CRM1-dependent, but not ARE-mediated, nuclear export of *IFN-α1* mRNA

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Summary

While the bulk of cellular mRNA is known to be exported by the TAP pathway, export of specific subsets of cellular mRNAs may rely on chromosome region maintenance 1 (CRM1). One line of evidence supporting this hypothesis comes from the study of mRNAs of certain early response genes (ERGs) containing the adenylate uridylate-rich element (ARE) in their 3′ untranslated regions (3′ UTRs). It was reported that HuR-mediated nuclear export of these mRNAs was CRM1-dependent under certain stress conditions. To further examine potential CRM1 pathways for other cellular mRNAs under stress conditions, the nuclear export of human *interferon-α1* (*IFN-α1*) mRNA, an ERG mRNA induced upon viral infection, was studied. Overproduction of human immunodeficiency virus type 1 Rev protein reduced the expression level of the co-transfected *IFN-α1* gene. This inhibitory effect, resulting from nuclear retention of *IFN-α1* mRNA, was reversed when *rev* had a point mutation that made its nuclear export signal unable to associate with CRM1. Leptomycin B sensitivity experiments revealed that the cytoplasmic expression of *IFN-α1* mRNA was arrested upon inhibition of CRM1. This finding was further supported by overexpression of ΔCAN, a defective form of the nucleoporin Nup214/CAN that inhibits CRM1 in a dominant-negative manner, which resulted in the effective inhibition of *IFN-α1* gene expression. Subsequent RNA fluorescence in situ hybridisation and immunocytochemistry demonstrated that the *IFN-α1* mRNA was colocalised with CRM1, but not with TAP, in the nucleus. These results therefore imply that the nuclear export of *IFN-α1* mRNA is mediated by CRM1. However, truncation of the 3′ UTR did not negatively affect the nuclear export of *IFN-α1* mRNA that lacked the ARE, unexpectedly indicating that this CRM1-dependent mRNA export may not be mediated via the ARE.

Key words: Nuclear export, *interferon-α1* mRNA, CRM1, HIV-1 Rev

Introduction

Nucleocytoplasmic transport occurs through nuclear pore complexes (NPCs) and is mediated by saturable transport receptors that shuttle between the nucleus and the cytoplasm. The vast majority of transport receptors are members of a conserved family of homologous proteins known as importins or exportins, and collectively known as karyopherins (reviewed by Conti and Izaurralde, 2001). The karyopherin chromosome region maintenance 1 (CRM1) mediates the nuclear export of a variety of protein and RNA substrates. These include Rev response element (RRE)-containing (Malim et al., 1989b; Mann et al., 1994), incompletely spliced human immunodeficiency virus type 1 (HIV-1) mRNAs (RRE mRNAs) via the virally encoded Rev protein (Fischer et al., 1995; Fornerod et al., 1997a; Kimura et al., 2000), several U-rich small nuclear RNAs (U snRNAs) (Fornerod et al., 1997a) and all rRNAs (Ho et al., 2000; Moy and Silver, 2002). CRM1 is, however, dispensable for the export of tRNA and most cellular mRNAs (Fornerod et al., 1997a; Herold et al., 2003).

Nuclear export of mRNA requires a number of coordinated processing events that must take place to form an export-competent mRNP (reviewed by Lei and Silver, 2002). Nuclear injection experiments demonstrated that some RNAs that undergo splicing are preferentially exported because of their specific association with the mRNA export factor REF (Aly in mice, Yra1p in *Saccharomyces cerevisiae*) (Luo and Reed, 1999; Zhou et al., 2000). It was later found that splicing resulted in the deposition of a large complex of proteins, including REF, 20-24 nucleotides upstream of an exon-exon junction (Le Hir et al., 2001). Therefore, selective export of spliced mRNAs may be explained by recruitment of mRNA export factors to the exon junction complex (Le Hir et al., 2001). Recently, it was demonstrated that recruitment of REF to spliced mRNAs depended on its interaction with the DEAD box helicase UAP56 (Luo et al., 2001; Strasser and Hurt, 2001). The regulated recruitment of UAP56, as well as its *S. cerevisiae* and *Drosophila* homologues Sub2p and HEL, respectively, onto the nascent transcript was also found to be required for mRNA export (Fleckner et al., 1997; Kistler and Guthrie, 2001; Kiesler et al., 2002).

On the basis of these data, it was therefore envisaged that UAP56 recruits REF to mRNAs during splicing (Cullen, 2003). In turn, REF binds to the major mRNA export receptor TAP (Mex67p in *S. cerevisiae*) (Strasser and Hurt, 2000; Stutz et al., 2000), which is analogous to a karyopherin in that it can interact with the FG repeats of nucleoporins (Bachi et al., 2000). TAP was originally identified as a host factor that facilitates the export of type D retroviral genomic RNAs.

However, it promotes the viral RNA export by binding directly to the constitutive transport element (CTE) of these mRNAs (Braun et al., 1999; Kang and Cullen, 1999).

Apart from this TAP-dependent pathway for bulk mRNA export, recent reports showed that the human RNA-binding nucleocytoplasmic shuttle protein HuR, a ubiquitously expressed member of the ELAV (embryonic lethal abnormal vision) family (Ma et al., 1996), was also involved in the nuclear export of a specific subset of mRNAs as an RNA adapter (reviewed by Lei and Silver, 2002). HuR affects the stability of certain early response gene (ERG) mRNAs by binding to an adenylate uridylate-rich element (ARE) in their 3′ untranslated regions (3′ UTRs) (Fan and Steitz, 1998; Peng et al., 1998) and subsequently by following these mRNAs into the cytoplasm (Brennan and Steitz, 2001). It was recently shown that leptomycin B (LMB) (Fornerod et al., 1997a; Kudo et al., 1999) blocked the HuR-dependent nuclear export of ERG mRNAs, such as c-*fos* mRNA (Brennan et al., 2000) and *COX-2* mRNA (Jang et al., 2003; Dixon et al., 2001) that were induced by serum stimulation or withdrawal, respectively. It is believed that the nuclear export of these mRNAs is based on the interactions of HuR with pp32 and APRIL, the leucine-rich nuclear export signal (NES)-containing ligands that interact with CRM1 (Brennan et al., 2000). It is therefore understood that access to the CRM1 pathway upon exposure to certain stress conditions may enhance the rapid expression of ERG proteins, thereby providing a prompt cellular response to the external stimuli (Gallouzi and Steitz, 2001).

IFNs serve as one of the principal components of the innate immunity against viruses. Viral infection is associated with the production of double-stranded RNA, which then stimulates the production of type I IFNs, i.e. IFN-αs and IFN-β (Alexopoulou et al., 2001), by infected cells within 48 hours of the infection (Brooks et al., 2001). It was shown that IFN-α production by peripheral blood mononuclear cells was reduced during the course of HIV-1 infection (Lopez et al., 1983; Siegal et al., 1986). This reduction correlates with the numerical and functional deficiencies in HIV-1-infected circulating plasmacytoid dendritic cells, i.e. natural interferon α-producing cells (Donaghy et al., 2003; Yonezawa et al., 2003). Since human *IFN-α* mRNA is an ERG transcript that contains the group II ARE cluster stretch in the 3′ UTR (Bakheet et al., 2001), it is of interest to examine whether Rev plays a role in the reduction of IFN-α production by down-regulating the expression of *IFN-α* mRNA through competition with a common export pathway component(s).

Indeed, overexpression of Rev blocked the nuclear export of human *IFN-α1* mRNA in a NES-dependent manner, suggesting that Rev and *IFN-α1* mRNA share some export pathway components. Experiments using LMB demonstrated that the cytoplasmic expression of *IFN-α1* mRNA was arrested upon inhibition of CRM1 function. This finding was further supported by the use of another CRM1-specific inhibitor, ΔCAN (Bogerd et al., 1998), that inhibited IFN-α1 expression in a dose-dependent manner. Subsequent RNA fluorescence in situ hybridisation (RNA-FISH) and immunocytochemistry revealed that *IFN-α1* mRNA was colocalised with CRM1, but not with TAP, in the nucleus. These results strongly implicate CRM1 in *IFN-α1* mRNA export, which may enable Rev to contribute to the previously reported suppression of IFN-α1 expression through competition with CRM1-dependent inhibition of *IFN-α1* mRNA export. Interestingly, truncation of the ARE-containing 3′ UTR did not negatively affect the *IFN-α1* mRNA export, unexpectedly suggesting that a new RNA adapter other than HuR may mediate this CRM1-dependent nuclear export of *IFN-α1* mRNA.

