Interferon-induced exonuclease ISG20 exhibits an antiviral activity against human immunodeficiency virus type 1

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Interferons (IFNs) encode a family of secreted proteins that provide the front-line defence against viral infections. It was recently shown that ISG20, a new 3’→5’ exoribonuclease member of the DEDD superfamily of exonucleases, represents a novel antiviral pathway in the mechanism of IFN action. In this report, it was shown that ISG20 expression is rapidly and strongly induced during human immunodeficiency virus type 1 (HIV-1) infection. In addition, it was demonstrated that the replication kinetics of an HIV-1-derived virus expressing the ISG20 protein (HIV-1NL4-3ISG20) was delayed in both CEM cells and peripheral blood mononuclear cells. No antiviral effect was observed in cells overexpressing a mutated ISG20 protein defective in exonuclease activity, suggesting that the antiviral effect was due to the exonuclease activity of ISG20. Paradoxically, despite the antiviral activity of ISG20 protein, virus rescue observed in HIV-1NL4-3ISG20-infected cells was not due to mutation or partial deletion of the ISG20 transgene, suggesting that the virus was able to counteract the cellular defences. In addition, HIV-1-induced apoptosis was significantly reduced in HIV-1NL4-3ISG20-infected cells suggesting that emergence of HIV-1NL4-3ISG20 was associated with the inhibition of HIV-1-induced apoptosis. Altogether, these data reflect the ineffectiveness of virus replication in cells overexpressing ISG20 and demonstrate that ISG20 represents a new factor in the IFN-mediated antiviral barrier against HIV-1.

INTRODUCTION

Interferons (IFNs) are a family of multifunctional secreted proteins characterized by their abilities to interfere with virus infection and replication (Espert et al., 2003b; Player & Torrence, 1998; Samuel, 2001). In particular, IFNs display a potent antiviral effect on primary and immortalized human immunodeficiency virus type 1 (HIV-1) permissive human T cells and monocytes/macrophages (Karpov, 2001; Pitha, 1991). IFNs act by interfering with the different steps of the virus life cycle, both in acute and chronic HIV-1 infections (Karpov, 2001; Pitha, 1994). For instance, during acute infection, IFNs inhibit virus cell penetration by downregulating both the CD4 receptor and the CXCR4 coreceptor of HIV-1 in peripheral blood mononuclear cells (PBMCs) and in monocytes (Dhawan et al., 1995; Shirazi & Pitha, 1998). In de novo HIV infection of monocytes, IFNs have been reported to interrupt early events in virus replication prior to provirus DNA integration (Gendelman et al., 1990b; Meylan et al., 1993; Shirazi & Pitha, 1992, 1993). In contrast, in chronically infected cells, IFN antiviral action seems to be mostly due to specific RNA and protein synthesis inhibition, as well as the inhibition of later stages of the HIV replication cycle leading to defective assembly, budding and release of virions (Coccia et al., 1994; Gendelman et al., 1990a; Hansen et al., 1992; Poli et al., 1989).

Two enzymes in the host-mediated antiviral response, the dsRNA-dependent protein kinase R (PKR) (Gale & Katze, 1998; Meurs et al., 1990, 1992) and the RNase L (Stark et al., 1998; Zhou et al., 1993), have been principally implicated in the IFN-induced antiviral response against HIV-1 (for reviews, see Espert et al., 2003b; Katze et al., 2002). After binding to dsRNA, PKR phosphorylates the protein synthesis initiation factor eIF2 and the inhibitor of NF-κB (1-κB) leading to a translational shut down and specific transcription regulation, both detrimental for virus replication.
development (Clemens & Elia, 1997; D’Acquisto & Ghosh, 2001; Williams, 2001). The overexpression of PKR has been shown to prevent reactivation of HIV-1 replication in latently infected cells (Benkirane et al., 1997; Muto et al., 1999). RNase L is a dormant cytosolic endoribonuclease activated by short oligoadenylates produced, in the presence of dsRNA, by the 2′-5′ oligoadenylate synthetase following viral infection or IFN exposure (Player & Torrence, 1998; Stark et al., 1998). Overexpression of RNase L has been reported to impair severely HIV replication associated with acceleration of death of infected cells (Maitra & Silverman, 1998). In addition, the IFN-induced 16 kDa inhibitory C/EBPβ isoform was reported to be involved in repression of HIV-1 replication by IFN-β in THP-1 cell-derived macrophages (Honda et al., 1998).

