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The response of mammalian cells to double-stranded RNA

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Abstract

Double-stranded RNA (dsRNA) has long been recognized as a central component of the interferon (IFN) system. It was originally characterized as a key mediator of IFN induction in response to virus infection. Subsequently, it was identified as a prime activator of the antiviral response. In recent years the discovery of RNA interference (RNAi) pathway in mammals has renewed interest in dsRNA-mediated cellular responses. This has coincided with the identification of key components of the IFN induction pathway. Here, we present an overview of the current knowledge of dsRNA-mediated pathways in mammalian cells and introduce a link between these pathways and application of RNAi.

Keywords

Double-stranded RNA; RNA interference; Innate immunity

1. Introduction: two pathways, one substrate

Historically, double-stranded RNA (dsRNA) has been considered as a byproduct of viral replication in mammalian cells for more than 30 years. This property of replicating viruses is exploited at the cellular level to signal infection, restrict virus growth and limit viral spread. Once cytoplasmic sensors detect dsRNA, a chain of events is activated promoting inhibition of protein synthesis, transcriptional induction of interferon and other cytokines, and ultimately, cell death. This response to viral dsRNA is a key component of the interferon (IFN) system, and constitutes the first line of defense to limit viral replication. Studies with gene-deleted mice show that it is an integral component of the host innate immune response, ensuring survival of the infected organism.

Although dsRNAs are clearly associated with viral sensing, it is now established that endogenous dsRNAs such as microRNAs (miRNAs) are also constantly synthesized by the cell. The recent discovery of the RNA interference (RNAi) pathway has brought to attention a vast post-transcriptional regulation by endogenous and foreign dsRNAs, conserved through evolution (reviewed by Rana [1]). RNAi however, is unlikely to be involved as an antiviral mechanism in mammals, in contrast to lower organisms (reviewed by Cullen [2]). Given the potential of the innate immune response to promote a generalized antiviral effect at a multicellular level, it is reasonable to hypothesize that the RNAi machinery has been conserved in mammals for other purpose(s), involving endogenous dsRNAs, including miRNAs. The current information on miRNAs clearly establishes the importance of these dsRNAs in the regulation of gene expression (reviewed by Ambros [3]), independent of the activation of the innate immune response. This changes the former paradigm of viral sensing and rather suggests

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that the cell differentiates between self and non-self dsRNAs on criteria other than the double-stranded structure.

2. Innate immune pathways activated by dsRNAs in mammalian cells

As mentioned above, dsRNA-activated innate immune pathways in mammalian cells promote inhibition of protein synthesis, transcriptional induction of antiviral genes, and ultimately, cell death (Fig. 1). The pathways responsible for each of these events can be largely separated, despite some degree of crosstalk between them.

2.1. dsRNAs in innate immunity

2.1.1. Viral dsRNAs—The origin of viral dsRNA is diverse. RNA viruses with double-stranded genomes induce the innate immune response through their genome itself. This property, however, is not exclusive to these viruses, and it is generally accepted that most viruses also produce dsRNAs when replicating (reviewed by Kumar and Carmichael [4] and Barber [5]). For instance, single-stranded RNA (ssRNA) viruses can produce dsRNA molecules during replication, or single-stranded transcripts or part of the genome can anneal and form dsRNA motifs. In adenoviruses, dsRNA arises from bidirectional transcription of overlapping genes. Interestingly, as recently suggested by the work of Weber *et al.*, not all viruses seem to produce dsRNA signals [6]. Using a dsRNA-specific antibody, this group confirmed that positive-strand RNA viruses (such as encephalomyocarditis virus - EMCV) and dsRNA viruses together with adenoviruses, were producing significant amounts of dsRNA. However, Weber *et al.* [6] were unable to detect significant amounts of dsRNA with several negative-strand RNA viruses (among which were Sendai virus and Newcastle disease virus - NDV). Given that Sendai virus and NDV are potent inducers of IFN and innate immunity, these results indicated that the synthesis of high amounts of dsRNA is not essential to activate the innate immune response.

