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The dsRNA protein kinase PKR: Virus and cell control☆

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Abstract

The IFN-induced double-stranded RNA-dependent protein kinase (PKR) is one of the four mammalian serine-threonine kinases (the three others being HRI, GCN2 and PERK) that phosphorylate the eIF2α translation initiation factor, in response to stress signals, mainly as a result of viral infections. eIF2α phosphorylation results in arrest of translation of both cellular and viral mRNAs, an efficient way to inhibit virus replication. The particularity of PKR is to activate by binding to dsRNA through two N terminal dsRNA binding motifs (dsRBM). PKR activation during a viral infection represents a threat for several viruses, which have therefore evolved to express PKR inhibitors, such as the *Vaccinia* E3L and K3L proteins. The function of PKR can also be regulated by cellular proteins, either positively (RAX/PACT; Mda7) or negatively (p58IPK, TRBP, nucleophosmin, Hsp90/70). PKR can provoke apoptosis, in part through its ability to control protein translation, but the situation appears to be more complex, as NF-κB, ATF-3 and p53 have also been implicated. PKR-induced apoptosis involves mainly the FADD/caspase 8 pathway, while the mitochondrial APAF/caspase 9 pathway is also engaged. As a consequence of the effects of PKR on translation, transcription and apoptosis, PKR can function to control cell growth and cell differentiation, and its activity can be controlled by the action of several oncogenes.

Keywords: Protein kinase; PKR; dsRNA-dependent enzyme; Antiviral; Anticellular; Translational control; Signal transduction; Modulation; Apoptosis

1. Introduction

Interferons (IFNs) have a wide range of biological functions, including antiviral, antiproliferative and immunomodulatory properties [1,2]. The cloning of the interferon genes, the structure of the ligand and their receptors, the signalling pathways and transcription of IFN-induced genes has been instrumental in the understanding of how these molecules exert their function in the cell [3,4]. This knowledge opened the way to the discovery of similar signalling pathways in the cytokine family. Among the molecules with important biological function induced by IFN is the doubled-stranded (ds) RNA-dependent protein kinase (PKR), an enzyme with multiple effects in cells, which plays a critical role in the antiviral defence mechanism of the host [5].

PKR was discovered after it was observed that IFN-treated vaccinia virus (VV) infected cells have a translational block of viral mRNAs and that cell extracts prepared from these IFN-treated VV-infected cells showed restricted translation of viral and cellular mRNAs [6,7]. At this time, VV was known to produce dsRNA [8] and dsRNA was also known to inhibit protein synthesis in animal cells or in cell free systems [9,10]. The group of Ian Kerr showed that extracts from IFN-pretreated cells had enhanced sensitivity to inhibition by dsRNA [11]. One reason for this inhibition was the generation of 2′-5′A oligonucleotides after activation by dsRNA of the IFN-induced 2′-5′A-oligoadenylate synthetase, leading to activation of the 2′-5′A/RNAse L pathway (see review by A.G. Hovanessian and J. Justesen in this issue). Another reason for this inhibition was the activation of a protein kinase. Whereas the discovery of 2′-5′A was unexpected, a kinase activity responsible for inhibition was under active search in different laboratories, after

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the demonstration that a dsRNA-dependent kinase could inhibit translation through phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2α) in rabbit reticulocyte lysates [12]. Similarly to the 2-5′A-oligoadenylate synthetase, the IFN-induced protein kinase could bind dsRNA. This facilitated the earlier studies on this protein, through partial purification on poly(I):poly(C)-Sepharose [13]. In addition to eIF2α, the kinase activity was found to phosphorylate a p68 protein in human cells and a p65 protein in murine cells [14]. The generation of polyclonal and monoclonal antibodies against the human p68 protein allowed one to determine that this was the kinase itself [15]. To achieve its cloning, which was performed at the Pasteur Institute [16], this kinase had to be first purified by immunoaffinity with specific monoclonal antibodies [17], then the purified protein was injected into mice, in presence of poly(A).poly(U), an adjuvant both very efficient and not toxic [18]. Different names were given to this kinase such as p68 protein kinase, DAI (Double stranded Activated Inhibitor), dsRNA-dependent protein kinase, P1/eIF2α kinase until the decision to give PKR as consensus name, for Protein Kinase dsRNA-dependent [19].

PKR is a serine-threonine kinase, composed by the kinase domain shared by the other eIF2α kinases, and two dsRNA binding domains (dsRBD) that regulate its activity. PKR autophosphorylation represents the activation reaction and leads to the phosphorylation of eIF-2α [20,21], impairing eIF-2 activity that results in inhibition of protein synthesis [22]. In addition to its translational regulatory function, PKR has a role in signal transduction and transcriptional control through the IκB/NF-κB pathway [23]. PKR, which is expressed constitutively in mammalian cells, has also been implicated in the control of cell growth and proliferation with tumour suppressor function [24–26].