There is now clear evidence that the effectiveness with which the host’s antiviral response can clear virus infections requires multiple and complementary antiviral pathways (Gale & Katze, 1998; Kumar & Carnmichael, 1998; Player & Torrence, 1998; Stark et al., 1998; Williams, 2001). We have isolated a human IFN-induced gene that we have termed ISG20 (Gongora et al., 1999). ISG20 encodes a 3′-5′ alkyltransferase isoenzyme with specificity for ssRNA (Nguyen et al., 2001). We showed that stable and constitutively expressed ISG20 conferred resistance to vesicular stomatitis virus (VSV), influenza virus and encephalomycocarditis virus (EMCV) infection in HeLa cells, providing an alternative antiviral pathway against RNA genomic viruses (Espert et al., 2003a). In this report, to investigate the potential of ISG20 for controlling HIV infection, we generated HIV-1 recombinant viruses expressing the ISG20 protein. This approach has been successfully used to analyse the effect of PKR and RNase L overexpression on HIV-1 replication (Benkirane et al., 1997; Maitra & Silverman, 1998). We demonstrated that ISG20 overexpression delays HIV-1 replication in both CEM cells and PBMCs. Our findings clearly demonstrate that ISG20 represents a novel antiviral pathway in the mechanism of IFN action against HIV-1.

**METHODS**

**Cell cultures and antibodies.** Human T-lymphoblastoid CEM cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (PAA Laboratories). Human embryonic kidney HEK293 and CD4+ HeLa P4 cells were cultured at 37°C in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% FBS. PBMCs were cultured in RPMI 1640 with 10% FBS containing 10 U interleukin 2 (IL2) ml⁻¹. Human α2bIFN (Hu-α2bIFN) was purchased from Schering-Plough. Polyclonal rabbit antisera against ISG20 was obtained by immunization of rabbit with recombinant purified protein. The human antisera against HIV-1 and the HIV-1 p24 rabbit antisera were obtained from the NIH AIDS Research & Reference Reagent Program.

**Construction of recombinant HIV-1 proviral DNAs and virus stocks.** The human ISG20 cDNA in sense or antisense orientation and the cDNA encoding an inactive mutated ISG20 protein were inserted into the modified HIV-1 proviral pNL4-3neo DNA (Benkirane et al., 1997; Huang et al., 1994) to generate the recombinant HIV-1 DNAs, pNL4-3ISG20, pNL4-3αISG20 and pNL4-3mutISG20 (Espert et al., 2003a; Nguyen et al., 2001), respectively (see Fig. 2). The different recombinant constructs were verified by sequencing. The different virus stocks were prepared from the culture supernatant of HEK293 cells 48 h after transfection with the appropriate HIV-1 recombinant proviral DNAs using Lipofectamine 2000 reagent, according to the manufacturer’s instructions (Invitrogen). Virus stocks were quantified by measuring their reverse transcriptase (RT) activities.