2.1.2. dsRNA substitutes—In the laboratory, most studies looking at the dsRNA-mediated-IFN antiviral response do not use purified viral RNA, but preferentially rely on T7-synthesized dsRNA, polyinosinic acid: polycytidylic acid (poly(I:C)), or more recently, chemically synthesized dsRNAs. Synthetic poly(I:C), which consists of stretches of complementary homopolymers of inosine and cytidine forming dsRNA-like motifs, has been extensively used to mimic dsRNA since its discovery half a century ago because of its ease of use compared to natural dsRNA [7]. However, because it is composed of stretches of ribonucleotides annealed together and forming dsRNA motifs of varied size, and because it relies on inosine (a relatively rare ribonucleotide involved in RNA editing), poly(I:C) is not a perfect substitute for viral dsRNA. With the recent discovery of RNA-sequence-dependent antiviral pathways (through toll-like receptors (TLR) 7 and 8) and its lack of complete dsRNA structure, the use of synthetic poly(I:C) can introduce a potential bias in the evaluation of antiviral pathways [8,9]. Another source of dsRNA relies on *in vitro* synthesis of RNA using bacteriophage RNA polymerases. As further developed in section 4, the triphosphate group added to the 5′ of bacteriophage polymerase transcripts has recently been implicated in direct activation of type-I IFNs [10, 11,12,13]; this suggests that in many previous reports, activation of innate immune response to *in vitro* synthesised dsRNAs was an artefact of the synthesis, independent of the double-stranded nature of the RNA. Finally, with the application of RNAi to mammalian cells, a new range of chemically synthesized dsRNAs, ranging from 19 to 27 bp, are now routinely used by many laboratories.

2.2. Historical background: dsRNA-induced translational inhibition mechanisms

The discovery of the link between dsRNA and antiviral activity of IFN can be traced to pioneering studies from the Kerr laboratory in the early 1970s [14], studying translation of

viral mRNA in extracts from IFN-treated cells. The biochemical pathways have now been well characterized (Fig. 1, see [15,16], **see also RH Silverman, this issue pp….**) and are constituted by the dsRNA-activated protein kinase (PKR) and the 2′-5′-oligoadenylate synthetases/RNase L systems. Although recently questioned (see section 2.3.3), the importance of these pathways is demonstrated by the lack of translational inhibition following treatment with dsRNA in mouse embryo fibroblasts derived from PKR/RNase L double knockout (KO) mice [17].

2.2.1, dsRNA-activated protein kinase, PKR—PKR is an IFN-inducible dsRNA-activated Ser/Thr protein kinase [18]. In the early 1970s, studies investigating the potential of dsRNA to trigger IFN activation, highlighted that dsRNA was a strong inducer of cell death [19]. Most interestingly, this effect was magnified by pre-treatment of the cells with IFN. Coincidently, studies of dsRNA-induced translation inhibition led to the identification, using poly(I:C) affinity columns, of a novel kinase activity present in cell lysates from IFN-treated cells [20]. This dsRNA-activated protein kinase (also known as Eif2ak2, Prkr, Tik, DAI, P1-eIF-2 and p68 kinase, but now commonly known as PKR), inhibits mRNA translation via the phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF-2α) (see Williams and Sadler [16]). PKR is composed of two dsRNA-binding domains (DRBDs) and a kinase domain [21]. The DRBDs bind specifically to dsRNAs and ssRNAs with extensive secondary structures, with no sequence specificity and no affinity for dsDNA or ssDNA [22]. dsRNA fragments shorter than 30 bp do not bind stably to PKR and do not activate the enzyme to any significant degree [23]; similarly, long dsRNAs do not activate PKR when used at high concentration but rather inhibit its activity. However, as little as 16 bp of dsRNA (with a ssRNA tail of ~10-15 nucleotides (nt)) is able activate PKR [24]. More recently we observed that small interfering RNAs (siRNAs) ranging from 21-27 nt could activate PKR *in vitro*, but are poor activators *in vivo* following transfection of siRNAs into cultured cells [25]. This suggests that PKR activity *in vivo* is likely to be regulated by co-factors and availability of the substrate.

