Characterization of the bipartite nuclear localization signal of protein LANA2 from Kaposi's sarcoma-associated herpesvirus

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LANA2 is a nuclear latent protein detected exclusively in Kaposi's sarcoma-associated herpesvirus-infected B cells. The protein inhibits p53-dependent transactivation and apoptosis, suggesting an important role in the transforming activity of the virus. To explore the molecular mechanisms of its nuclear localization, fusion proteins of green fluorescent protein (EGFP) and deletion constructs of LANA2 were expressed in HeLa cells. Only the fragment comprising amino acid residues 355–440 of LANA2 localized in the cell nucleus. This fragment contains two closely located basic domains and forms a putative bipartite nuclear localization signal (NLS). The putative LANA2 NLS was able to target EGFP to the nucleus consistently. Site-directed mutation analyses demonstrated that LANA2 contains a functional bipartite NLS between amino acid positions 367 and 384. In addition, analysis of cells transfected with a cytoplasmic LANA2 mutant revealed that an appropriate subcellular localization may be crucial to regulate p53 activity.

Key words: EGFP fusion proteins, LANA2, protein localization, site-directed mutants.

INTRODUCTION

KSHV (Kaposi's sarcoma-associated herpesvirus) or HHV8 (human herpesvirus 8) is a gammaherpesvirus implicated in the pathogenesis of KS (Kaposi's sarcoma) [1] and other proliferative disorders, such as PEL (primary effusion B-cell lymphoma) [2] and multicentric Castleman's disease [3]. The KSHV genome displays an unusual degree of genetic piracy compared with other herpesviruses [4]. Many of its viral genes encode proteins whose cellular homologues are known to be involved in growth control, signal transduction and other regulatory processes [5]. Thus a region of the genome contains a cluster of viral sequences with limited homology to the IRF (interferon regulatory factor) family of proteins [4]. One of these genes (ORFK10.5) encodes for a protein with homology to cellular IRF-4, LANA2 (initially called vIRF3). LANA2 has been shown to function as a dominant-negative mutant of both IRF-3 and IRF-7 and inhibits virus-mediated transcriptional activity of the IFNA promoter, suggesting an effect which diminishes the early inflammatory response [6]. In addition, LANA2 acts as an inhibitor of p53-induced transcription and antagonizes apoptosis mediated by p53 [7]. KSHV can persist in a latent form in most tumour cells and lymphoma-derived cell lines, but only a small number of KSHV genes are routinely found to be expressed in tumour tissues and tissue-specific gene expression patterns may exist. In accordance with this theory, LANA2 is a latent protein expressed in the nucleus in virtually all KSHV-infected cells in PEL and in the majority of KSHV-infected cells in Castleman's disease tumours but not in KS-infected cells [7]. Since herpesviruses are associated with transformation of cells, it has been suggested that the latent protein LANA2 may play a role in KSHV-mediated oncogenesis in haematopoietic tissues via its activity as a transcriptional regulator.

A large number of studies have shown that most proteins are imported into the nucleus in an energy- and signal-dependent manner [8–10]. In brief, general protein import is determined by the presence of a single, short stretch of several basic amino acids or two separate clusters of basic residues (monopartite and bipartite motif of classical NLSs respectively) [11,12]. Proteins entering the nucleus require importin molecules to recognize NLS sequences, allowing nuclear pore docking. This recognition permits transport through the nuclear pore, followed by release inside the nucleus [13,14].

In the present study, we examined the nuclear localization of LANA2 using EGFP (green fluorescent protein)-LANA2 hybrids. We provide evidence for a functional bipartite NLS and identify the sequence requirements for efficient nuclear localization of LANA2. In addition, we demonstrate that the presence of LANA2 into the cell nucleus may be necessary to inhibit the apoptosis mediated by p53 activation.