**HIV-1 infections.** For massive infections, 5 x 10⁶ CEM cells were co-cultured with plated HEK293 cells previously transfected with 5 µg of each modified HIV-1 proviral DNA construct using the Lipofectamine 2000 reagent. For CEM cell infections, 5 x 10⁵ cells were infected with the recombinant viruses in a final volume of 130 µl and incubated at 37°C. After 1 h, the cells were collected by centrifugation to remove the viruses and then resuspended in 1 ml culture medium on six-well plates. For PBMC infections, cells were activated for 24 h in medium containing 10% FBS, 10 U IL2 ml⁻¹ and 5 µg phytohaemagglutinin (PHA) ml⁻¹. Then, the cells were washed twice in fresh medium and resuspended to a concentration of 2 x 10⁶ cells ml⁻¹ in RPMI 1640 containing 10% FBS and 8 µg polybrene ml⁻¹ and incubated overnight at 37°C in the presence of virus. The cells were then washed twice with PBS to remove the viruses and resuspended in RPMI 1640 with 10% FBS and IL2.

**RT assays.** RT assays were performed as previously described (Huang et al., 1994). Each reaction contained 5 µl viral supernatant in 25 µl RT cocktail [60 mM Tris/HCl pH 8, 75 mM KCl, 5 mM MgCl₂, 0.1% NP-40, 1 mM EDTA, 5 µg poly(rA) ml⁻¹, 0.16 µg oligo(dt) ml⁻¹, [α-32P]dTTP (1 µCi ml⁻¹; 37 kBq)]. The reaction was incubated for 3 h at 37°C. Then 10 µl of each reaction was spotted on to DEAE paper, washed three times in 2× SSC, dried and quantified using an Instant Imager (Packard).

**Detection of apoptotic cells and flow cytometry analysis.** Apoptotic cells were detected by using the fluorescein isothiocyanate-labelled annexin V method (Boehringer Mannheim). Cells were washed, labelled with annexin V–Fluos according to the manufacturer’s recommendations and analysed by flow cytometry.

**Western blotting analysis.** Cells (3 x 10⁶) were lysed in 50 mM Tris/HCl pH 7.5, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EDTA, 0.1% NP-40, 10% glycerol, 1 mM PMSF, 5 mM NaF and complete mini protease inhibitor cocktail (Roche). Lysate was cleared by centrifugation at 10,000 g for 10 min. Proteins were fractionated by 12% SDS-PAGE and transferred on to PVDF membrane. After a blocking step, the membrane was hybridized with the appropriate antibody and developed using a chemiluminescent detection system (ECL Plus; Amersham Pharmacia Biotech).

**β-Galactosidase activity assay.** CD4⁺ HeLa P4 cells were infected with each recombinant virus and β-galactosidase activity was measured using the β-Galactosidase enzyme assay system with reporter lysis buffer (Promega) according to the manufacturer’s instructions.

**RESULTS**

**ISG20 protein expression is induced during HIV-1 infection of CEM cells**

Host-cell factors are required for both virus replication and the establishment of an efficient antiviral response. In this way, HIV-1 infections activate the transcription of a large number of cellular genes (Corbeil et al., 2001; van’t Wout et al., 2003). Activation occurs either directly through...
activation of cellular transcription factors or indirectly through prior production of type I IFN (Bolt et al., 2002; Chang & Laimins, 2001; Cheung et al., 2002; Korth & Katze, 2002; Simmen et al., 2001; van ’t Wout et al., 2003). To analyse the effect of HIV-1 infection on ISG20 gene expression, the human T-lymphoblastoid cell line CEM was subjected to a massive HIV-1 infection. To this aim, CEM cells were incubated in the presence of human embryonic kidney HEK293 cells transfected with the HIV-1 proviral DNA (pNL4-3Δnef) to generate HIV-1NL4-3Δnef virus, as described in Methods. This HIV-1-derived recombinant virus has previously been shown to have similar in vitro infectivity to the HIV-1NL4-3 wild-type virus (Benkirane et al., 1997; Huang et al., 1994; Maitra & Silverman, 1998). At various times after infection, CEM cells were collected and protein extracts prepared and analysed by immunoblotting with a specific rabbit polyclonal antibody directed against the ISG20 recombinant protein. The sizes (kDa) of the molecular mass markers and the position of the ISG20 protein are indicated. Expression of α-tubulin was used as an invariant control.