Materials and Methods

Recombinant plasmids

The human IFN-α1 gene (*IFNA1*) was amplified from HeLa cell genomic DNA by PCR using the high-stringency protocol described by Nishizawa and co-workers (Nishizawa et al., 2000). The gene-specific primers were M241, 5′-cccaagcttAGAACCTAGAGCCC-AAGGTTCAGAGTCAC-3′ (human chromosomal *IFN-α1* coding segment nt 1-29) and M242, 5′-tgctctagaGAGTAAATATAAGGAA-CATGTTTTATTAC-3′ (human chromosomal *IFN-α1* coding segment nt 876-848) (the flanking restriction sites are in lower case type). A 3′ UTR-deletion mutant was similarly generated using M241 and M227, 5′-tgctctaga-TTATTCCTTCCTCCTTAATCTTTC-3′ (human chromosomal *IFN-α1* coding segment nt 637-614). The amplicons were digested with *Hd*III/*Xba*I and cloned into the *Hd*III/*Xba*I sites of the pSI vector (Promega) to generate full length (nt 1-876; phuIFN-α1) and 3′ UTR-truncated (nt 1-637; phuIFN-α1/Δ3′ UTR) *IFN-α1* gene expression plasmids. The sequences of the amplicons were verified by DNA sequencing (data not shown).

The effect of coexpressed HIV-1 Rev on the nuclear export of *IFN-α1* mRNA was examined by cotransfection with pCG-HA-Rev (Kimura et al., 2000). M10, which acts as a trans-dominant repressor of Rev function (Malim et al., 1989a) (a kind gift from Dr B. R. Cullen, Duke University Medical Center), was expressed under the control of the CMV immediate early region promoter in the pCG-HA vector (Kimura et al., 2000). pCG-HA-Rev$^{27\text{-}29A}$ expresses a recessive-negative mutant of Rev, which is efficiently transported into the nucleus but lacks RRE mRNA binding activity (Hope et al., 1990; Zapp et al., 1991) (T.K., I.H., M. Nishikawa and J.-I.F., unpublished). Oligonucleotide-directed mutagenesis to produce *rev*$^{27\text{-}29A}$ was performed using the gene assembly method including splicing by overlap extension (Clackson et al., 1991). The generated mutation was confirmed by DNA sequencing (data not shown). An HA-tagged truncated form of CAN/Nup214 (ΔCAN; amino acids 1864-2090 of human CAN/Nup214) (Bogerd et al., 1998) was expressed using a pCG-HA vector and initially isolated by PCR amplification of the human *CAN/Nup214* gene (nt 5684-6364 of hg02228; provided by the DNA Bank, Kazusa DNA Research Institute, Chiba, Japan). The nucleotide sequence data of hg02228 has been deposited to the DDBJ, EMBL and GenBank nucleotide sequence databases under accession number AB159230. pRSVluc-CTE was generated by inserting the full-length SRV-1 CTE (Tang et al., 1997) (a kind gift from Dr F. Wong-Staal, University of California, San Diego through Dr K. Taira, University of Tokyo) at the unique *Xba*I site immediately downstream of the luciferase cDNA in pRSVluc (de Wet et al., 1987). pCRRE is a defective provirus derived from the infectious molecular clone of HIV-1$_{NL4\text{-}3}$ (Adachi et al., 1986) that has been described previously (Kimura et al., 1996).

Cells and transfections

HeLa cells (ATCC CCL2), maintained in Dulbecco's MEM containing 10% heat-inactivated foetal calf serum (D10), were transfected with the indicated expression plasmids by the calcium phosphate method as described previously (Kimura et al., 2000). The cells were transfected at 37°C for 16 hours, and further incubated for an additional 16 hours. HeLa cells were then subjected to either an IFN-α ELISA assay, western blotting, a luciferase assay, or RT-PCR analysis as described below.

IFN-α1 secreted into the culture supernatants of phuIFN-α1- or

phuIFN-α1/Δ3′ UTR-transfected HeLa cells was quantified using a human IFN-α ELISA kit (BioSource International Inc.). The luciferase enzymatic activity per 1 μg of total cytoplasmic protein was assayed using the manufacturer's suggested protocol (Promega). When HA-tagged Rev wild-type or Rev mutant proteins were expressed in order to compare their effects on *IFN-α1* gene expression, they were separated in a 15% SDS-polyacrylamide gel and electroblotted. The HA-tagged Rev proteins were then visualised with a mouse monoclonal anti-HA epitope antibody (clone SCP-12CA5; BAbCO) in order to confirm an equal level of gene expression among the Rev expression plasmids. $p55^{Gag}$ expressed in pCRRE-transfected HeLa cells was separated in a 7.5% SDS-polyacrylamide gel, electroblotted and visualised using pooled AIDS patient sera (Kimura et al., 1994) as described previously (Kimura et al., 2000). The relative amounts of Rev and HIV-1 $p55^{Gag}$ proteins were determined using a Bio-imaging analyser, MacBAS (Fuji Film), according to the manufacturer's instructions.

Microinjections

HeLa cells were plated onto Cellocate gridded coverslips (Eppendorf) pre-treated with poly-L-lysine hydrobromide (M_r: 70-150×10^3; Sigma). Twenty-four hours later, the growth medium was removed and replaced with D10 containing 25 mM Hepes, and the nuclei of the cells were injected with the indicated expression plasmids that had been adjusted to a concentration of either 365 μg/ml (phuIFN-α1 expression plasmids and pRSVluc-CTE) or 73 μg/ml (pCRRE) of microinjection buffer (10 mM Tris-HCl pH 7.5, 0.25 mM EDTA). Microinjection was performed at 37°C for 30 minutes using an Eppendorf automatic microinjection system that consisted of an automatic micromanipulator (Model 5171) and a microinjector (Model 5242). The cells were further incubated at 37°C for 4 hours, and then fixed, permeabilised and subjected to RNA-FISH alone or together with immunofluorescence as described below. When required, the injected HeLa cells were incubated at 37°C for 30 minutes (cells expressing *IFN-α1* or *luc*-CTE mRNAs) or 45 minutes (cells expressing HIV-1 *gag* mRNA), and subsequently treated for an additional 3 hours at 37°C with 20 nM LMB (Sigma). The cells were then subjected to RNA-FISH analysis as described below.

RNA-FISH and immunocytochemistry

RNA-FISH was performed as described previously (Kimura et al., 2000). The probes were digoxigenin (DIG)-11-dUTP (Roche Diagnostics)-labelled antisense oligonucleotides for *IFN-α1* mRNAs and *luciferase* mRNA fused with a CTE transcript (*luc*-CTE) [complementary to nucleotides 2538-2656 of the SV40 genome corresponding to the region that encodes the SV40 polyadenylation signal in both the pSI vector and pRSVluc-CTE (Portela et al., 1986)], or HIV-1 *gag* mRNA (complementary to nucleotides 798-964 of HIV-1_{NL4-3}), used at 5 ng/ml with a sheep antibody to DIG Fab-FITC (50 μg per ml) (Roche Diagnostics) and then with a 1:200 dilution of dichlorotriazinyl aminofluorescein-conjugated donkey anti-sheep IgG antibodies (Chemicon International Inc.). Following hybridisation and visualisation of the probes, the cells were processed for immunolabelling as previously described (Kimura et al., 2000). The primary antibodies were a rabbit anti-CRM1 antiserum (1:600 dilution in D10) and a mouse monoclonal anti-TAP antibody (10 μg/ml in D10; clone 31; BD Transduction Laboratories). The rabbit anti-CRM1 antiserum was raised against a peptide corresponding to the C-terminal 20 amino acids [Glu^{1024}-Arg^{1043} (Kudo et al., 1997) a kind gift from Dr M. Yoshida, Graduate School of Agriculture and Life Sciences, The University of Tokyo]. TRITC-conjugated swine anti-rabbit IgG antiserum (1:20 dilution in D10) and TRITC-conjugated rabbit anti-mouse IgG antiserum (1:20 dilution in D10) (both from Dakopatts) were used as the secondary antibodies. Visualisation was performed with an Olympus Fluoview confocal laser scanning microscope as previously described (Kimura et al., 2000). The FITC and TRITC images were obtained individually through separate channels and merged using the Adobe Photoshop software. No signals were obtained when the fixed cells were treated with RNase before the hybridisation or with the secondary antibody alone (data not shown). The merged images were used for measuring the colocalisation coefficients, M_1 and M_2, and Pearson's correlation coefficient, R_p (Manders et al., 1993), by the Zeiss LSM510 image examiner software (v. 3.2) according to the manufacturer's instructions. The colocalisation coefficients are defined as follows:

$$M_1 = \frac{\sum_i Ch1_{i,coloc}}{\sum_i Ch1_{i,total}} \quad \text{and} \quad M_2 = \frac{\sum_i Ch2_{i,coloc}}{\sum_i Ch2_{i,total}}$$