MATERIALS AND METHODS

Antibodies and reagents

Mouse monoclonal sera specific for LANA2 was kindly provided by Dr Yuan Chang and Dr Patrick Moore (Hillman Cancer Center, University of Pittsburgh School of Medicine, PA, U.S.A.). Monoclonal β-actin antibody was obtained from Sigma.

Cell lines and transfections

The human epithelial cell line HeLa (A.T.C.C. no. CCL2) was cultured in RPMI 1640 medium supplemented with 10% foetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml) and 2 mM L-glutamine. U2OS cells were maintained in Dulbecco's modified Eagle medium (Gibco BRL) with 10% foetal bovine serum. HeLa and U2OS cells were transfected at 50–70% confluence with 5 and 10 μg of DNA respectively, by using the cationic polymer transfection system (jetPEI, Polytransfection, Genomed, Bad Oeynhausen, Germany), according

Abbreviations used: EGFP, green fluorescent protein; IRF, interferon regulatory factor; KS, Kaposi's sarcoma; KSHV or HHV8, Kaposi's sarcoma-associated herpesvirus; NLS, nuclear localization signal; PEL, primary effusion B-cell lymphoma; TUNEL, terminal transferase deoxytidyl uridine end labelling.

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Table 1 Sequences of oligonucleotides used in plasmid construction

Oligonucleotide	Sequence
NLSLANA2EcoRIF	5′-AATTCTCGGAGACACGAGAGGCCTACCACGAGGCGCATTCGCCACAGGAAGCTT-3′
NLSLANA2NotIR	5′-GGCCGGATCTAAGCTTCCTGTGGCGAATGCGCCTCGTGGTAGGCCTCTCGTGTC-3′
NLSMUTRR1F	5′-GGGATGATGGGCCAGCGGCACACGAGAGGCCTACC-3′
NLSMUTRR1R	5′-GGTAGGCCTCTCGTGTGCCGCTGGCCCATCATCCC-3′
NLSMUTRR2F	5′-GAGAGGCCTACCACGGCGGCCATTCGCCACAGG-3′
NLSMUTRR2R	5′-CCTGTGGCGAATGGCCGCCGTGGTAGGCCTCTC-3′
NLSMUTRR3F	5′-CATTCGCCACGCGAAGCTTAGATCCGC-3′
NLSMUTRR3R	5′-GCGGATCTAAGCTTCGCGTGGCGAATG-3′
NLSMUTR4F	5′-GGGCCACGGAGACACGAGGCGCCTACCACGAGGCGC-3′
NLSMUTR4R	5′-GCGCCTCGTGGTAGGCGCCTCGTGTCTCCGTGGCCC-3′
NLSMUTR5F	5′-CCACGAGGCGCATTGCCCACAGGAAGC-3′
NLSMUTR5R	5′-GCTTCCTGTGGGCAATGCGCCTCGTGG-3′
NLSMUTH6F	5′-GCCACGGAGAGCCGAGAGGCCTACC-3′
NLSMUTH6R	5′-GGTAGGCCTCTCGGCTCTCCGTGGC-3′
NLSMUTH7F	5′-GCGCATTCGCGCCAGGAAGCTTAGATCCGC-3′
NLSMUTH7R	5′-GCGGATCTAAGCTTCCTGGCGCGAATGCGC-3′
NLSMUTK8F	5′-CGCATTCGCCACAGGGCGCTTAGATCCGC-3′
NLSMUTK8R	5′-GCGGATCTAAGCGCCCTGTGGCGAATGCG-3′

to the manufacturer's instructions. The subcellular localization of the EGFP fusion proteins in living cells was visualized 24 h post-transfection by fluorescence microscopy using a Nikon EclipseTE2000-U microscope.