Characterization of HIV-1-derived virus expressing ISG20 protein

We have recently shown that ISG20 is an active player in the antiviral response of IFNs against various RNA viruses (Espert et al., 2003a). As it was induced during HIV-1 infection, we decided to determine the effect of its overexpression on HIV-1 replication. To this end, we subcloned human ISG20 cDNA into the modified HIV-1 proviral pNL4-3Δnef DNA (Fig. 2a). To synthesize ISG20 coincidently with peak periods of viral gene expression, ISG20 cDNA was inserted in-frame for expression into the nef gene of the modified HIV-1 proviral pNL4-3Δnef DNA. As negative controls, we generated HIV-1-derived proviral DNAs expressing either the ISG20 cDNA in an antisense orientation (pNL4-3asISG20) or an ISG20-inactive RNase mutant (pNL4-3mutISG20) as previously described (Espert et al., 2003a; Nguyen et al., 2001). The different proviral DNAs were then transfected into the HEK293 cells and the recombinant viruses produced were collected in the culture supernatant 48 h after transfection. To ascertain that the pNL4-3ISG20 and pNL4-3mutISG20 HIV-1-derived proviral constructs were able to express wild-type or mutated ISG20 protein, whole cellular extracts from transfected HEK293 cells were prepared and analysed by Western blotting with specific antibodies for ISG20 and HIV-1 proteins. The major HIV-1 proteins Pr55Gag, IN(32) and p24, as well as the ISG20 protein, are indicated.
transgene. No detectable differences in the HIV-1 protein pattern expressed by the four different proviral DNAs were observed, suggesting that the insertion of DNA into the HIV-1 nef gene did not influence viral protein expression. In addition, purified virions exhibited similar protein patterns, indicating that viral integrity was preserved for all the recombinant viruses produced (data not shown). The amount of virus obtained from the proviral DNAs was determined by measuring the level of RT activity, as described in Methods. The different virus stocks were normalized for RT activity and then used to infect CEM cells.

Expression of ISG20 protein from a recombinant HIV-1 severely delays virus replication in CEM cells

CEM cells were infected with the four different recombinant viruses, as described in Methods. At regular intervals after infection, virus replication was monitored by measuring the RT activity in the culture supernatant. A typical experiment, presented in Fig. 3(a), showed that the RT peaks for HIV-1NL4-3Δnef, HIV-1NL4-3ΔISG20, and HIV-1NL4-3mutISG20 were observed at day 7 post-infection. In contrast, the RT peak for HIV-1NL4-3ISG20 was strongly delayed and occurred at day 14, demonstrating that ISG20 expression was detrimental for HIV-1 replication. Similar data were obtained in various independent experiments performed with different preparations of virus stocks (data not shown). Concurrently, ISG20 expression was monitored for each virus, at the RT peak, by Western blot analysis (Fig. 3b). As expected on the basis of the data presented in Fig. 1, induction of endogenous ISG20 was observed in CEM cells infected with HIV-1NL4-3Δnef and HIV-1NL4-3ΔISG20. In addition, Nef-ISG20 fusion protein was strongly expressed at the RT peak (day 16) in CEM cells infected with HIV-1NL4-3ISG20, suggesting that the delayed emergence of virus was not viral rescue due to inactivation of Nef-ISG20 expression by the virus. The fact that strong expression of Nef-mutISG20 fusion protein was also observed in CEM cells infected with HIV-1NL4-3mutISG20 (day 7) demonstrated that the antiviral effect of ISG20 did not result from overexpression of a foreign protein by the virus and suggested that this effect was dependent on its exonuclease activity. To determine whether the viral resistance observed was dependent on the number of viral particles used for infection, an additional experiment was performed using 100-fold more virus. As expected, the replication kinetics of all viruses were faster when the cells were infected with higher virus concentrations. However, a significant delay between the RT peaks of HIV-1NL4-3ISG20 and the three other viruses was still observed (Fig. 3c).