PKR is constitutively expressed at low levels in the cell cytoplasm in an unphosphorylated state, associated with ribosomes; a small fraction of PKR is also present in the nucleus [26]. When dsRNA binds to PKR, a conformational change, is induced resulting in unmasking of the kinase domain and autophosphorylation [27]. Subsequently, PKR can phosphorylate different substrates, the most well characterized of which is eIF-2α [28] (Fig. 1). Phosphorylation of eIF-2α on serine 51 results in the strong binding of eIF-2α to eIF-2B, subsequently inhibiting protein synthesis [29], even when as little as 20% of eIF-2α is phosphorylated [27]. Consequently, the prevalent role of activated PKR is protein synthesis inhibition, conferring an antiviral state. PKR can also promote phosphorylation of IκB via the IKK complex, thus activating transcription factor nuclear factor κB (NF-κB) [30]. PKR has also been involved in the activation of IFN regulatory factor 1 (IRF1) [31], and in pathways involving STAT-1 [5]. Finally, PKR activated by different stimuli including dsRNA promotes apoptosis in a variety of cell types [15,27].

2.2.2. 2′-5′-Oligoadenylate synthetase—The IFN-inducible 2′-5′-oligoadenylate synthetases (2′-5′OAS) are activated by binding of dsRNAs longer than 15 bp, and were among the first antiviral proteins characterized (reviewed by Rebouillat and Hovanessian [32]). We have observed that small RNAs ranging from 21 to 27 bp are very poor activators of OAS *in vitro* (JT Marques, R Hartmann and BRG Williams, unpublished observations). In mammals, this family of proteins is composed of four members, namely OAS1, OAS2, OAS3 and OASL. Crystallographic analysis of porcine 2′-5′OAS revealed that two domains assemble to form a dsRNA activation site; upon binding of dsRNA, a conformational change promotes activation of the enzyme [33]. Activated 2′-5′OAS enzymes convert ATP to PP_i and 2′-5′-linked oligoadenylates (2-5A) ranging from dimers up to 30-mers [33]. 2-5A binding to RNase L activate its dimerization. When dimerized, the cytoplasmic RNase L catalyses the degradation of RNAs (both self and non-self), resulting in a generalized drop of protein synthesis [34] (**see**

also Silverman RH page …. this issue). As with PKR, activated RNase L has also been implicated in the induction of apoptosis [35].

2.3. dsRNA-activated pathways involved in transcriptional response

Type-I IFNs were the first genes identified as dsRNA regulated [36]. It is now known that following interactions with host cell sensors, viral dsRNA promotes direct or indirect transcriptional induction of many genes via activation of a few essential transcription factors, the IRF family being of prime importance. This family of transcription factors has nine members (recently reviewed by Honda *et al.* [37]) four of which, IRF1, 3, 5 and 7, have been shown to induce type-I IFN. The production of type-I IFNs results in an induction of about one thousand genes through interferon-stimulated response elements by the signal transducer and activator of transcription family (STAT) [38,39]. Independent to the activation of interferon-stimulated genes (ISGs), dsRNA sensing by the cells also stimulates direct induction of several genes, via IRFs or NF-κB. These have been termed viral stress inducible genes [40] and different dsRNA sensors are involved in their induction.

2.3.1. TLR3—TLRs are a family of proteins recognizing different pathogen-associated molecular patterns (PAMPs). Once engaged to the TLRs, PAMPs trigger the expression of specific genes, the products of which are engaged to remove and eliminate the invading pathogen (reviewed by Schroeder and Bowie [41]). TLR3, which mediates responses to both viral dsRNA and synthetic poly(I:C), is expressed in the cytoplasm of peripheral blood mononuclear cell (PBMC) subtypes, including monocyte-derived immature dendritic cells [42], or at the cell surface of selected cell types [43]. Engagement of the TLR3 signaling pathway leads to the activation of NFκB and IRF3 transcription factors in a TRIF (toll/IL-1 receptor domain-containing adaptor inducing IFN-β) dependent manner [44], promoting expression of type-I IFNs and pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) (Fig. 2). The discovery of TLR3 raised new questions about dsRNA-mediated type-I IFN activation and identified the existence of dsRNA sensors other than PKR. This was consistent with the observation that PKR^{-}null mice retained certain poly(I:C)-induced IFN responses [45].