Plasmid constructions and site-directed mutagenesis

Plasmids expressing EGFP-LANA2 fusion proteins were generated by cloning the DNA encoding LANA2 excised from the pcDNA-LANA2 plasmid (kindly provided by Dr Yuan Chang and Dr Patrick Moore) and inserted into the EGFP vector (Clontech) as a *Kpn*I/*Apa*I fragment. LANA2 deletion constructs comprising 1–430, 430–1066, 1066–1321, 1321–1706 bps fused to EGFP were generated by excision from the pcDNA-LANA2 (1–430 bp), pcDNA-LANA2 (430–1066 bp), pcDNA-LANA2 (1066–1321 bp), and pcDNA-LANA2 (1321–704 bp) plasmids (kindly provided by Dr Yuan Chang and Dr Patrick Moore), and inserted into the EGFP vector as *Kpn*I/*Apa*I fragments. To make EGFP-NLSLANA2, double-stranded oligonucleotides encoding the putative NLS of LANA2 (^{367}RRHERPTTRRIRHRKLRS^{384}) were generated by annealing the complementary oligonucleotides encoding the LANA2 NLS with an *Eco*RI site at one end and an *Apa*I site at the other end (NLSEcoRI-F and NLSApaI-R oligonucleotides are listed in Table 1, with the *Eco*RI and *Apa*I sites underlined) and insertion into the *Eco*RI/*Apa*I site of the EGFP vector. The expression plasmids for mutants 1, 2, 3, 4, 5, 6, 7 and 8, encoding LANA2 NLS mutants harbouring point mutations at specific basic residues within the putative NLS coding region, were created by PCR according to the instructions of the QuickChange Site-directed mutagenesis kit (Stratagene), using the primers listed in Table 1 and the wild-type EGFP-LANA2 plasmid as template. The mutagenic primers used were NLSMUTRR1F and NLSMUTRR1R for mutant 1, NLSMUTRR2F and NLSMUTRR2R for mutant 2, NLSMUTRR3F and NLSMUTRR3R for mutant 3, NLSMUTR4F and NLSMUTR4R for mutant 4, NLSMUTR5F and NLSMUTR5R for mutant 5, NLSMUTH6F and NLSMUTH6R for mutant 6, NLSMUTH7F and NLSMUTH7R for mutant 7 and NLSMUTK8F and NLSMUTK8R for mutant 8. This resulted in the mutations described in Table 2. All plasmid constructs were verified by DNA sequencing.

Table 2 Summary of LANA2 NLS mutants

Bold letters indicate basic residues that have been mutated in each mutant. Amino acid residues are represented using the single-letter code.

Code	Amino acid sequence (LANA2 residues 367–384)
WILD-TYPE	**RRH**E**R**PTT**RR**I**RHRK**LRS
MUTANT 1	**AA**HERPTTRRIRHRKLRS
MUTANT 2	RRHERPTT**AA**IRHRKLRS
MUTANT 3	RRHERPTTRRIRH**A**KLRS
MUTANT 4	RRHE**A**PTTRRIRHRKLRS
MUTANT 5	RRHERPTTRRI**A**HRKLRS
MUTANT 6	RR**A**ERPTTRRIRHRKLRS
MUTANT 7	RRHERPTTRRIR**A**RKLRS
MUTANT 8	RRHERPTTRRIRHR**A**LRS

Analysis of protein synthesis

For immunoblot analysis, protein samples were fractionated by SDS/PAGE, transferred to nitrocellulose and analysed by immunoperoxidase staining after reactivity with different sera.

FACS analysis

U2OS cells transfected with EGFP, EGFP-LANA2 or the cytoplasmic EGFP-LANA2 mutant 1 were treated with doxorubicin 18 h post-transfection. U2OS cells were washed 30 h later with PBS and fixed at 4 °C in 80 % ethanol in PBS for 1 h. Cells were washed again three times with PBS and incubated for 30 min at 37 °C in 0.1 % Triton X-100/0.1 % trisodium citrate/0.5 μg/ml RNase A/50 μg/ml propidium iodide. The DNA content of cells was then analysed using a FACScan flow cytometer.

