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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 the 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 by-product 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 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

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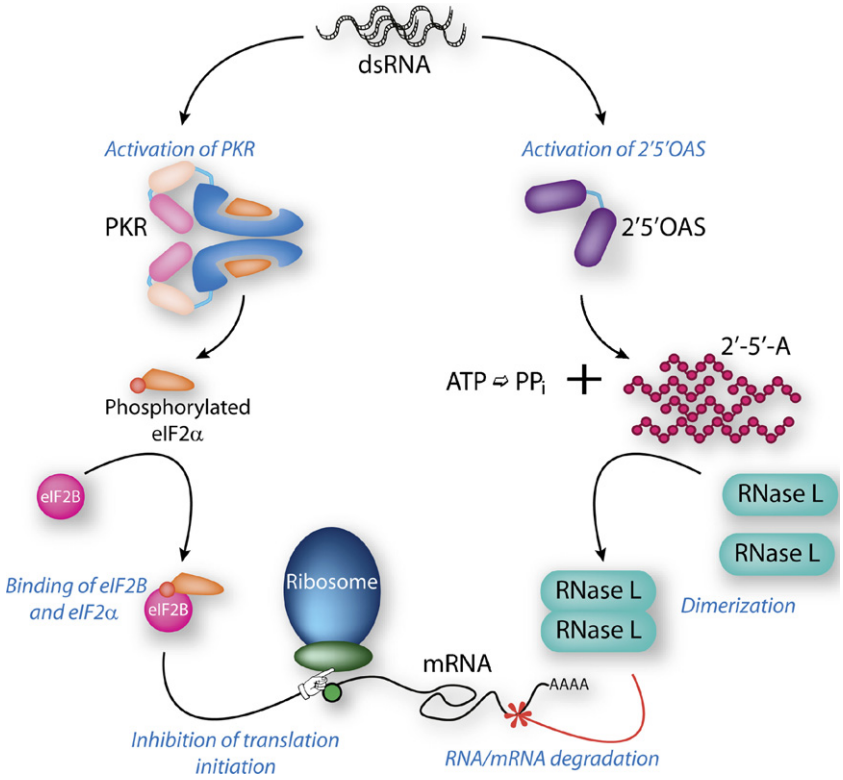

Fig. 1. dsRNA-induced translation inhibition mechanisms. dsRNA promotes activation of PKR and 2′-5′OAS. PKR phosphorylates eIF2α, which results in sequestration of eIF2B and inhibition of translation initiation by the ribosomal complex [29]. Activated 2′-5′OAS promotes RNaseL dimerization via the formation of 2′-5′oligoadenylates [33]. RNaseL subsequently degrades RNAs non-specifically. These two events fortify the antiviral state by stopping protein synthesis.

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