Expression of ISG20 protein from a recombinant HIV-1 does not alter transcription of the long terminal repeat (LTR) promoter of HIV-1

To determine whether the integration of ISG20 affected the first steps of viral infection before viral LTR promoter expression, the HIV-1-derived viruses were used in a single-round virus infection to challenge CD4+ HeLa P4 cells, which contain an integrated β-galactosidase reporter gene

Fig. 3. ISG20 overexpression strongly delays HIV replication in CEM cells. (a) HIV-1NL4-3Δnef, HIV-1NL4-3ΔISG20, HIV-1NL4-3ΔmutISG20, and HIV-1NL4-3mutISG20 produced from transfected HEK293 cells were normalized for RT activity and used to infect CEM cells. Virus replication was monitored at regular intervals after infection by measuring the accumulation of RT activity in the culture supernatant. (b) Protein extracts prepared from CEM cells infected with the indicated recombinant viruses were analysed for ISG20 expression at the RT peak by Western blotting with specific antibodies against ISG20. Expression of α-tubulin was used as an invariant control. The Nef-fused ISG20 recombinants and endogenous ISG20 proteins are indicated. (c) The experiments described in (a) were reproduced using 100-fold more virus.
Expression of ISG20 protein from a recombinant HIV-1 severely delays virus replication in PBMCs

To assess the physiological relevance of our observations, we next examined the efficiency of HIV-1NL4-3Δ nef and HIV-1NL4-3ISG20 replication in PBMCs. To this aim, PBMCs obtained from healthy donors were cultured and activated in the presence of 4 μg PHA ml⁻¹ and 10 U IL2 ml⁻¹ for 24 h. Infections were performed as described above and virus replication was monitored by measuring RT activity in the culture supernatant. Similar to the results observed for CEM cells, the replication kinetics for HIV-1NL4-3ISG20 were strongly delayed compared with HIV-1NL4-3Δ nef; the RT peak occurred at day 8 for HIV-1NL4-3Δ nef and at day 21 for HIV-1NL4-3ISG20 (Fig. 5). These data strengthened our findings on the antiviral activity of ISG20 against HIV-1.

Expression of ISG20 protein from a recombinant HIV-1 results in inhibition of HIV-induced apoptosis of CEM cells

Strategies used by HIV-1 to escape cellular antiviral processes include rapid mutations and gene deletions. In particular, the negative selective pressure imposed by a transgene present in the viral genome frequently results in mutation or partial deletion of the transgene. To determine whether the viral rescue resulted from alteration of the nef–ISG20 transgene by the virus, viral recombinant ISG20 gene was PCR amplified and sequenced. Interestingly, neither deletion nor mutation was observed (data not shown), indicating that ISG20 protein was not altered, contrary to what has been described for RNase L (Maitra & Silverman, 1998) and PKR (M. Benkirane, unpublished data). In accordance with the data presented in Fig. 3(b), these findings demonstrated that the emergence of virus at late times following infection with HIV-1NL4-3ISG20 was not due to mutation or partial deletion of the ISG20 transgene. Despite the antiviral activity of the ISG20 protein, its cDNA appeared to be stable in HIV-1NL4-3ISG20, suggesting that the virus is able to bypass the mechanism of ISG20 action.

It has been suggested that HIV-1 has evolved mechanisms of blocking or delaying the cellular suicide programme at least until high levels of progeny virus are produced (Selliah & Finkel, 2001; Gougeon, 2003). We observed that HIV-1NL4-3ISG20-infected cells died less frequently at the RT peak compared with cells infected with HIV-1NL4-3Δ nef HIV-1NL4-3Δ ISG20 or HIV-1NL4-3mutISG20 (Fig. 6a), although, they produced similar amounts of infectious particles, monitored by measuring the accumulation of RT activity in the culture supernatant of the infected cells (Fig. 6b).
The percentage of apoptotic cells was then evaluated in massively infected cells by flow cytometry analysis with the annexin V staining method. A typical experiment presented in Fig. 6(c) showed that apoptosis was significantly reduced in HIV-1\_NL4-3\_ISG20\_infected cells compared with cells infected with the other recombinant viruses, suggesting that the delayed emergence of HIV-1\_NL4-3\_ISG20 was associated with inhibition of HIV-1-induced apoptosis.