These coefficients show the sum of the intensities of colocalising pixels in channels 1 (FITC/mRNAs) and 2 (TRITC/CRM1 or TAP), respectively, compared to the overall sum of the pixel intensities above the thresholds in channels 1 and 2, respectively. The thresholds were set to exclude the pixel intensities present in the nucleoli.
Pearson's correlation coefficient can be calculated from

$$R_p = \frac{\sum_i (Ch1_i - Ch1_{aver}) \times (Ch2_i - Ch2_{aver})}{\sqrt{\sum_i (Ch1_i - Ch1_{aver})^2 \times \sum_i (Ch2_i - Ch2_{aver})^2}}$$

where $Ch1_i$ and $Ch2_i$ are the grey values of voxel *i* of the FITC component and the TRITC component of a dual-colour image, respectively. $Ch1_{aver}$ and $Ch2_{aver}$ are the average values of $Ch1_i$ and $Ch2_i$, respectively.

Image analysis

Quantification of cytoplasmic and nuclear mRNA signals in the injected cells was performed using an Olympus BX50 fluorescence microscope equipped with a cooled Quantix CCD camera (Photometrics). Twelve bit images were captured for a constant exposure time. Image analysis and presentation were performed using the IPLab software and Adobe Photoshop. For quantitative analysis, 25-50 cells were analysed on one Cellocate coverslip for each experiment. Experiments were performed with three coverslips for each variable, and each experiment was repeated at least three times.

Analysis of mRNA produced from transfected cells

Total cytoplasmic and nuclear RNAs were extracted from the transfected HeLa cells by the modified NP40 method as described previously (Tsuboi et al., 1998). The RNA samples were digested with DNase I (Amplification Grade; Gibco BRL Life Technologies) to remove any contaminating plasmid DNA. First-strand synthesis was performed as previously described (Kimura et al., 2000) and the cDNA synthesis reaction mixtures were then appropriately diluted to ensure linear amplification of the cDNAs in the subsequent PCR reactions. The amplified fragments were randomly labelled with [α^{32}P]dCTP (111 TBq/mmol; NEN Life Science), separated in a 6% denaturing polyacrylamide gel and then visualised by autoradiography. The relative amounts of *IFN-α1* and *GAPDH* transcripts were determined with MacBAS as described above. The *IFN-α1* transcript levels were then normalised to those of the corresponding *GAPDH* transcripts, and the ratios of the normalised *IFN-α1* transcript levels in the presence of Rev proteins to those in the absence of Rev proteins were determined.

For the detection of *IFN-α1* mRNA, the primers were M258, 5′-

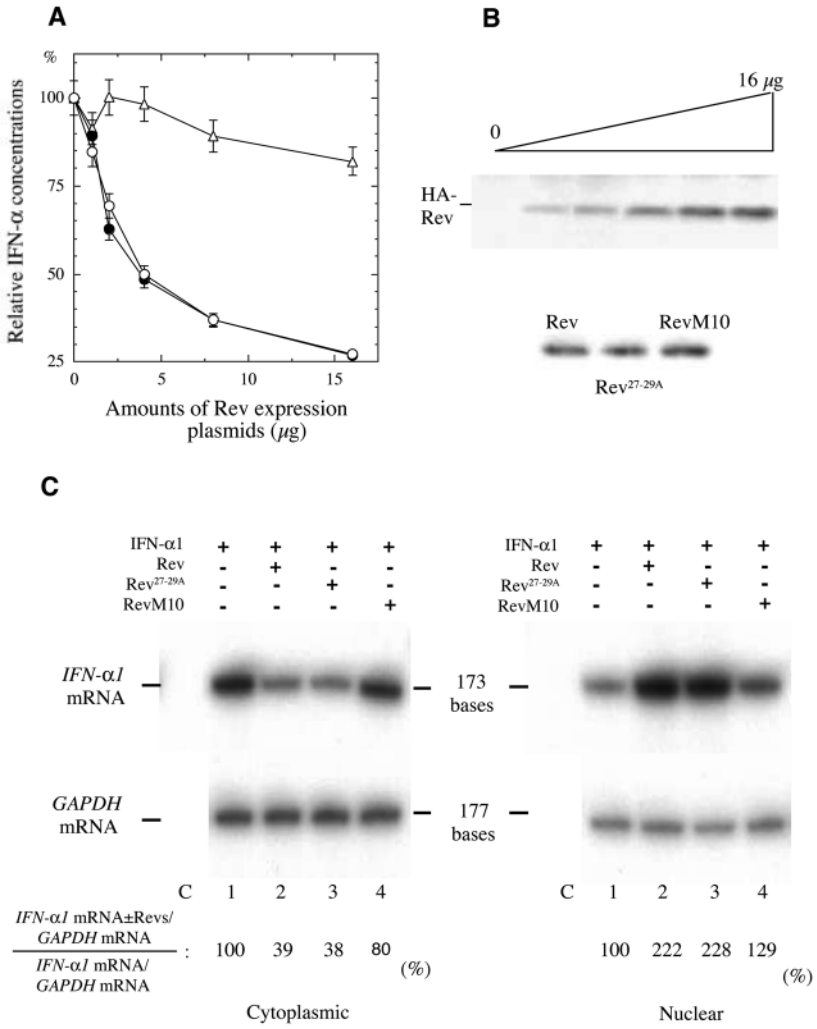

Fig. 1. HIV-1 Rev inhibits the nuclear export of *IFN-α1* mRNA in a NES-dependent manner. (A) Rev NES-dependent inhibition of *IFN-α1* gene expression. HeLa cells were cotransfected with various amounts of either pCG-HA-Rev (open circles), pCG-HA-Rev$^{27\text{-}29A}$ (closed circles) or pCG-HA-RevM10 (open triangles), and 1 μg of phuIFN-α1 for 16 hours at 37°C. pCG-HA, the empty parental vector, was included to normalise the amounts of the vector-based expression plasmids. pUC12 was included to give a total DNA amount of 20 μg. 32 hours after the DNA addition, the culture supernatants were collected to quantify the IFN-α1 secreted from the transfected cells as described in the Materials and Methods. Values are presented as the percentage IFN-α1 concentration relative to that secreted from phuIFN-α1 alone transfected HeLa cells. Values of a representative experiment of three independent transfection experiments are shown; mean±s.e.m. of triplicate samples. (Bars cannot be seen when they are smaller than the graph symbols.) (B) The top western blot shows the plasmid dosage-dependent increase in HIV-1 Rev protein expression. The bottom western blot indicates the comparable amounts of Rev and Rev mutant proteins expressed in HeLa cells transfected with the corresponding expression plasmids (16 μg each). (C) Coexpression of Rev results in a NES-dependent reduction in the cytoplasmic *IFN-α1* mRNA levels, but an increase in the nuclear *IFN-α1* mRNA levels. HeLa cells were cotransfected with pCG-HA-Rev, pCG-HA-Rev$^{27\text{-}29A}$ or pCG-HA-RevM10, and phuIFN-α1. Cytoplasmic and nuclear total RNAs were collected at 32 hours after the DNA addition and subjected to RT-PCR analysis as described in the Materials and Methods. The cDNA synthesis reaction mixtures were appropriately diluted to ensure linear amplification of the cDNAs in the PCR reactions. The ratio of each *IFN-α1* transcript level normalised to the corresponding *GAPDH* transcript level in the presence of Rev proteins, to that in the absence of Rev proteins, is shown below each lane. C, mock-transfected control. The sizes of the amplicons of the *IFN-α1* and *GAPDH* transcripts are indicated. The autoradiographs shown were obtained after 3 hours of exposure at –70°C. The results are from a representative experiment of three independent experiments.

GATGGCCTCGCCCTTTGC-3′ (human chromosomal *IFN-α1* coding segment nt 67-84), and M259, 5′-CATGTCTGTCC-ATCAGAC-3′ (human chromosomal *IFN-α1* coding segment nt 239-222). The primer pair used for the detection of human *GAPDH* mRNA were described previously (Kimura et al., 2000).

