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Signalling networks in focus

Control of mRNA splicing by noncoding intragenic RNA elements that evoke a cellular stress response

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ABSTRACT

Once activated by double-helical RNA, mammalian RNA-dependent stress protein kinase, PKR, phosphorylates its substrate, translation initiation factor eIF2α, to inhibit translation. eIF2α phosphorylation is critical for mounting a cellular stress response. We describe short, 100–200 nucleotide elements within cellular genes that, once transcribed, form RNA structures that potently activate PKR in the vicinity of the RNA and thereby tightly regulate gene expression in cis. Intragenic RNA activators of PKR can (a) attenuate translation of the encoded mRNA by activating PKR and inducing eIF2α phosphorylation, exemplified by the IFN-γ gene, or (b) greatly enhance mRNA splicing efficiency by activating PKR and inducing nuclear eIF2α phosphorylation, thus enabling efficient early spliceosome assembly, exemplified by the adult and fetal globin genes and the TNF-α gene that activates PKR through an RNA pseudoknot conserved from fish to humans. These opposite outcomes considerably extend the potential scope of gene regulation by these novel RNA elements.

Signalling Network Facts

- Activation of PKR greatly increases mRNA splicing efficiency
- Once activated, PKR directly controls gene expression by phosphorylating eIF2α on Serine51
- PKR activation and eIF2α phosphorylation control *TNF-α* mRNA splicing in human peripheral blood mononuclear cells
- PKR activation and eIF2α phosphorylation control *globin* mRNA splicing in nuclear extract and in human cells
- PKR activation and eIF2α phosphorylation are needed for an early step in spliceosome assembly, formation of Complex A
- Further insight into PKR signalling in splicing can be found at https://www.ncbi.nlm.nih.gov/pubmed/28683312 and https://www.ncbi.nlm.nih.gov/pubmed/28374749.

1. Introduction

1.1. The RNA-dependent protein kinase PKR

Cells respond to environmental stress by rapidly changing their capacity to translate specific mRNAs. Translational control occurs primarily at the initiation step. Stress induces a transient, reversible phosphorylation of the α chain of eukaryotic initiation factor 2 (eIF2α), blocking GDP/GTP exchange needed for recycling of eIF2 between rounds of translation and thus inhibiting protein synthesis (Sonenberg and Hinnebusch, 2009). Phosphorylation of eIF2α is critical for mounting the integrated cellular stress response (Harding et al., 2003; Muaddi et al., 2010). A prominent eIF2α kinase is PKR, a serine/threonine kinase expressed in latent form in most cells. PKR plays an important part in signal transduction, apoptosis, cell growth and differentiation. PKR has an essential role in the interferon (IFN)-mediated antiviral response. The action of IFNs, including that of IFN-γ, involves induction of high levels of PKR in the cell (Stark et al., 1998). However, the kinase is not yet activated. Activation of PKR requires its *trans*-autophosphorylation and depends on double-stranded RNA produced during virus replication (Meurs et al., 1990). PKR contains tandem double-stranded RNA binding motifs. Activation of PKR requires highly ordered double-stranded RNA structures rather than specific sequences (Bevilacqua and Cech, 1996). Phosphorylation of eIF2α by PKR, once activated by double-stranded RNA, leads to inhibition of translation in the cell, blocking virus spread and inducing apoptosis of infected cells (Stark et al., 1998).

1.2. Cis-acting intragenic elements that activate PKR

As just reviewed, the classical view has been that viral double-

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[1] https://medicine.ekmd.huji.ac.il/en/home/pages/home.aspx.

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stranded RNA produced during infection causes the activation of PKR, resulting in inhibition of global protein synthesis that constitutes a major mechanism of host defense against viral infection. We have discovered novel, short noncoding elements within cellular genes. Once transcribed into single-stranded RNA, these elements fold into structures that activate PKR far more potently than does linear double-helical RNA and use this property to greatly enhance splicing (Osman et al., 1999; Ilan et al., 2017; Namer et al., 2017) or to repress translation (Ben-Asouli et al., 2002; Cohen-Chalamish et al., 2009) of the cellular gene harboring the element. Both regulatory outcomes depend on phosphorylation of eIF2α, which is a hallmark of the integrated cellular stress response. Intragenic RNA activators of PKR thus constitute a novel class of regulatory RNA elements.

2. Intragenic element controls *IFN*-γ mRNA translation by activating PKR

In line with the classical function of PKR in negative control of translation, we found, through a genetic approach, that human *IFN*-γ mRNA uses activation of PKR in the cell to control its own translation yield (Supplementary Fig. S1). *IFN*-γ mRNA locally activates PKR through a 203-nucleotide element having a pseudoknot RNA fold, thereby inducing local eIF2α phosphorylation and inhibiting its translation in *cis* by over an order of magnitude (Ben-Asouli et al., 2002; Cohen-Chalamish et al., 2009). IFN-γ, a prominent inflammatory cytokine, is an immunomodulator vital for protective immunity and for the antitumor response but is also a major mediator of inflammatory disease when produced in excess. By activating PKR, *IFN*-γ mRNA attenuates its own translation through a negative feedback loop, to avoid hyperinflammation (Ben-Asouli et al., 2002). Extensive genetic analysis, including gain-of-function mutations, showed that the *IFN*-γ mRNA element, covering the 5′-UTR and start of the open reading frame, undergoes dynamic refolding to enable its dual function as PKR activator and as translation template, rendering its ability to activate PKR exquisitely sensitive to even single-nucleotide mutations (Cohen-Chalamish et al., 2009). The intragenic element acts to adjust translation of *IFN*-γ mRNA to the level of PKR in the cell (Ben-Asouli et al., 2002).