2.3.2. TLR7 and 8—The structurally highly conserved TLR7 and TLR8 recognize dsRNAs as short as 19-21 bp [46-48], in addition to ssRNA (reviewed by Schlee *et al.* [49]). TLR7/8 are both constitutively expressed at high levels in specific PBMC subsets compared to other tissues. Human TLR7 is highly expressed in plasmacytoid DCs (pDCs), whereas human TLR8 is mainly found in monocytes [8,50]. Unlike TLR3 (and TLR4), which signals viral detection through TRIF adaptor, TLR7 and 8 signal via the MyD88 adaptor which can directly associate with IRF7 in pDCs (Fig. 2) [51]. Human TLR8 is mainly expressed in cells devoid of constitutive IRF7, and principally signals through NF-κB, inducing pro-inflammatory cytokines and chemokines (including TNF-α, IL-6, IL-12 and IL-1β). The antiviral properties of these two TLRs have been highlighted by the findings that ssRNAs from HIV can trigger TLR7/8 [9], and that TLR7 is required for pDC recognition of live or heat-inactivated influenza [8]. More recently, Schlaepfer *et al*. [52] have suggested that TLR7/8 agonists are able to exert potent anti-HIV effects.

2.3.3. RIG-I, MDA5 and LGP2: helicases modulating innate immunity The finding that TLR3^{-}null cells retained responses to poly(I:C), similar to PKR^{-}null cells, further demonstrated that other cytoplasmic receptors were involved in IFN induction in response to dsRNA [53]. The recently discovered cytoplasmic RNA helicase RIG-I (retinoic acid inducible gene I) and melanoma differentiation associated gene-5 (MDA5) are key elements in this recognition [54], as confirmed by the involvement of RIG-I and MDA5 in type-I IFN signaling by cytoplasmic dsRNAs (Fig. 2) [55-59].

Interferon inducible RIG-I is a cytoplasmic DExD/H box RNA helicase, whose ATPase activity is dependent on dsRNA [54]. Full-length RIG-I has one RNA helicase domain and two caspase recruitment domains (CARDs) through which RIG-I promotes type-I IFN activation [54]. Several studies have further qualified the activation pathways downstream of RIG-I, highlighting the role of MAVS/IPS-1/VISA/CARDIF as the main connector of RIG-I activation to IFN-β activation (reviewed by Hiscott *et al.* [60]). Activation of RIG-I by cytoplasmic dsRNAs results in activation of IRF-3 and NF-κB [54,57,58], thus promoting IFN-β expression. The type-I IFN responses to the ssRNA virus NDV were abrogated in RIG-I null mouse embryonic fibroblasts and conventional non-plasmacytoid DCs (cDCs) but not in $MyD88^{-/-}TRIF^{-/-}$ double KO, giving credence to the concept that RIG-I is the *bona fide* cytoplasmic sensor of some viral dsRNAs in certain cell types such as fibroblasts and cDCs [57].

MDA5 (also referred to as Helicard) was first suggested to play a role in the cytoplasmic sensing of viral dsRNA because of its sequence homology with RIG-I [54] and its ability to activate IFN-β expression (via IRF3 and NF-κB) upon overexpression and cell treatment with poly(I:C) [61]. MDA5 is composed of one DExD/H box RNA helicase and two CARD-like domains [62]. MDA5 is expressed ubiquitously at low levels and is induced by IFN-β and TNF-α. Studies of $MDA5^{-/-}$ KO mice have recently demonstrated that this protein, like RIG-I, is a key mediator of virally induced type-I IFN signaling [59]. In accordance with an antiviral role for MDA5, V proteins of paramoxyviruses were shown to bind MDA5 and inhibit poly(I:C)-induced IFN-β activation [61]. MDA5 has been proposed to be the prime sensor of poly(I:C)-mediated type-I IFN induction, together with the detection of EMCV independently of RIG-I [59,63].