TUNEL (terminal transferase deoxytidyl uridine end labelling) assay

HeLa cells transfected with EGFP, EGFP-LANA2 or the cytoplasmic EGFP-LANA2 mutant 4 were treated with doxorubicin 18 h post-transfection. A TUNEL assay was performed 30 h

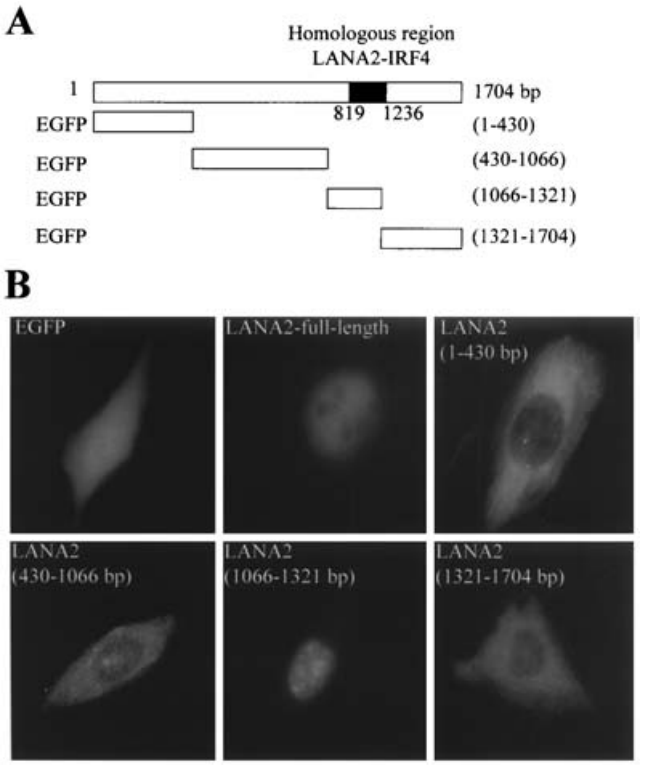

Figure 1 Region encompassing the nucleotides 1066–1321 is important for LANA2 nuclear localization

(**A**) Schematic representation of the full-length EGFP-LANA2 and a series of LANA2 deletion constructs fused to EGFP. The location of the IRF-like motif is indicated. (**B**) Subcellular localization of the indicated EGFP-LANA2 deletion mutants. HeLa cells were transiently transfected with constructs expressing the fusion proteins indicated and analysed 24 h post-transfection by fluorescence microscopy without fixation. Only the deletion mutant containing 1066–1321 bp mediates LANA2 nuclear localization, exhibing the typical speckle pattern. A control experiment demonstrated cytoplasmic and nuclear localization of EGFP alone and nuclear localization of full-length EGFP-LANA2.

later by using the In situ Cell Death Detection Kit, TMR Red (Roche) according to the manufacturer's instructions. Cells were photographed under a fluorescence microscope after a 50.2 ms exposure.

RESULTS

The fragment of LANA2 comprising 1066–1321 bp localized into the nucleus of the cell

Previous studies have shown that LANA2 is localized in the nuclei of B cells latently infected with KSHV [7]. To identify the regions of LANA2 that are responsible for its nuclear distribution, we analysed the localization of full-length and truncated forms of LANA2 fused in-frame to a gene encoding EGFP in living cells. These constructs were transiently transfected into HeLa cells and analysed for EGFP expression 24 h post-transfection. We found that, in contrast with EGFP alone, which is distributed diffusely in both the cytoplasm and the nucleus, as previously described [15], EGFP-LANA2 is located mainly in the nucleus of cells (Figure 1B). Proteins larger than 45 kDa cannot enter into the nucleus through the nuclear pore complex by passive diffusion [16]. Since EGFP-LANA2 is too large to diffuse into the nucleus, it seemed logical to assume that LANA2 could