3. Intragenic elements potently enhance mRNA splicing by activating PKR

3.1. Splicing of tumor necrosis factor mRNA

Induction of inflammatory cytokine gene expression is under tight control. Tumor necrosis factor (TNF)-α, pivotal to protective immunity and a major mediator of inflammatory diseases, is expressed promptly during the cellular immune response. In human peripheral blood mononuclear cells, *TNF-α* mRNA levels reach a maximum within 3 h after stimulation (Jarrous et al., 1996). The human *TNF-α* gene employs a remarkable strategy towards this goal, activation of the RNA-dependent stress kinase PKR. The first hint for this unexpected result was the finding that splicing of all three *TNF-α* introns is blocked by 2-aminopurine, a known inhibitor of PKR (Jarrous et al., 1996). While the classical role of activated PKR is to inhibit translation, splicing of *TNF-α* pre-mRNA is not only fully dependent on its ability to activate PKR but is rendered highly efficient through this property (Osman et al., 1999; Namer et al., 2017). Efficient splicing of *TNF-α* pre-mRNA is mediated by the 104-nucleotide 2-aminopurine response element (2-APRE), located within the 3′ untranslated region (Fig. 1). This RNA element activates PKR more effectively than double-stranded RNA, the canonical PKR activator (Osman et al., 1999). The 2-APRE confers a regulatory advantage: it enhances splicing of *TNF-α* mRNA by as much as twentyfold when expression of PKR is increased (Osman et al., 1999). Activation of PKR by this *cis*-acting RNA element is required for splicing of *TNF-α* mRNA and increases splicing efficiency without reducing

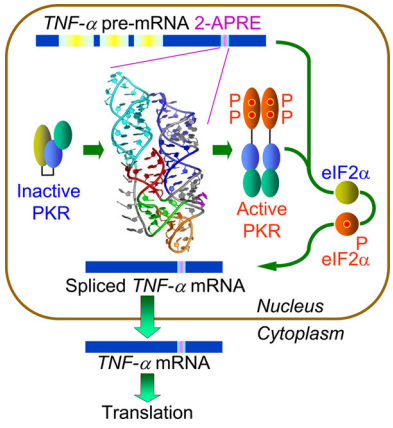

Fig. 1. The RNA activator of PKR in *TNF-α* pre-mRNA upregulates splicing via eIF2α phosphorylation. The *TNF-α* RNA activator of PKR (2-APRE) in the 3′-UTR folds into a pseudoknotted structure having two parallel helices, each of sufficient length to bind a PKR monomer, facilitating kinase dimerization on the RNA and activation. Activated PKR induces eIF-2α phosphorylation, a strict prerequisite to efficient splicing of *TNF-α* mRNA (Namer et al., 2017).

subsequent translation. The phenotype of 2-APRE mutations that impair PKR activation, including compensatory mutations that restore base pairing and secondary RNA structure, shows that greater activation of PKR leads to enhanced splicing and more TNF synthesis, rather than to greater translational repression (Namer et al., 2017). The element is transposable; its insertion into the closely related *TNF-β* (lymphotoxin) gene, which naturally lacks a PKR activator element, converts the pre-mRNA from being spliced poorly to being spliced highly efficiently (Osman et al., 1999; Namer et al., 2017). Hence, the 2-APRE functions as an autonomous splicing control element.

Extensive genetic analysis resolved the structural features of the 2-APRE element that enable it to activate PKR so effectively and thereby to enhance mRNA splicing (Namer et al., 2017). PKR must form a homodimer on the activating RNA; *trans*-autophosphorylation of the dimer then leads to kinase activation (Zhang et al., 2001; Dey et al., 2005). Binding of PKR requires at least 16–18 base pairs (bp) of double-helical RNA and activation of PKR at least 33 bp, optimally around 80 bp (Bevilacqua and Cech, 1996; Manche et al., 1992), raising the question how the RNA element, having only 104 nucleotides, could activate PKR. The *TNF-α* RNA activator is generated from helical domains, each too short to activate PKR directly, that fold into a pseudoknot critical for PKR activation and mRNA splicing, validated by gain-of-function mutations. The pseudoknot constrains the RNA into two double-helical stacks with parallel axes (Fig. 1), each of sufficient length to accommodate a PKR monomer, permitting facile kinase dimerization and activation (Namer et al., 2017). This compact structure, conserved from teleost fish to humans over 400 million years, provides a molecular basis for the exceptional ability of the 2-APRE to activate PKR and thus to achieve high mRNA splicing efficiency. The turbot *TNF-α* gene element can replace the human element, being fully functional both in activating the human kinase PKR and in rendering human *TNF-α* mRNA splicing highly efficient (Namer et al., 2017). Despite the tight and compact structure of the pseudoknotted *TNF-α* RNA activator of PKR, even single-nucleotide mutations can abrogate its ability to activate PKR, showing that this structure remains highly sensitive to perturbation (Namer et al., 2017).

Next, we addressed the question, how PKR activation promotes *TNF-α* mRNA splicing. As exemplified above by the *IFN*-γ gene (Ben-Asouli et al., 2002; Cohen-Chalamish et al., 2009), phosphorylation of eIF2α by activated PKR represses translation. Whereas eIF2α phosphorylation resulting from activation of PKR plays a central and classical role in downregulating protein synthesis, our finding is that this mechanism can upregulate splicing of individual mRNAs. Through