucleotide at a specific position in the genome, the most common type of genetic variation among people.

Vitiligo
An autoimmune disease where the immune system attacks the melanocytes in the skin.

Psoriasis
An autoimmune disease that speeds up the growth cycle of skin cells and causes red, itchy scaly patches over the body.

Studies of dietary and genetic obesity mouse models suggest that excessive nutrient leads to aberrant activation of PKR in adipose and liver tissues, and PKR inhibition ameliorates inflammation triggered by metabolic stress[172].

Mutations in the coding and non-coding regions of NLRP1 have been linked to different types of disease, including metabolic disorders, cancer and autoimmune disorders[173,174]. NLRP1 has seemingly opposing effects by attenuating or augmenting certain cellular processes in a tissue-specific manner. For example, NLRP1 promotes inflammation and exacerbates neurological disorders, cardiopulmonary diseases and cancer, but NLRP1 also protects the gastrointestinal tract and decreases inflammation by modulating the microbiota composition[173]. Polymorphisms that lead to constitutive activation of NLRP1 have been associated with an increased risk of autoimmune diseases, including vitiligo, psoriasis and rheumatoid arthritis[173]. In these cases, however, the potential role of endogenous dsRNA remains unclear, given that NLRP1 can be activated by other PAMPs besides dsRNAs.

Unlike the situations described above, sterile immune activation can also occur without any alteration in dsRNA sensors, but through alteration in endogenous dsRNAs. For example, loss-of-function mutations in ADAR1 lead to an increase in the structural integrity of cellular dsRNAs and thus their stimulatory activity against MDA5, PKR and OASes[17,67,80,82,83], causing Aicardi–Goutières syndrome[175]. Consistent with the importance of A-to-I editing, knock-in of catalytic-deficient ADAR1 in mice also results in MDA5 activation and a similar inflammatory phenotype as in ADAR1 depletion[80]. Although the mechanism is less clear, diseases caused by deficiency of SKIV2 and Dicer (trichohepatoenteric syndrome and age-related macular degeneration, respectively) can be considered in a similar disease category, where altered RNA leads to abnormal cellular response. Finally, tumorigenic inflammation caused by hyperactivation of Pol III and RIG-I is yet another case where altered cellular dsRNA population leads to disease pathogenesis, in this case breast cancer pathogenesis[125].

Therapeutic applications. Whereas chronic inflammation can promote tumour formation, it has become increasingly clear that acute inflammation can be an effective way to promote anticancer immunity. Studies suggest that some cancer cells are particularly susceptible to dsRNA sensor-mediated immune response[176–179]. Additionally, traditional chemotherapy and radiotherapy, which cause epigenetic dysregulation[63–66] and genotoxic stress[17,67,136,149], were also shown to activate multiple dsRNA receptors, including MDA5, RIG-I, PKR, OASes and TLR3 (as described in the section entitled Endogenous sources of dsRNA). Most importantly, the clinical efficacy of radiotherapy and chemotherapy correlates with the level of dsRNA sensor activation, and this innate immune activity synergizes with other immunotherapies[63,64,136]. Therefore, therapies that specifically leverage the acute activation of dsRNA sensors or acute induction of cellular dsRNAs, for example by developing agonists for RLRs[180] or antagonists for ADAR1, would have the potential to contribute to existing cancer immunotherapies.

Conclusions and perspective

Over the last decade or so, our understanding of innate immune sensors for dsRNA and their signalling pathways has greatly improved at the level of structure and biochemical reconstitution. At the same time, there has been a rapid expansion of the list of human diseases and biological processes that involve sterile activation of dsRNA sensors. These studies identified new links connecting antiviral innate immune responses to diverse cellular processes, from the DNA damage response to neuronal excitation. Collectively, these observations support a model that dsRNAs are not simply PAMPs but are a new form of cellular signalling molecules that can alert cells to the presence of 'danger' or other biological processes that require involvement of antiviral immune response.

The diverse array of cellular sources for dsRNAs also implies that there are at least equally diverse and complex regulatory mechanisms at the level of both endogenous RNA and dsRNA sensors to prevent constitutive and uncontrolled activation of the dsRNA sensors. While multiple regulators of the dsRNA sensing pathways have been identified, it remains unclear exactly how these individual regulatory nodes work together and whether their dysregulation contributes to human diseases. Additionally, several dsRNA-binding proteins, such as ZBP1 (REFS[181,182]) and DHX9 (REF.[183]), have emerged as important regulators of cellular response to dsRNAs, but the detailed molecular mechanisms and their interactions with cellular dsRNAs need further investigation. Finally, it is also important to note that the precise identity of cellular dsRNAs that mediate sterile activation of the dsRNA sensors remain unclear in many cases. For instance, RNAs from repetitive elements have often been shown to associate with dsRNA sensors, but their functional connection has been difficult to probe, largely due to the lack of feasibility to genetically perturb the repetitive elements. Biochemical approaches to demonstrate the importance of such interactions beyond binding, for example in a biochemically reconstituted signalling pathway[17], are necessary. Altogether, these future efforts will enable us to create a complete blueprint for cell biology of dsRNA.

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