PKR is involved in signalling various pathways that activate and engage a number of transcription factors (Fig. 1). Since these transcription factors regulate the expression of many cellular genes, it is anticipated that PKR control the expression of multiple genes.

2. PKR activation

PKR is activated in response to dsRNA of cellular, viral, or synthetic (such as polyrI:polyrC, pIC) origin, with a size greater than 30 nucleotides. PKR mediates a critical role in response to dsRNA, acting as a sensor of viral infections. Moreover, PKR is stimulated by a set of other activators such as pro-inflammatory stimuli, growth factors, cytokines and oxidative stress. A wide range of different cell stresses can activate PKR independently of dsRNA or other molecules through PACT (PKR-associated activator) and its mouse orthologue RAX that are cell proteins that bind to and activate PKR [27,28]. PKR is also an intermediary in TLR signalling [29]. PKR is engaged in dsRNA-activated TLR3 signalling, recruited by a TAK1-containing complex in response to dsRNA binding to the TLR3 receptor [30]. In addition, PKR integrates and transmits these signals not only to eIF-2α and the translational machinery, but also to various factors such as STAT,

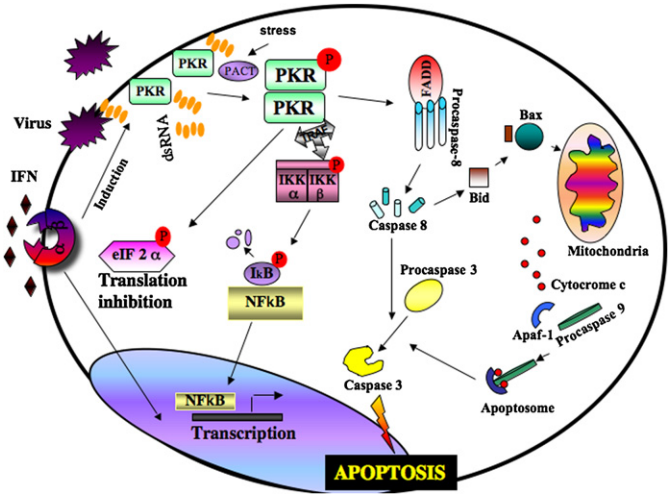

Fig. 1. PKR: induction, activation and its role in different cellular pathways. Viral infections provoke induction and secretion of IFN, which then binds to specific cellular receptors and triggers induction of hundreds of genes, including the dsRNA-activated protein kinase PKR. PKR gets activated through homodimerisation upon binding to viral dsRNA structures via its dsRNA binding domains. In the absence of virus infection, other conditions of stress can lead to PKR activation through the cellular PACT protein. As a kinase, PKR phosphorylates the protein synthesis initiation factor eIF2α and provokes an important inhibition in protein translation. Through adapters, such as TRAF, PKR can activate the kinase complex IKKα/IKKβs, which provokes liberation of the NF-κB transcription factor by phosphorylating its IκBα inhibitor. As a result, the induction of several genes can be promoted. Through interaction with FADD, PKR activates caspase-8, which in turn activates the conversion of Procaspase-3 and provokes Bid/Bax interaction, release of cytochrome *c* from the mitochondria and formation of the apoptosome (Apaf-1/cytochrome *c*/caspase-9). Both pathways result in activation of caspase-3 and degradation of DNA, thus resulting in programmed cell death or apoptosis.

IRF1, p53, JNK and p38, as well as engaging the NF-κB pathway [5,31,32].

In non-stressed cells, PKR is in a monomeric latent state due to the autoinhibitory effect of its dsRBD, which occlude the KD and regulate activation of the kinase. The different dsRNA molecules are recognised and bound by PKR through the two N-terminal dsRBM, resulting in PKR activation and autophosphorylation [33]. The structure of the PKR dsRNA binding domain was determined by nuclear magnetic resonance (NMR); it consists of two identical α–β–β–α folds, whereas the 20-amino-acid linker is entirely in a random coil conformation [34]. This allows the dsRBD to wrap around the dsRNA molecule for optimal protein-RNA interactions, and offers a satisfactory explanation for length requirements in dsRNA molecules to be effective PKR activators.

Most natural dsRNA activators of PKR are synthesised in virus-infected cells as by-products of viral replication or transcription. For RNA viruses, dsRNA replicative forms (RF) are obligatory intermediates for the synthesis of new genomic RNA copies. Complex DNA viruses such as vaccinia virus (VV), adenovirus or herpes simplex virus (HSV) have open reading frames (ORFs) in opposite orientation; they produce overlapping mRNA transcripts that can fold to form dsRNA stretches responsible for PKR activation in infected cells [5]. After binding dsRNA, PKR undergoes