Numbering of sequences

The sequence numbering presented in this report is based on the following GenBank sequences: XM_005504 for *Homo sapiens IFN-α1* mRNA; NC_001669 for the SV40 complete genome; and M19921 for HIV-1$_{NL4\text{-}3}$.

Results

Coexpressed HIV-1 Rev inhibits the cytoplasmic expression of *IFN-α1* mRNA in a NES-dependent manner

In order to test whether the nuclear export pathway for *IFN-α1* mRNA shared common components with that for HIV-1 Rev, we examined the effect of Rev overexpression on the cytoplasmic expression of *IFN-α1* mRNA (Fig. 1). Overexpression of *rev* and inactive *rev* NES mutants was previously applied to show that the Rev NES and heat shock mRNAs shared some export pathway components in *S. cerevisiae* (Saavedra et al., 1997b). As shown in Fig. 1A, an expression plasmid encoding the human *IFN-α1* gene was co-transfected into HeLa cells with increasing amounts of expression vectors for Rev or Rev mutants. The increasing level of Rev expression (Fig. 1B, top panel) caused a progressive reduction in *IFN-α1* gene expression. Under the conditions in which Rev and the Rev mutant proteins were all expressed at a comparable level (Fig. 1B, bottom panel), this inhibitory effect was reversed when *rev* had a point mutation that made its NES unable to interact with the cellular transport factors complex, including CRM1 (Rev M10; Fig. 1A, triangles) (Malim et al., 1989a) (reviewed by Kimura et al., 2003). In contrast, coexpression of Rev$^{27\text{-}29A}$ resulted in a reduction in IFN-α1 expression to a level comparable with that in Rev-transfected cells (Fig. 1A, open and closed circles). Rev$^{27\text{-}29A}$ is a recessive-negative mutant that is efficiently transported into the nucleus but lacks RRE mRNA binding activity due to its arginine-rich domain (Hope et al., 1990;

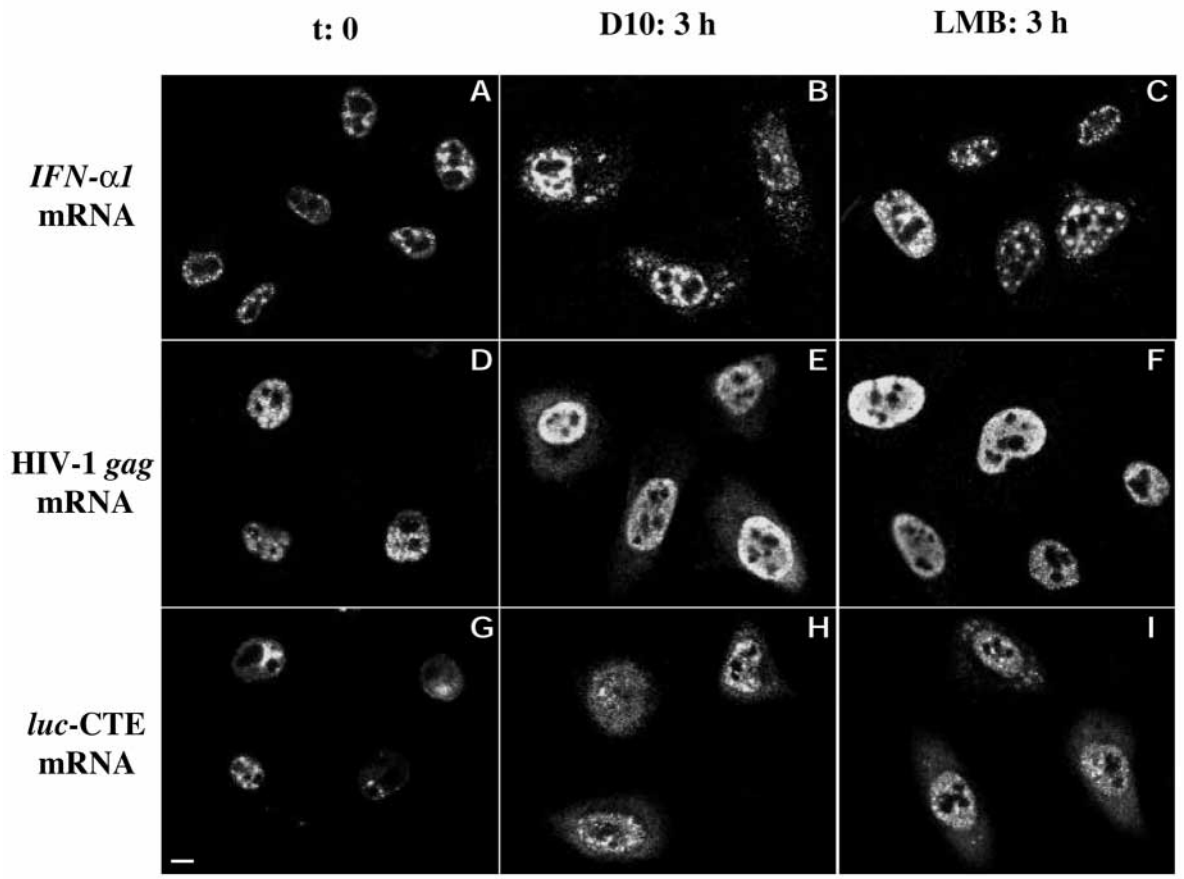

Fig. 2. LMB inhibits the nuclear export of *IFN-α1* mRNA. HeLa cell nuclei were microinjected with either phuIFN-α1, pCRRE or pRSVluc-CTE at 37°C for 30 minutes as described in the Materials and Methods. The cells were further incubated at 37°C for 30 minutes (A,G; t: 0 for cells expressing *IFN-α1* mRNA and *luc*-CTE mRNA, respectively) or 45 minutes (D; t: 0 for cells expressing HIV-1 *gag* mRNA), and then treated for an additional 3 hours with 20 nM LMB (C,F,I; LMB: 3 h) as described in the Materials and Methods. (B,E,H; D10: 3 h) Mock-treated cells. The cells were subsequently fixed, permeabilised and subjected to RNA-FISH as described in the Materials and Methods. Representative examples of each type of injected cell are shown. Scale bar: 10 μm.

Zapp et al., 1991). These results indicate that Rev blocks *IFN-α1* gene expression in a NES-dependent manner.

In order to further study the mechanism of the inhibitory Rev effect, we used PCR to analyse the subcellular localisation of *IFN-α1* mRNA in the cotransfected cells described in Fig. 1A (Fig. 1C). The mRNA quantification (shown underneath each lane) revealed that both Rev- and Rev$^{27\text{-}29A}$-mediated reductions in the *IFN-α1* gene expression level were reflected by corresponding decreases in the cytoplasmic mRNA level (approximately 70% reduction by both Rev proteins; Fig. 1C, Cytoplasmic/lanes 2 and 3) together with increases in the nuclear mRNA level (222 and 228%, respectively; Fig. 1C, Nuclear/lanes 2 and 3). In contrast, the NES mutant, RevM10, failed to inhibit the cytoplasmic expression of *IFN-α1* mRNA to the degree observed in both the Rev- and Rev$^{27\text{-}29A}$-transfected cells (Fig. 1C, Cytoplasmic and Nuclear/lanes 4, respectively). These results therefore suggest that the decrease in the amount of cytoplasmic *IFN-α1* mRNA relative to the increase in the nuclear fraction upon Rev overexpression is consistent with inhibition of the nuclear export of *IFN-α1* mRNAs, as was observed for heat shock mRNAs (Saavedra et al., 1997b). This finding then raises the possibility that the *IFN-α1* mRNA transport pathway may share common components with that for the Rev-RRE mRNA complex. A high level of Rev expression could therefore titrate these components, thereby reducing the export of *IFN-α1* mRNAs.