LGP2 is another RNA helicase related to RIG-I and MDA5 through its helicase domain, but lacking the CARD-like domain [54]. Overexpression of LGP2 counteracts RIG-I activation by Sendai virus and NDV, by inhibiting IRF and NF-κB activation, probably via an interaction with CARDIF [64,65]. Even though the role of this helicase is yet to be fully understood, its negative regulatory effect on RIG-I activity, together with its inducibility, suggests it acts as a negative feedback of RIG-I sensing during the late response of IFN signaling (Fig. 2).

2.4. Programmed cell death

The sensing of viral infection by dsRNAs byproducts activates a complex signaling mechanism affecting both infected and neighboring cells. This signaling relies on specific cytokines aiding the differentiation of the cells involved in adaptive immunity. However, once infected, the most efficient way to block further propagation of the pathogen is programmed cell death [66]. The potential of apoptosis to contain viruses has been demonstrated in many instances, following the principle that the higher the sensitivity to apoptosis, the less severe the infection [17]. In accordance with this, an artificial model of vaccinia virus infection of *Caenorhabditis elegans* demonstrated that the worm programmed cell death pathway is an important component of antiviral defense [67]. Furthermore, several viruses encode a variety of anti-apoptotic molecules, underlining the importance of apoptosis in antiviral defense [68]. A role for dsRNA produced during viral infection of mammalian cells in apoptosis has been proposed, but the details of the mechanism underlying this response remain under investigation [69] (JT Marques *et al.*, submitted). Death receptor signaling, via caspase-8 activation, plays a role in dsRNA-induced apoptosis independently of the mitochondrial pathway [70]. Moreover, PKR, RNase L, IRF3 and c-Jun N-terminal kinase are some of the components of the pro-apoptotic pathways activated by dsRNA, while NF-κB promotes survival [71-74,75,76].

Therefore dsRNA signals both viral infection and induces apoptosis, highlighting its converging antiviral roles. From this perspective, it is hardly surprising that dsRNA has almost

exclusively been associated with viral infections in mammalian cells. However, the discovery of RNAi has increased interest and shed new light on the biology surrounding dsRNAs.

3. dsRNAs, RNAi and innate immunity

Since its discovery in 1998, the RNAi pathway has already been the subject of numerous reviews (see Rana for a recent review [1]); here we will briefly focus on the discovery of RNAi in mammals, and its relationship to innate immunity.

3.1. Discovery of a functional RNAi pathway in mammalian cells

In mammalian cells, the initial attempts at RNAi and gene-specific down-regulation were unsuccessful. For instance, Tuschl *et al.* reported in 1999 non-specific mRNA degradation when *in vitro* synthesized long dsRNAs (> 30 bp) were added to rabbit reticulocyte lysates [77]. This was attributed to the activation of RNase L by the long dsRNA, previously demonstrated *in vitro* in similar cellular extracts [78]. Caplen *et al.* [79] later found that *in vitro* synthesized long dsRNAs were potent triggers of a non-specific gene down-regulation in BHK21 cells but not in human embryonic kidney 293 cells. These studies raised the issue of dsRNA-induced translational inhibition, through PKR or 2′-5′OAS and activation of the innate immune response as a potential limit to the use of RNAi in mammalian cells [77]. Wianny *et al.* and Svoboda *et al.* were first to report conservation of RNAi in mammals [80,81]. They showed specific gene silencing with transfected long dsRNAs in preimplantation embryos and mouse oocytes, introducing the concept that RNAi could also be used for loss-of-function studies in mammals in cells lacking an IFN response [80-83].

