Available online at www.sciencedirect.com
ScienceDirect
Virology 363 (2007) 166–173
VIROLOGY
www.elsevier.com/locate/yviro

Interferon-induced inhibition of parainfluenza virus type 5; the roles of MxA, PKR and oligo A synthetase/RNase L

T.S. Carlos [a], D. Young [a], S. Stertz [b], G. Kochs [b], R.E. Randall [a,*]

[a] *School of Biology, University of St. Andrews, Fife KY16 9ST, Scotland, UK*
[b] *Department of Virology, University of Freiburg, Hermann-Herder-Str. 11, 79104, Freiburg, Germany*

Received 14 November 2006; returned to author for revision 21 December 2006; accepted 10 January 2007
Available online 16 February 2007

Abstract

We have previously reported that the addition of interferon (IFN) to the culture medium of Vero cells (which cannot produce IFN) that were infected with the CPI− strain of parainfluenza virus 5 (PIV5, formally known as SV5), that fails to block IFN signaling, rapidly induces alterations in the relative levels of virus mRNA and protein synthesis. In addition, IFN treatment also caused a rapid redistribution of virus proteins and enhanced the formation of cytoplasmic viral inclusion bodies. The most studied IFN-induced genes with known anti-viral activity are MxA, PKR and the Oligo A synthetase/RNase L system. We therefore examined the effects of these proteins on the replication cycle of PIV5. These studies revealed that while these proteins had some anti-viral activity against PIV5 they were not primarily responsible for the very rapid alteration in virus protein synthesis observed following IFN treatment, nor for the IFN-induced formation of virus inclusion bodies, in CPI− infected cells.

Keywords: Parainflueza virus type 5; Interferon; PKR; OAS; MxA

Introduction

Parainfluenza virus type 5 (previously known as simian virus 5; SV5) (Chatziandreou et al., 2004), is a prototype member of the *Rubulavirus* genus in the *Paramyxovirinae* subfamily of the family *Paramyxoviridae* (Lamb and Kolakofsky, 2001). PIV5, like all other paramyxoviruses, is an enveloped, non-segmented negative-stranded RNA virus. The helical nucleocapsid (rather than free genomic RNA) acts as a template for all RNA synthesis. The viral polymerase complex transcribes, in a sequential manner, the NP, V/P, M, F, SH, HN and L genes, after polymerase entry at the single 3′ promoter of the template, by a "stop–start" transcription mechanism. Efficiency of transcription decreases with increasing distance of the genes from the promoter generating a transcriptional gradient, with the NP gene transcribed most frequently and the L gene transcribed the least frequently (reviewed in Whelan et al., 2004).

Interferon (IFN)-α/β are produced by cells in direct response to virus infection and are characterized by their ability to induce antiviral responses and cell growth inhibitory effects. The secreted IFN-α/β bind to the IFN-α/β receptor on the surface of the infected cells and neighboring cells to initiate an intracellular signaling cascade that ultimately activates the expression of hundreds of IFN-inducible genes. Some of the genes up-regulated by IFN stimulation are involved in the establishment of an antiviral state. Well characterized examples of IFN-induced antiviral proteins include protein kinase R (PKR), the MxA GTPases and the family of 2′-5′ oligoadenylate synthetases (OAS) that activate the latent endoribonuclease L (RNase L). PKR is a dsRNA activated, serine–threonine protein kinase normally present in the cell in an inactive form (Williams, 1999). PKR can mediate inhibition of protein synthesis through phosphorylation of eukaryotic initiation factor-2α (eIF-2α), which, in the course of viral infection, provides a defence mechanism for restricting viral protein translation and, ultimately, viral replication (Clemens and Elia, 1997; Meurs et al., 1990). Mx proteins are dynamin-like large GTPases that have antiviral activity and inhibit the multiplication of several RNA viruses, in contrast to other IFN-stimulated genes, they are not constitutively expressed in cells. The importance of Mx proteins for host survival has been amply demonstrated (Arnheiter et al.,

* Corresponding author. Fax: +44 1334 463397.
E-mail address: rer@st-and.ac.uk (R.E. Randall).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.01.014

1996; Hefti et al., 1999; Kochs et al., 2002; Pavlovic et al., 1995), but the mechanism of MxA antiviral action is still not completely understood. The viral target recognised by MxA is virus- and cell type-specific, and it can inhibit virus transcription or mRNA translation, or interfere with viral ribonucleocapsid protein complexes or transportation of virus nucleocapsids (Haller and Kochs, 2002). The OAS system consists of enzymes that, when activated by dsRNA, catalyze the synthesis of oligoadenylates whose function is to activate latent RNase L, which degrades ssRNA, including both viral and cellular mRNA, thereby blocking protein synthesis and leading to viral inhibition (Zhou et al., 1997).