LMB inhibits the nuclear export of *IFN-α1* mRNA

The important feature of the Rev-dependent nuclear export pathway for RRE mRNA, but not of the bulk cellular mRNA export pathway, is the involvement of CRM1, the receptor for short leucine-rich Rev-type NESs (Fornerod et al., 1997a; Fukuda et al., 1997; Stade et al., 1997). In order to test whether CRM1 was involved in *IFN-α1* mRNA export, we performed LMB sensitivity assays on the mRNA export. LMB is a *Streptomyces* metabolite that selectively binds to and blocks CRM1-mediated nuclear transport (Wolff et al., 1997). However, CRM1 is also involved in the nuclear export of U snRNAs (Fornerod et al., 1997a) and rRNAs (Ho et al., 2000; Moy and Silver, 2002), and LMB therefore shows long-term toxicity in tissue culture (Wolff et al., 1997). We therefore employed nuclear injection rather than transfection of plasmids to minimise the incubation period required for gene expression. Thus, an *IFN-α1* gene expression plasmid, phuIFN-α1, was microinjected into the nuclei of HeLa cells,

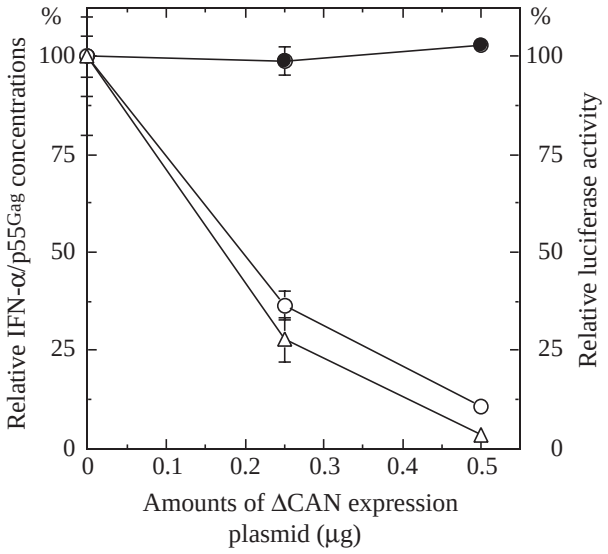

Fig. 3. Coexpressed ΔCAN inhibits *IFN-α1* gene expression in a plasmid dosage-dependent manner. HeLa cells were cotransfected at 37°C for 16 hours with a constant amount of either phuIFN-α1 (open circles), pRSVluc-CTE (closed circles) or pCRRE (open triangles), and various amounts of pCG-HA-ΔCAN that are shown on the *x*-axis. At 32 hours after the DNA addition, cytoplasmic lysates and culture supernatants were obtained to assay the IFN-α1 concentrations (open circles) and luciferase activities (closed circles), or to analyse the $p55^{Gag}$ expression by western blotting, as described in the Materials and Methods. The concentrations of IFN-α1 and $p55^{Gag}$, as well as the luciferase activities measured, are given as the percentage protein concentrations or enzymatic activities relative to those from phuIFN-α1, pCRRE or pRSVluc-CTE alone transfected HeLa cells. Values of a representative experiment of three independent transfection experiments are shown; mean±s.e.m. of triplicate samples. (Bars cannot be seen when they are smaller than the graph symbols.)

and the cells were incubated for 30 minutes at 37°C to allow transcription (Fig. 2A, t: 0). After an additional 3 hours of incubation with (Fig. 2C, LMB: 3 h) or without (Fig. 2B, D10: 3 h) 20 nM LMB, the nuclear export of *IFN-α1* mRNA was evaluated using RNA-FISH. As shown in Fig. 2C, treatment with LMB caused rapid cessation of *IFN-α1* mRNA export, resulting in the nuclear retention of the mRNA (compare Fig. 2C with B). The same concentration of LMB blocked Rev-dependent nuclear export of HIV-1 *gag* mRNA, as the positive control, as efficiently as it blocked *IFN-α1* mRNA export (Fig. 2E,F). In contrast, the nuclear export of *luc* mRNA fused with the CTE (*luc*-CTE mRNA) was not affected by LMB (Fig. 2G-I), which is consistent with previous reports that the CTE does not depend on a leucine-rich NES for its function (Bogerd et al., 1998; Otero et al., 1998). This result further indicates that the LMB-dependent inhibition of *IFN-α1* mRNA export does not result from general toxic effects on the cell viability.

ΔCAN is the dominant-negative form of CAN/Nup214, and has been shown to specifically inhibit CRM1 (Bogerd et al., 1998). This effect is based on formation of a complex between ΔCAN and CRM1, enabling the nucleoporin fragment to compete with authentic nucleoporins for binding to CRM1 (Fornerod et al., 1997b). In order to further substantiate the results of the LMB sensitivity experiments shown in Fig. 2, we performed a dose-response experiment that measured the effect of ΔCAN on *IFN-α1* gene expression. As shown in Fig. 3, ΔCAN produced a clear dose-dependent inhibition of *IFN-α1* gene expression (open circles) to a degree that was comparable to that of the Rev/RRE-dependent *gag* expression (open triangles). As expected from previous reports (Bogerd et al., 1998; Otero et al., 1998), CTE-dependent *luc* expression was not affected (Fig. 3, closed circles).

Table 1. Colocalisation and correlation coefficients of *IFN-α1* mRNA and CRM1[a]

mRNA	M_1[b]	M_2[c]	R_p[d]
IFN-α1 mRNA	0.83	0.91	0.43
HIV-1 *gag* mRNA	0.91	0.98	0.52
luc-CTE mRNA	0.29	0.88	–0.12

[a]The nuclei of HeLa cells labelled for *IFN-α1* mRNA, *gag* mRNA or *luc*-CTE mRNA, and CRM1 (shown in Fig. 4C,F,I) were analyzed using the Zeiss LSM 510 image examiner software (v. 3.2) as described in Materials and Methods. The colocalisation coefficients of each mRNA[b] and CRM1[c] as well as the Pearson's correlation coefficients[d] were then measured using the software functions.

IFN-α1 mRNA is colocalised with CRM1, but not with TAP, in the nucleus

The above data strongly implicated CRM1 in *IFN-α1* mRNA export. To confirm the involvement of CRM1 in the mRNA export and further examine the relationship between the two molecules, we studied the subnuclear localisation of *IFN-α1* mRNA and CRM1. PhuIFN-α1 was microinjected into HeLa cell nuclei (Fig. 4A-C). After 4 hours at 37°C, to allow transcription, processing and export of *IFN-α1* transcripts, the cells were subjected to analysis by RNA-FISH (Fig. 4A) and immunocytochemistry (Fig. 4B). As shown in Fig. 4C, merging of the two colours resulted in a yellow signal, indicating that the majority of nuclear *IFN-α1* mRNA was colocalised with CRM1. Quantitative analysis of these cells using the Zeiss LSM510 image examiner software revealed that the colocalisation coefficients (see Materials and Methods) of *IFN-α1* mRNA and CRM1 signals were 0.83 and 0.91, respectively (Table 1). Similar results were obtained when Rev-dependent HIV-1 *gag* mRNA was analysed as a positive control. As expected, most of the nuclear viral mRNA (Fig. 4D) was colocalised with CRM1 (Fig. 4E), resulting in a yellow signal in the merged image (Fig. 4F), and the colocalisation coefficients were 0.91 (*gag* mRNA) and 0.98 (CRM1) (Table 1). The high colocalisation coefficients for each component of the dual colour staining and the almost identical subnuclear distribution patterns for each mRNA and CRM1 were further supported by the high Pearson's correlation coefficients (*Rp*) shown in Table 1, i.e. 0.43 for *IFN-a1* mRNA/CRM1 and 0.52 for *gag* mRNA/CRM1. The *Rp* is one of the standard procedures in pattern recognition for matching one image with another, in which positive or negative values close to nil indicate almost no overlapping of the signals (Manders et al., 1993). The similar colocalisation and correlation profiles of both *gag* mRNA and *IFN-α1* mRNA with CRM1 thus suggest that *IFN-α1* mRNA interacts with CRM1 in the nucleus.