3.2. Transfected siRNAs can mediate specific gene silencing

Tuschl and colleagues [84] reported that 21-23 nt dsRNAs are the specific ‘probes’ used by the RNAi machinery to promote specific mRNA degradation. Using Drosophila cell extracts, they showed that the introduction of chemically synthesized small interfering RNAs (siRNAs), i.e. 21-22 nt dsRNAs with 2 nt overhangs at their 3′ end, could potently promote specific mRNA degradation [84]. Subsequently, they assessed whether or not siRNAs could bypass the ‘dsRNA-sensors’ of the innate immune response [85]. One of the key assumptions underlying this work originated from the concept that PKR was the major sensor of the type-I IFN response to non-self dsRNAs in mammals, and that PKR was not activated by dsRNAs shorter than 30 bp [23]. In addition, 2′-5′OAS isozymes, the other key components of the translational arm of the innate response, were speculated not to be activated by short dsRNAs [86]. Subsequently it was shown that transfected siRNAs were potent inducers of RNAi in several mammalian cultured cell lines and sequence-specific down-regulation of target proteins was demonstrated [85]. Moreover, they confirmed that *in vitro* synthesized long dsRNAs were potent inducers of a non-specific protein down-regulation, although the data also implied simultaneous processing of the long dsRNAs and activation of RNAi by long dsRNAs [85]. Importantly, these studies were carried out before the discovery of TLR3, RIG-I and MDA5.

4. Overlapping of the pathways

With the discovery of the key role for RIG-I and MDA5 in the detection of cytoplasmic dsRNAs and the revision of the role of PKR and 2′-5′OAS as major facilitators of the antiviral response, it is now clear that short RNAs (such as siRNAs and miRNAs) can be differentiated from viral dsRNAs by innate immune sensors on criteria other than their double-stranded structure or their short size.

It is now apparent that dsRNAs can induce type-I IFNs differentially, depending on whether they are endogenous, *in vitro* synthesized, synthetic or of viral origin. Mammalian cells do not detect all dsRNAs and dsRNA analogs in the same way. Moreover, the discovery of TLR3,

RIG-I and MDA5 has provided a better understanding of how cells deal with foreign or non-self dsRNAs. It is noteworthy that none of these proteins has the evolutionarily conserved double-stranded RNA binding domain present in different dsRNA-binding proteins including PKR, Dicer and TRBP [87]; this lack of DRBDs results in poor affinity for dsRNA substrates, and underlines that activation of innate immunity by these receptors is not uniquely related to the double-stranded structure of dsRNAs. Indeed, RIG-I and MDA5 have a poor affinity for dsRNAs through their helicase domain when compared to traditional DRBDs [25]. Yet, in an ATP-independent fashion, RIG-I binds to chemically synthesized dsRNA products as short as 21-23 bp, independently of the 3′ termini [25]. Depending on the presence of ATP and the structure of the 3′ termini, RIG-I then unwinds the two strands of the dsRNA, resulting in an exposure of the CARD and further signaling through IRF3/7 activation [25]. We have shown that conventional Dicer products, i.e. small dsRNAs of 21-23 bp with a 3′-2 nt overhang, bind to RIG-I but do not activate its helicase activity in RIG-I-expressing cell lines [25]. However, similar dsRNA products with a minimum of one 5− blunt end and a 3′ single-stranded extension are potent inducers of RIG-I helicase activity [25].

These findings suggest that RIG-I can differentiate between self dsRNAs such as pre-miRNAs (with a 3′ overhang and a stem loop) and non-self dsRNAs from viral replication, based on the presence of protruding motifs at the end of the dsRNAs. Thus the end configuration of dsRNAs is a new parameter in their induction of innate immunity.

Other recent studies have further highlighted that the specific features of non-self dsRNA are of prime importance in the activation of innate immunity. In their work, Hornung *et al.* and Pilchmair *et al.* [11,12] provided supporting evidence that RIG-I preferentially binds to 5′-triphosphate RNAs, regardless of the double-stranded structure. In addition, using injection into $MDA5^{-/-}$ whole mice, poly(I:C) was shown to signal IFN-α/β induction through MDA5 but not RIG-I [59,63]. Following these observations, it would be expected that these distinct abilities of RIG-I and MDA5 would selectively be recruited according to the virus infecting the cell and its ability to produce dsRNA .