Many paramyxoviruses have been shown to at least partially circumvent the IFN response by blocking IFN signaling and limiting IFN production. PIV5 blocks IFN signaling by targeting STAT1, a host cell transcription factor essential for both IFN-α/β and IFN-γ signaling, for proteasome-mediated degradation (Didcock et al., 1999a, 1999b). The V protein of PIV5 also helps to limit IFN production by interacting with, and inhibiting the action of, mda-5, an intracellular signaling molecule which plays a key role in at least one intracellular signaling pathway that leads to the induction of IFN (Andrejeva et al., 2004). However, the ability of PIV5 to circumvent the IFN response is not absolute as infected cells still release some IFN, which can induce an antiviral state in neighboring uninfected cells, thereby restricting the replication of PIV5 (Andrejeva et al., 2002; Chatziandreou et al., 2004; Didcock et al., 1999a; Wansley et al., 2005). In support of these observations, PIV5 replication was shown to be enhanced in cells that have been engineered to be non-responsive to IFN (Young et al., 2003). We have recently described a model in which it is possible to study the kinetics of IFN-induced effects on PIV5 transcription, protein synthesis and the distribution of virus proteins, in the absence of virus countermeasures. In this model, CPI−, a canine strain of PIV5 that fails to block IFN signaling, was used to infect Vero cells, which are unable to produce IFN due to spontaneous gene deletions (Desmyter et al., 1968; Mosca and Pitha, 1986) but can respond to exogenous IFN supplemented to culture medium. It was shown that addition of IFN to CPI− infected cells, once virus replication was established, rapidly changed the profile of virus transcription and protein synthesis. IFN increased the steepness of the virus mRNA transcription gradient and the production of virus mRNAs with longer poly(A) tails, which suggests that the virus polymerase processivity may be altered in cells in an IFN-induced antiviral state (Carlos et al., 2005). Although not in complete concordance with mRNA levels, IFN also caused an alteration in the protein synthesis pattern such that there was a marked down-regulation in the expression levels of genes downstream of the V/P gene. Furthermore, IFN treatment led to a redistribution of virus proteins within infected cells that resulted in the formation of inclusion bodies (Carlos et al., 2005). In this report we attempt to further define how IFN mediates these effects, and demonstrate that although PKR, oligo A/RNase L and MxA have some anti-viral activity none of them appears to be primarily responsible for the rapid IFN-induced alterations in the PIV5 replication cycle.

Results

Comparison of the effects of IFN on CPI− virus protein synthesis in Hep2 and Vero cells

To ascertain whether similar IFN-induced changes in the pattern of protein synthesis observed in Vero cells infected with CPI− occurred in cells that can produce and respond to IFN, we examined the replication of CPI− in human Hep2 cells. Vero and Hep2 cells were mock infected or infected with CPI− or CPI+ (the parental virus that blocks IFN signalling) and either treated with IFN at 12 h p.i. or left untreated. Six hours after IFN treatment, cells were metabolically labeled with [^{35}S] methionine for 1 h and the relative levels of newly synthesized viral proteins were estimated by immunoprecipitation (Figs. 1A and B). It was

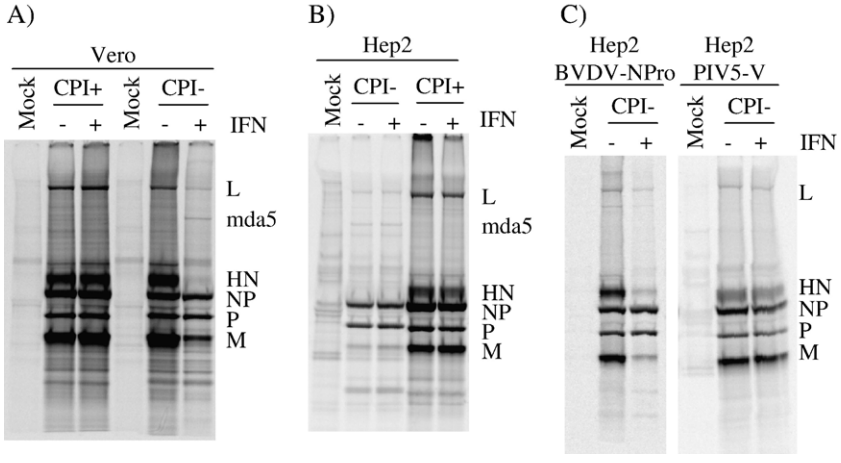

Fig. 1. CPI− protein synthesis profile in Vero, Hep2 naive, Hep2/BVDV-NPro and Hep2/PIV5-V cells, in the absence and presence of IFN. Vero (A) and Hep2 naïve cells (B) were infected with CPI− or CPI+, and Hep2/BVDV-NPro and Hep2/PIV5-V cells (C) were infected with CPI−. Cells were infected at m.o.i. of 50 pfu/cell and either treated with exogenous rHuIFN-α at 12 h p.i. or left untreated. Cells were metabolically labeled with [^{35}S]-methionine for 1 h, 6 h after addition of IFN. Virus proteins were immunoprecipitated from extracts of these cells with a pool of antibodies to the NP, P, M, HN and L proteins. The precipitated proteins were subsequently separated on a 4–12% gradient PAG and visualized by phosphorimager analysis.