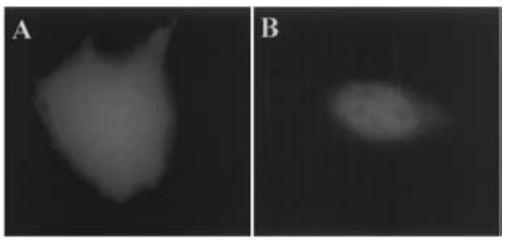

Figure 2 LANA2 contains a functional NLS

Localization of chimaeric EGFP-NLSLANA2 fusion protein demonstrating that the ^{367}RRHERPTTRRIRHRKLRS^{384} motif is a functional NLS. HeLa cells were transiently transfected with constructs expressing EGFP alone (**A**) or the fusion protein (**B**). At 24 h post-transfection the subcellular localization of the indicated proteins was determined. While EGFP localized to the nucleus and cytoplasm of the cells (**A**), fusion of amino acid residues 367 to 384 of LANA2 to EGFP directed the resulting fusion protein into the cell nucleus (**B**).

contain a functional NLS or be transported via interaction with a partner protein containing a NLS. Thus to map the region(s) of LANA2 required for nuclear localization, deletion constructs were made in the context of EGFP fusion proteins (Figure 1A). Plasmids expressing the different LANA2 fragments were transfected into HeLa cells, and the localization of the different EGFP-fusion proteins in living cells by fluorescence microscopy was determined. Analysis of this localization revealed that only the region encompassing the nucleotides 1066–1321 of LANA2 mediated efficient nuclear localization of the EGFP fusion protein, concentrated in nucleoplasmic speckles. The punctate nuclear staining is characteristic of LANA2 and might represent accumulation of LANA2 at small dense nuclear bodies. This fragment contained two stretches of basic amino acids, slightly similar to the typical bipartite NLS, suggesting that this putative NLS of LANA2 was in fact responsible for the nuclear localization.

The putative bipartite NLS of LANA2 can transport a heterologous protein to the cell nucleus

To demonstrate the functionality of LANA2 NLS, we next tested the capacity of this domain to act as a signal for active nuclear import of EGFP. We constructed a plasmid expressing EGFP fused to the LANA2 NLS sequence (EGFP-NLSLANA2) and the subcellular distribution of this fusion protein was analysed in transiently transfected HeLa cells 24 h post-transfection.

As reported previously [15], EGFP itself localized in both the nucleus and the cytoplasm of the cell, but after fusion of the putative NLS of LANA2 to EGFP, the protein was transported to the nucleus of the cell (Figure 2), indicating that this region (amino acids 367–384) indeed contains a functional signal sequence for nuclear import.

Analysis of the basic residues required for NLS functionality

The amino acid composition of the LANA2 NLS weakly resembles the bipartite NLS present in nucleoplasmin. This type of NLS is characterized by two basic amino acids that are followed by a 10-amino-acid spacer sequence and four essential basic residues [11,12]. LANA2 NLS sequence is constituted by three basic amino acids followed by a 5-amino-acid spacer sequence containing a basic amino acid, followed by two clusters of two and four basic residues separated only by one amino acid. To identify critical residues for LANA2 NLS function, we next