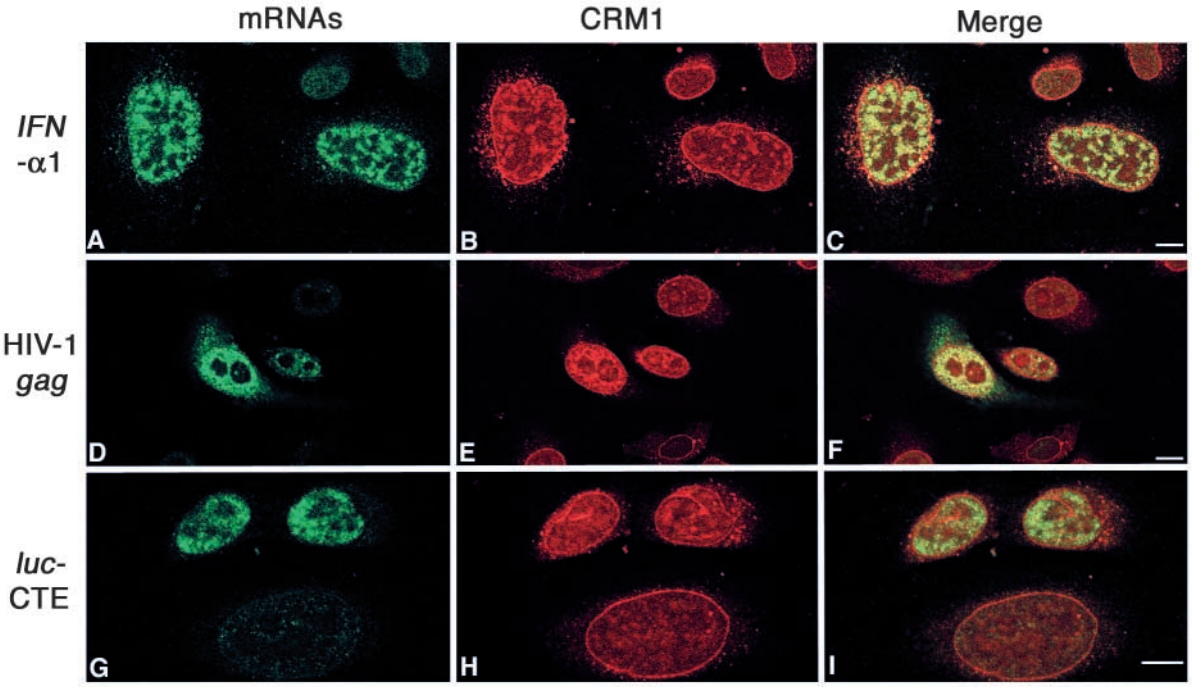

Fig. 4. Colocalisation of *IFN-α1* mRNA with CRM1 in HeLa cell nuclei. HeLa cell nuclei were microinjected with either phuIFN-α1 (A-C), pCRRE (D-F) or pRSVluc-CTE (G-I) as described in the legend for Fig. 2. The cells were further incubated for 4 hours at 37°C, and then fixed, permeabilised and subjected to RNA-FISH (A,D,G) and immunochemistry (B,E,H) as described in the Materials and Methods. The mRNAs and CRM1 immunofluorescence images were merged electronically (C,F,I). Representative examples of each type of injected cell are shown. Scale bar: 10 μm.

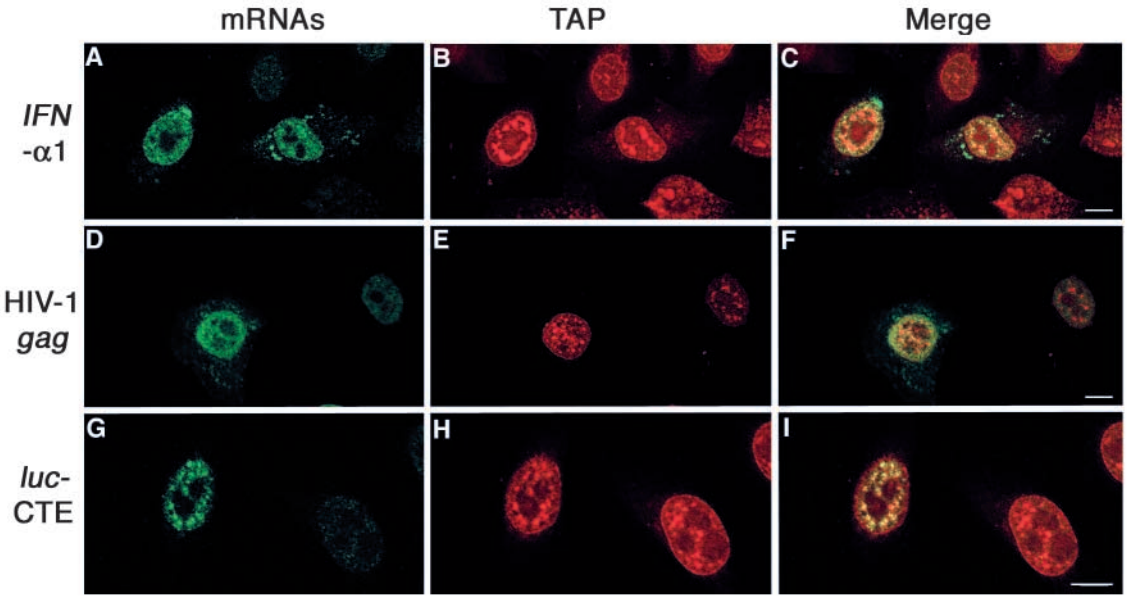

Fig. 5. *IFN-α1* mRNA is not colocalised with TAP in HeLa cell nuclei. HeLa cell nuclei were microinjected with either phuIFN-α1 (A-C), pCRRE (D-F) or pRSVluc-CTE (G-I) as described in the legend for Fig. 2. The cells were further incubated for 4 hours at 37°C, and then fixed, permeabilised and subjected to RNA-FISH (A,D,G) and immunochemistry (B,E,H) as described in the Materials and Methods. The mRNAs and TAP immunofluorescence images were merged electronically (C,F,I). Representative examples of each type of injected cell are shown. Scale bar: 10 μm.

In contrast, the colocalisation analysis of the negative control cell nuclei (Fig. 4I) showed that the colocalisation coefficients were 0.29 (*luc*-CTE mRNA) and 0.88 (CRM1) (Table 1). The relatively high colocalisation coefficient of the TRITC signal to the FITC signal does not necessarily mean partial colocalisation of CRM1 with the CTE transcript, since the *Rp* for *luc*-CTE mRNA/CRM1 was –0.12 (Table 1). Indeed, the merged image in Fig. 4I showed that most of the CRM1

had a distinct localisation from that of *luc*-CTE mRNA (compare Fig. 4H with G).

However, when the microinjected cells prepared for Fig. 4 were subjected to immunocytochemistry for TAP, most nuclear *luc*-CTE mRNA was localised with TAP, resulting in a yellow signal in the merged image (Fig. 5G-I). The following quantitative colocalisation analysis of the cells in Fig. 5I demonstrated that the colocalisation coefficients were 0.80 for *luc*-CTE mRNA and 0.90 for TAP, and the *Rp* for *luc*-CTE mRNA/TAP was 0.47 (Table 2). In contrast, the *Rp* for *gag* mRNA/TAP or *IFN-α1* mRNA/TAP were negative or close to nil (Table 1). These observations are consistent with the notion that a TAP-mediated CTE-dependent RNA export pathway is shared by the cellular mRNAs, but is distinct from the CRM1-mediated Rev-dependent RRE RNA export system (Pasquinelli et al., 1997; Saavedra et al., 1997a; Grüter et al., 1998). Thus, the subnuclear distributions of both *gag* mRNA (Fig. 5D) and *INF-α1* mRNA (Fig. 5A), which are distinct from those of TAP (Fig. 5C,F) further reinforce the previous conclusion (Fig. 4) that nuclear *IFN-α1* mRNA interacts functionally with a CRM1-dependent, but not TAP-mediated, export pathway.

The ARE in the 3′ UTR may not be relevant to the nuclear export of *IFN-α1* mRNA

It was recently reported that inhibition of the CRM1 function by LMB resulted in the selective nuclear accumulation of certain mRNAs that contained AREs in the 3′ UTR (Brennan et al., 2000; Jang et al., 2003). The nuclear export of these ARE-containing mRNAs was mediated through the interactions between HuR and CRM1-dependent ligands for HuR (Brennan et al., 2000). It was also reported that the ARE from an *IFN-α* gene, when appended in cis to a heterologous reporter gene, enhanced the export of the entrapped chimeric transcript from resealed nuclear envelope vesicles in the presence of a Jurkat cell cytosolic extract expressing ARE-binding activity (Müller et al., 1992).

In order to test whether the ARE motif present in the 3′ UTR (nt 638-876) of the *IFN-α1* gene (Bakheet et al., 2001) was involved in the nuclear export of *IFN-α1* mRNA, expression plasmids carrying either the full-length or 3′ UTR-truncated *IFN-α1* gene were microinjected into HeLa cell nuclei. After 4 hours at 37°C, the cells were subjected to RNA-FISH analysis using the probe corresponding to the region encoding the SV40 polyA signal (see Fig. 6A and the Materials and Methods). Quantitative image analysis of the RNA-FISH experiments revealed that 3′ UTR truncation did not abolish, but rather slightly enhanced, the nuclear export of *IFN-α1* mRNA (Fig. 6A, top panel; compare 1-876 with 1-637). This positive effect may result from the increase in the total cellular *IFN-α1* mRNA signal observed in phuIFN-α1/Δ3′ UTR-injected cells (Fig. 6A, bottom panel; compare 1-876 with 1-637). Fig. 6B shows representative images of both the full-length (1-876) and 3′ UTR-truncated (1-637) *IFN-α1* mRNA-expressing cells used for the analysis shown in Fig. 6A. Since the ARE motifs are only present in the 3′ UTR (see GenBank XM_005504 for *Homo sapiens IFN-α1* mRNA), these results suggest that the nuclear export of *IFN-α1* mRNA is mediated through sequences other than the ARE sequence.