To date, it has been demonstrated that RIG-I is essential for the type-I IFN response to negative-strand RNA viruses such as NDV, Sendai virus, influenza virus, vesicular stomatitis virus, and positive-strand Japanese encephalitis virus or Hepatitis C virus [59,88,89]. On the other hand, the response to positive-strand EMCV [59,63], and negative-strand measles virus [90] is mainly mediated via MDA5.

Collectively, these findings infer that in the case of non-self RNAs, RIG-I binds preferentially to genomic RNAs with 5′-triphosphate RNA, while high levels of dsRNA products generated during viral replication (e.g. from EMCV) would be detected through MDA5. This model of RIG-I/MDA5 sensing of dsRNA is supported by the findings that some *in vitro* synthesized RNAs can be potent inducers of type-I IFNs in certain cell types [10,25,91,92], and the suggestion that this results from a 5′-triphosphate group added by the bacteriophage RNA polymerase, not the double stranded structure of the RNA [10-12]. However, the ability to detect IFN-β activation with short dsRNAs lacking 5'-triphosphate [25] with the fact that some endogenous RNA polymerase III RNAs with 5'-triphosphate (such as 7SL RNA [93,94]) are present in high amounts in the cell cytoplasm underlines the complexity of dsRNA sensing by RIG-I.

5. Concluding remarks

Double-stranded RNAs have moved from interest as viral by-products, to playing critical roles as endogenous regulators of cell development. The potential involvement of dsRNAs in the regulation of gene expression at the epigenetic level emphasizes their importance in the regulation of gene expression [95,96]. We propose a model where the distinction between self

and non-self dsRNA relies on different structural features including 5′-triphosphate, 3′ protruding end or other specific motifs (Fig. 2). With the discovery of RNA helicases RIG-I and MDA5, and TLRs 7/8, a new paradigm emerges where different dsRNAs are selectively recognized by different sensors of the innate immune response. Furthermore, differential responses can be mounted in a cell-type specific manner, with the repertoire of innate antiviral responses closely related to the detection of the pathogens. This implies that the innate immune response is more *adapted* than previously envisioned, and that sensing of the pathogens has a strong impact on the outcome of the adaptive immune response itself.

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Biographies

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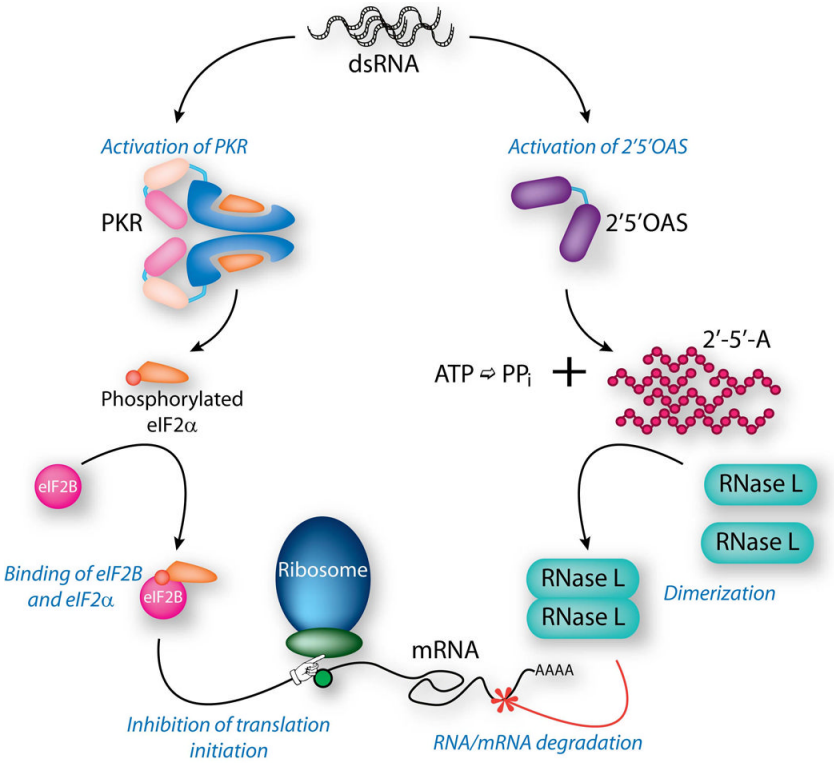