**DISCUSSION**

Mammalian cells have developed several antiviral pathways to interfere efficiently with viral multiplication (Espert et al., 2003b; Gale & Katze, 1998; Kumar & Carmichael, 1998; Player & Torrence, 1998; Samuel, 2001; Stark et al., 1998; Williams, 2001). Among the gene products known to contribute to the antiviral activity of IFN, the 2’-5’ adenylate/RNase L system and PKR are reported to be involved in cellular protection against HIV-1 virus (for review, see Karpov, 2001). Indeed, a recombinant HIV-1 provirus encoding PKR has been shown to suppress virus replication of wild-type HIV-1 provirus in co-transfection experiments (Benkirane et al., 1997; Maitra & Silverman, 1998). In the same way, a recombinant HIV-1 provirus expressing RNase L replicated less efficiently than its wild-type counterpart in Jurkat cells and peripheral blood lymphocytes (Maitra & Silverman, 1998). We have recently shown that ISG20, an IFN-induced 3’→5’ exonuclease specific for ssRNA, confers cell resistance to various RNA viruses, including VSV, EMCV and influenza virus, suggesting that ISG20 represents a novel antiviral pathway in the mechanism of IFN action (Espert et al., 2003a; Gongora et al., 1997; Nguyen et al., 2001). In the present report, we analysed the potential antiviral activity of ISG20 against HIV-1.

We showed that ISG20 expression was rapidly and strongly induced during HIV-1 infection. These data are not surprising because it is now clearly established that HIV can induce expression of cellular genes involved in the host-mediated antiviral response, independently of IFN secretion (Baca et al., 1994; Corbeil et al., 2001; de Veer et al., 2001). More recently, it has been shown that HIV-1 infection or expression of Tat alone, using an adenovirus-mediated gene transfer system (adeno-Tat), induces IFN-responsive gene expression in immature human dendritic cells (Izmailova et al., 2003). Interestingly, ISG20 induction was observed both in HIV-1 and adeno-Tat infections, in the absence of detectable IFNs in the culture supernatants, suggesting that ISG20 overexpression is mediated by Tat. As, the transcriptional regulators of IFN-inducible genes, interferon regulatory factor-7 (IRF-7) and signal transducer and activator of transcription 1 (STAT1), were also induced by adeno-Tat, the authors speculated that they could be responsible for the induction of the other set of IFN-induced genes (Izmailova et al., 2003). Accordingly, we have previously shown that the enhancer sequence element involved in the response to these transcription factors is present in the ISG20 promoter region (Gongora et al., 2000).

To investigate whether ISG20 could interfere with HIV-1 infection, we developed the same approach successfully used to analyse the effect of PKR and RNase L (Benkirane et al.,...
1997; Maitra & Silverman, 1998). We showed that the replication kinetics of an HIV-1-derived virus expressing the ISG20 protein was strongly delayed in both T cells and PBMCs. These data demonstrated that ISG20 can function as a potent suppressor of HIV-1 replication when it is overexpressed in infected cells and represents a new factor in the IFN-mediated antiviral barrier against HIV. Interestingly, the exonuclease activity of ISG20 seems to be required for its antiviral effect, since the HIV-1NL4-3mutISG20 and control viruses replicated with similar kinetics. Unfortunately, the ISG20 antisense HIV-1-derived virus was unable to downregulate endogenous ISG20 expression and exhibited the same phenotype as HIV-1NL4-3Anef and HIV-1NL4-3mutISG20.