To confirm that 3′ UTR truncation did not alter the pathway of choice for *IFN-α1* mRNA export, we examined the NES-dependent inhibitory effect of Rev on the expression of the *IFN-α1* gene lacking the 3′ UTR. The expression plasmid for the 3′ UTR-truncated *IFN-α1* gene was co-transfected into HeLa cells with increasing amounts of expression vectors for Rev or Rev mutants. As shown in Fig. 6C, increasing amounts of Rev (open circles) or Rev$^{27\text{-}29A}$ (closed circles) plasmids caused progressive reductions in the truncated *IFN-α1* gene expression to levels similar to that observed with the wild-type gene (see also Fig. 1A). In contrast, RevM10, the NES mutant, again failed to inhibit expression of the truncated *IFN-α1* gene to the degrees observed with both wild-type Rev- and Rev$^{27\text{-}29A}$-transfected cells (Fig. 6C, open triangles). These results indicate that Rev blocks *IFN-α1* gene expression irrespective of the 3′ UTR in a NES-dependent manner, suggesting that *IFN-α1* mRNA lacking the ARE sequence also shares a common export pathway with HIV-1 Rev. This further implies that an as-yet-unidentified motif(s) other than the ARE may account for the CRM1-dependent export of *IFN-α1* mRNA.

Table 2. Colocalisation and correlation coefficients of *IFN-α1* mRNA and TAP[a]

mRNA	M_1[b]	M_2[c]	R_p[d]
IFN-α1 mRNA	0.38	0.91	0.09
HIV-1 *gag* mRNA	0.25	0.94	–0.08
luc-CTE mRNA	0.80	0.90	0.47

[a] The nuclei of HeLa cells labelled for *IFN-α1* mRNA, *gag* mRNA or *luc*-CTE mRNA, and TAP (shown in Fig. 5C,F,I) were analyzed using the Zeiss LSM 510 image examiner software (v. 3.2) as described in Materials and Methods. The colocalisation coefficients of each mRNA[b] and TAP[c] as well as the Pearson's correlation coefficients[d] were then measured using the software functions.

Discussion

The increasing number of nucleocytoplasmic shuttle proteins harbouring Rev-like NESs makes it clear that Rev, and hence the RRE-containing HIV-1 RNA, is exported via a cellular protein export pathway (reviewed by Kjems and Askjaer, 2000). However, cellular factors utilising the Rev export pathway could also potentially be involved in RNA transport. Cross-competition experiments in microinjected *Xenopus* oocytes and subsequent use of reagents that specifically inhibit CRM1 function, have revealed that apart from Rev-dependent RRE mRNA export, CRM1 is also essential for the export of several U snRNAs and all rRNAs, but largely dispensable for the export of cellular mRNAs and tRNA (Jarmolowski et al., 1994; Fischer et al., 1995; Fornerod et al., 1997a) (for a review, see Cullen, 2003). More recently, an extensive genome-wide analysis of mRNAs expressed in *Drosophila* cells treated with LMB showed that the nuclear export of the vast majority of mRNAs was unaffected (Herold et al., 2003). Nevertheless, the export of specific subsets of cellular mRNAs may rely on CRM1. One line of evidence supporting this hypothesis comes from the study of mRNAs of certain ERGs containing the ARE in the 3′ UTR. Many AREs bind to HuR (Ma et al., 1996; Myer et al., 1997) (reviewed by Brennan and Steitz, 2001), whose nucleocytoplasmic shuttling is CRM1-dependent under certain stress conditions (Brennan et al., 2000; Jang et al., 2003). c-*fos* and *COX-2* mRNAs are such ERG transcripts (Brennan et

al., 2000; Dixon et al., 2001) and their export is LMB-sensitive upon induction by serum stimulation or withdrawal, respectively (Brennan et al., 2000; Jang et al., 2000; Jang et al., 2003). It is believed that access to the CRM1 pathway is based on the association of HuR with the other nucleocytoplasmic shuttle proteins, pp32 and APRIL. Both pp32 and APRIL contain leucine-rich NESs and interact with CRM1 (Brennan et al., 2000).

IFN-α is an ERG protein whose production by peripheral blood mononuclear cells is reduced during HIV-1 infection (Lopez et al., 1983; Siegal et al., 1986). It was recently reported that both the numerical and functional deficiencies in circulating plasmacytoid dendritic cells induced by HIV-1 infection caused the decreased IFN-α production in AIDS patients (Donaghy et al., 2003; Yonezawa et al., 2003). These findings in combination with the information that *IFN-α1* mRNA contains the group II ARE cluster stretch in the 3′ UTR (Bakheet et al., 2001) led us to examine whether Rev protein might suppress the expression of *IFN-α1* mRNA at the level of nuclear export via competition with CRM1, a possible common pathway component.

As expected from a previous report demonstrating that overexpression of Rev, but not Rev NES mutants, led to a block in the export of heat shock mRNAs following stress (Saavedra et al., 1997b), coexpressed Rev also caused a clear dose-dependent inhibition of *IFN-α1* gene expression (Fig. 1A). This inhibitory effect was due to Rev NES-dependent inhibition of the nuclear export of *IFN-α1* mRNA (Fig. 1C). These findings thus raise the possibility that, similar to heat shock mRNAs, the *IFN-α1* mRNA export pathway may share common components with the export pathway for Rev, and hence for the RRE-containing HIV-1 mRNA.

Subsequent LMB sensitivity experiments demonstrated that this was indeed the case. LMB is an antifungal antibiotic that binds covalently to a cysteine residue in CRM1 (Kudo et al., 1999), thereby preventing the binding of both the GTP-bound form of Ran and export cargo to CRM1, thus arresting the export (Fornerod et al., 1997a; Kudo et al., 1998). The RNA-FISH assay in Fig. 2 showed that LMB inhibited the nuclear export of both Rev-