Fig. 1. dsRNA-induced translation inhibition mechanisms

dsRNA promotes activation of PKR and 2'5'OAS. PKR phosphorylates eIF2α, which results in sequestration of eIF2B and inhibition of translation initiation by the ribosomal complex [29]. Activated 2'5'OAS promotes RNaseL dimerization via the formation of 2'-5'oligoadenylates [33]. RNaseL subsequently degrades RNAs non-specifically. These two events fortify the antiviral state by stopping protein synthesis.

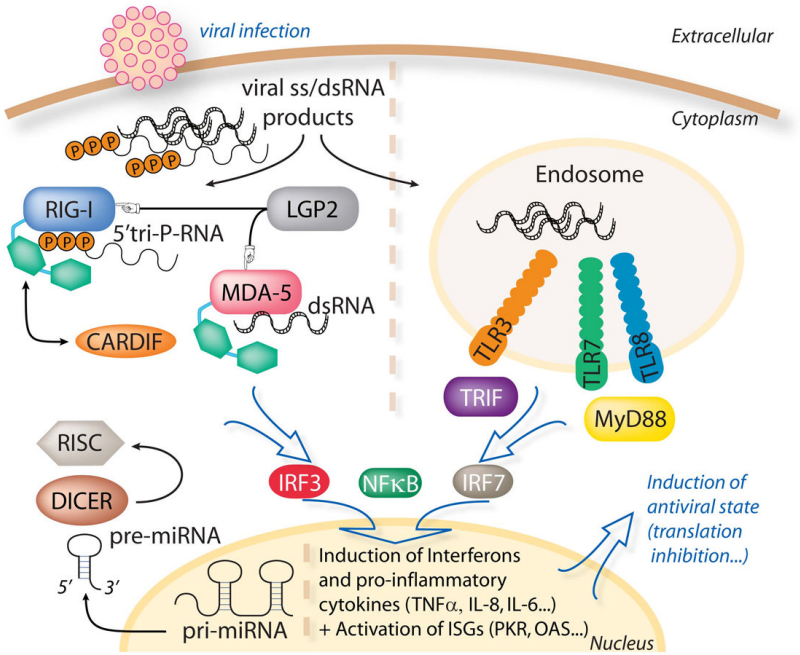

Fig. 2. Sensing of ssRNA/dsRNA by the immune system

The detection by viral ss/dsRNA products by the cell is cell-type dependent. On the left of the diagram, sensing in non-immune cell types is represented. This relies on RIG-I (for RNAs with 5'-triphosphate) and MDA-5 (for dsRNAs), and is negatively regulated by LGP2 [11,12,59, 65,97]. CARDIF transduces the activation of RIG-I, resulting in activation of transcription factors [60]. On the right of the schematic, innate immune sensing of ss/dsRNAs by endosomal TLRs in immune cells is represented. Through activation and recruitement of adaptors (TRIF and MyD88), TRL3, 7 and 8 activate IRFs and NF-κB transcription factors [98]. These transcription factors induce expression of IFNs and pro-inflammatory cytokines. This further results in the induction of ISGs such as PKR/2'5'OAS and confers a general antiviral state by affecting cell growth and ultimately provoking cell death. In parallel, endogenous cytoplasmic dsRNAs such as pre-miRNAs are specifically recognised by DICER because of their protruding 3'-end, preventing potential recognition by RIG-I [25].