Viruses have developed diverse non-immune strategies to counteract host-mediated antiviral mechanisms. For example, the HIV-1 Tat protein is able to bind directly to PKR and inhibit its function (Brand et al., 1997; Cai et al., 2000; Demarchi et al., 1999; McMillan et al., 1995). Similarly, the cellular RNA-binding protein, identified by its ability to cooperate with HIV-1 Tat protein for binding to the 5’-termini TAR sequence of HIV-1 RNA, blocks the anti-HIV effect of PKR (Benkirane et al., 1997; Daher et al., 2001; Gatignol et al., 1991). In the same way, HIV-1 inhibits the RNase L pathway by upregulating expression of the RNase L inhibitor (Martinand et al., 1999), also known as the multifunctional cellular protein (HP68) (Zimmerman et al., 2002). Finally, the virally encoded Vif protein turns away antiviral defences during the late stages of virus production through proteasome-mediated degradation of the antiviral protein CEM15 (Marin et al., 2003; Mehle et al., 2004; Sheehy et al., 2002, 2003; Yu et al., 2003). Thus, we cannot exclude the possibility that the emergence of HIV-1NL4-3ISG20 virus was due to inactivation of ISG20 antiviral function. However, we observed that HIV-1NL4-3ISG20 virus reactivation did not result in mutation or deletion in the virally integrated ISG20 cDNA contrary to what has been reported for the reactivation of HIV-1NL4-3RNaseL virus (Maitra & Silverman, 1998). Accordingly, HIV-1NL4-3ISG20 produced in the first-round infection was able to reinfest cells with a similar delayed replication (data not shown). These data suggested that the virus is able to bypass the antiviral activity of ISG20.

On the basis of its RNase activity, it is tempting to imagine that ISG20 acts directly by specifically degrading viral RNA. However, we previously showed that ISG20 specifically degrades ssRNA but not RNA sharing a stem-loop structure at the 3’ end. Thus, it is rational to speculate that the genomic HIV-1 RNA with the TAR structure is not a favourable substrate for ISG20, suggesting that ISG20 probably affects the expression of viral proteins or the late steps of virus replication such as budding and release. However, we cannot exclude the possibility that ISG20 acts indirectly on virus by global or specific degradation of cellular RNAs. Indeed, widespread activation of ISG20 leading to global degradation of RNA and resulting in cell death would be detrimental for cell survival but also for virus replication. In this way, IFN has been shown to be an essential mediator of the apoptosis induced during viral infection (Tanaka et al., 1998). Thus, it is conceivable to imagine that early ISG20-mediated destruction of infected cells might greatly reduce the ability of virus to replicate and might represent a major component of the IFN-induced host antiviral response (Barber, 2001; Gil & Esteban, 2000; Pantaleo & Fauci, 1995; Samuel, 2001; Varela et al., 2001). The fact that HIV-1 rescue is associated with the reduction of HIV-1-induced apoptosis in HIV-1NL4-3ISG20-infected cells is in accordance with this hypothesis. Because the establishment of a functional HIV-1 life cycle requires a dynamic interplay between viral and host factors, we can also postulate that ISG20 specifically affects the stability of cellular RNAs encoding cellular factors required for virus replication or transcription (Gurer et al., 2002; Ott et al., 1996, 2000). In accordance with this, we have previously demonstrated that ISG20 strongly inhibited VSV replication without any apparent global alteration in the cellular RNA profile (Espert et al., 2003a). The exact mechanism by which ISG20 expression resulted in inhibition of virus replication remains unclear. More generally, the control of RNA turnover is involved in the regulation of critical functions such as cell cycling, apoptosis and stress response, suggesting that ISG20 might be involved in these processes. The identification of cellular targets of ISG20 remains a main challenge for the comprehension of the molecular mechanism of ISG20 and IFN action.

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