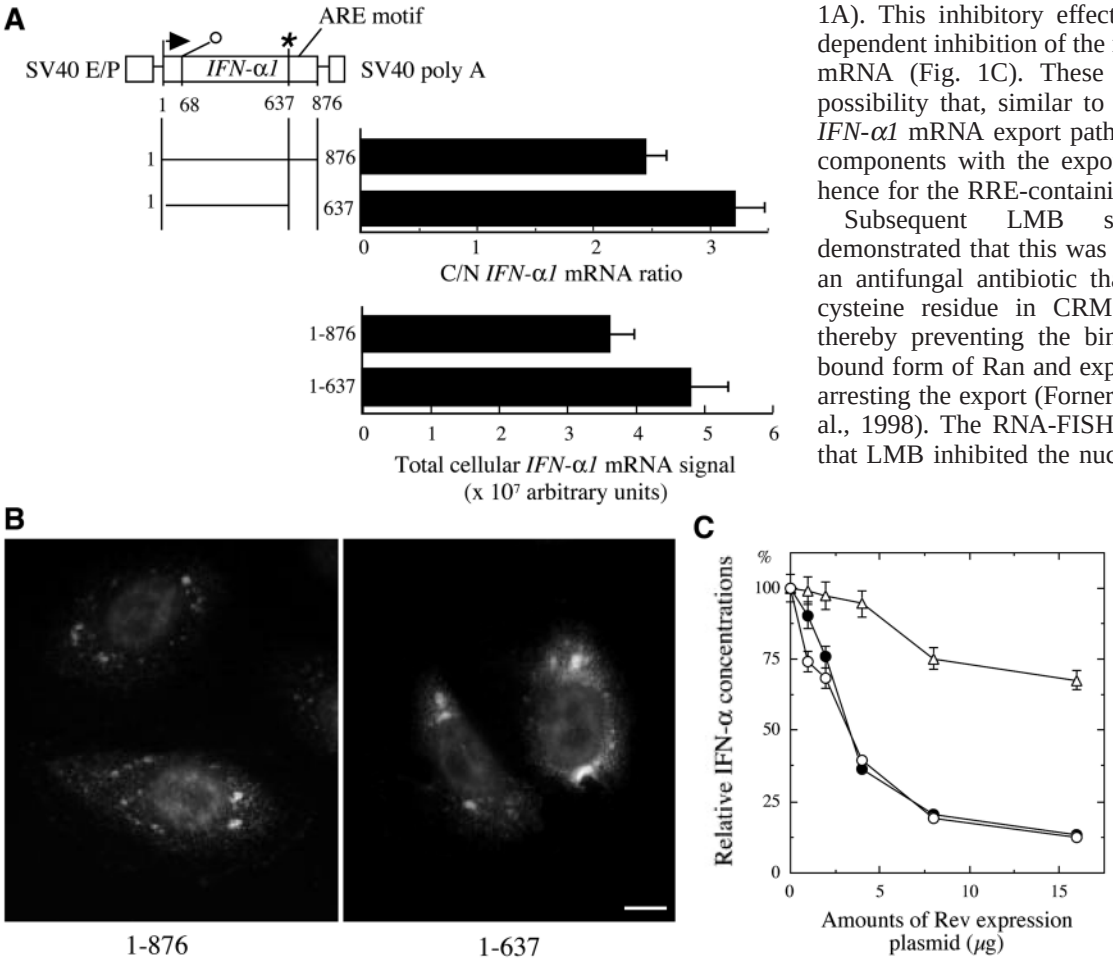

Fig. 6. 3′ UTR truncation does not negatively affect the nuclear export of *IFN-α1* mRNA. (A) HeLa cell nuclei were microinjected with either phuIFN-α1 or phuIFN-α1/Δ3′ UTR at 37°C for 30 minutes. The cells were further incubated at 37°C for 4 hours and then fixed, permeabilised and subjected to RNA-FISH as described in the legend for Fig. 2. Upon image analysis of full length *IFN-α1* mRNA- or 3′ UTR-truncated *IFN-α1* mRNA-expressing cells, the cytoplasmic versus nuclear ratios of each *IFN-α1* mRNA signal were determined and the total cellular mRNA signals were quantified as described in the Materials and Methods. The mean±s.e.m. values obtained from a representative experiment are shown. For the analyses, 25-50 cells were analysed on one Cellocate coverslip and three coverslips were examined for each plasmid; each experiment was repeated three times. The structure of the full length *IFN-α1* gene (nt 1-876) expression vector is depicted at the top left. The arrow, open circle and star show the transcription start site, and translation start and stop codons, respectively. Nt 1-67, 68-637 and 638-876 are the 5′ UTR, coding region and 3′ UTR, respectively. The ARE present in the 3′ UTR is also shown. (B) Representative images used for the analyses are shown. 1-876: phuIFN-α1-injected; 1-637: phuIFN-α1/Δ3′ UTR-injected cells. (C) Rev NES-dependent inhibition of the expression of the 3′ UTR-truncated *IFN-α1* gene. HeLa cells were cotransfected with various amounts of either pCG-HA-Rev (open circles), pCG-HA-Rev$^{27\text{-}29A}$ (closed circles) or pCG-HA-RevM10 (open triangles), and 1 μg of phuIFN-α1/Δ3′ UTR as described in the legend for Fig. 1A. At 32 hours after the DNA addition, the culture supernatants were collected to quantify the IFN-α1 secreted from the transfected cells as described in the legend for Fig. 1A. Values are presented as the percentage IFN-α1 concentrations relative to that secreted by phuIFN-α1/Δ3′ UTR alone transfected HeLa cells. Values of a representative experiment of three independent transfection experiments are shown; mean±s.e.m. of triplicate samples. (Bars cannot be seen when they are smaller than the graph symbols.)

dependent *gag* mRNA and *IFN-α1* mRNA, but not that mediated by CTE. Thus, the similar sensitivity and export inhibition profiles for *IFN-α1* and RRE-containing mRNAs imply that the nuclear export of *IFN-α1* mRNA is CRM1-dependent. This conclusion was further supported by employing ΔCAN, another selective inhibitor of CRM1 function (Bogerd et al., 1998) by blocking the ability of CRM1 to interact with NPC (Fornerod et al., 1997b). Overexpression of ΔCAN inhibited *IFN-α1* gene expression to a level that was comparable to that of the Rev-dependent HIV-1 *gag* expression, whereas the CTE-dependent *luc* gene expression, as the negative control, was not affected (Fig. 3).

On the basis of these data, we conclude that CRM1 is involved in *IFN-α1* mRNA export. However, the exact nature of the relationship between *IFN-α1* mRNA and CRM1 is currently unclear. We have been unable to detect direct interactions between the *IFN-α1* mRNA and CRM1 (data not shown). Nevertheless, the subnuclear localisation of *IFN-α1* mRNA with CRM1, but not with TAP (Figs 4, 5), with similar colocalisation and correlation coefficients to those shown by HIV-1 *gag* mRNA, strongly suggests that the recruitment of CRM1 to nuclear *IFN-α1* mRNA probably depends on an as-yet-unidentified adapter molecule that has an analogous role to Rev. The disruption of CRM1 function by either LMB or ΔCAN would prevent the shuttling of this putative adapter, resulting in inhibition of the mRNA export. Therefore, the most likely scenario for the relationship is that a leucine-rich NES-containing cellular adapter binds to a target RNA sequence and mediates the export of *IFN-α1* mRNA via the CRM1 pathway. Hence, it will be interesting to map the sequence on the mRNA and identify the adapter protein.

The *IFN-α1* gene is an ERG that encodes the ARE in the 3′ UTR (Bakheet et al., 2001). It has previously been reported that the ARE from the *IFN-α14*-related *λ2h* gene (Henco et al., 1985), when appended in cis to a heterologous RNA reporter, increased the RNA export from resealed nuclear envelope vesicles in the presence of a Jurkat cell cytosolic extract expressing ARE-binding activity (Müller et al., 1992). However, the experiments shown in Fig. 6 unexpectedly demonstrated that 3′ UTR truncation did not inhibit, but rather slightly enhanced, the nuclear export of truncated *IFN-α1* mRNA. Since the subsequent overexpression of Rev blocked 3′ UTR-truncated *IFN-α1* gene expression as efficiently as it blocked the wild-type gene in a NES-dependent manner, it is likely that the *IFN-α1* mRNA is exported through a CRM1-dependent pathway, even in the absence of the ARE-containing 3′ UTR.

Our observations argue that *IFN-α1* mRNA, a naturally intronless transcript (Nagata et al., 1980), is exported via the CRM1 pathway, whereas the key factors so far identified for mRNA export, UAP56/Sub2p, REF/Yra1p and TAP-p15, also affect the export of non-intron-containing mRNAs (Gatfield et al., 2001; Rodrigues et al., 2001; Kiesler et al., 2002; Huang et al., 2003). While the dominant export pathway is readily available, it is currently unclear why CRM1 is the receptor of choice for *IFN-α1* mRNA export.

The human subgroup C adenoviral E1B 55-kDa and E4 Orf6 proteins have been implicated in the induction of selective export of viral late mRNAs from the nucleus, with concomitant inhibition of the nuclear export of the majority of newly synthesised cellular mRNAs (reviewed by Flint and Gonzalez, 2003). Although the molecular mechanisms by which these viral proteins subvert cellular pathways are not yet clear (Flint and Gonzalez, 2003), it is not unreasonable to suppose that, in order to take control of the viral infection, *IFN-α1* mRNA may require a means to access another export pathway other than the TAP pathway which the adenovirus usurps for viral late mRNAs. Alternatively, in a more general sense, perhaps access to the CRM1 pathway, which is not routinely utilised for mRNA export, enables the rapid expression of IFN-α1 protein, thereby providing a prompt initial response to the viruses that prevents infection of the host. Indeed, in response to serum stimulation, access to the CRM1 pathway (Brennan et al., 2000) enhanced the rapid expression of c-*fos* mRNA (Andrews et al., 1987), probably facilitating the reinitiation of the cell cycle during recovery from stress (Andrews et al., 1987).

Naturally intronless transcripts often contain specific sequences that recruit export factors (Liu and Mertz, 1995; Huang and Steitz, 2001; Popa et al., 2002; Huang et al., 2003). Progressive 5′ and 3′ deletions mapped the putative export element within the coding region of the *IFN-α1* gene (T.K., I.H. and J.-I.F., unpublished). It is therefore intriguing to examine whether this export element is conserved and utilised for the posttranscriptional regulation of the *IFN-α* gene family, which comprises 20 or so separate genes (Henco et al., 1985), further suggesting that HIV-1 Rev may be able to block the innate host defence regulated by this gene family by posttranscriptionally suppressing the gene expression.

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