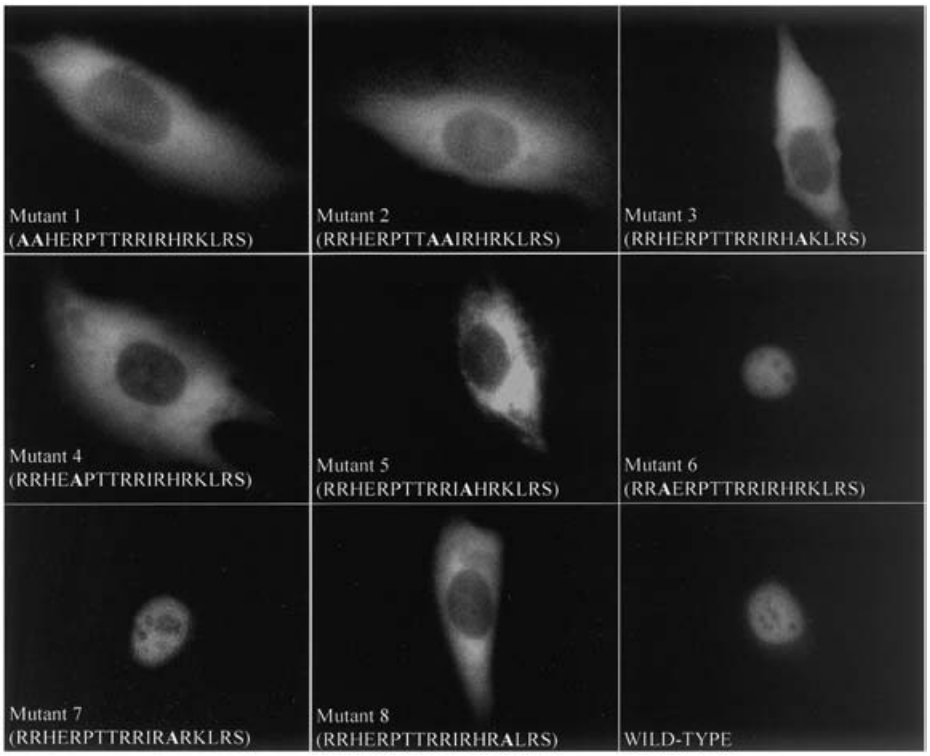

Figure 3 The basic amino acids essential for LANA2 NLS activity

The functionality of a series of LANA2 NLS domain mutants was tested by analysing the localization of the EGFP-fusion proteins. Substitution of basic amino acids in the NLS of LANA2 by alanine revealed that the basic residues RR–R—RR-R-RK, altered in mutants 1, 2, 3, 4, 5 and 8, are functionally important in directing EGFP into the nucleus. In contrast, the mutation of basic residues in bold, RR**H**-R—RR-R**H**RK, altered in mutants 6 or 7, did not negatively affect nuclear localization of the respective hybrid proteins.

generated site-directed mutants in the basic residues of LANA2 NLS (summarized in Table 2) fused to EGFP, and the localization of mutant proteins was examined by fluorescence microscopy. As shown in Figure 3, the conversion of the arginine residues at positions 367 and 368 into alanine (mutant 1) negatively affected nuclear import function, indicating the bipartite character of the NLS. Similarly, substitution of the arginine residues at position 375 and 376 with alanine (mutant 2) abrogates the nuclear localization of LANA2. Other basic residues required for the functionality of the NLS are the arginine residues at position 371, 378 and 380, as well as the lysine located in position 381. However, the conversion of the histidine residues at positions 369 or 379 into alanine did not affect nuclear import function. These data demonstrated which basic amino acid residues are important in LANA2 NLS function and revealed the high variability in the number of amino acid residues between the two clusters of basic residues among the bipartite NLS of different nuclear proteins (Table 3).

Table 3 Comparison of the NLS of LANA2 defined in this work with known bipartite NLS found in other nuclear proteins

Amino acid residues of representative NLSs are represented using the single-letter code. Bold letters indicate the basic amino acid residues required for nuclear import. LANA2 NLS weakly resembles the classical bipartite nucleoplasmin NLS

Protein	Amino acid sequence
LANA2	**RR**HE**R**PTT**RR**I**R**H**RK**LRS
Human IL5	**KK**YIDGQKKKCGEE**RRR**
Human pRb	**KR**SAEGSNPPKPL-**KK**L**R**
Human p53	**KR**ALPNNTSSSPQP**KKK**P
Nucleoplasmin	**KR**PAATKKAGQA–**KKKK**
ICP22 NLS1	**RR**PALRSPPLGT–**RKRK**
MAPKAP Kinase 2	**KK**IEDASNPLLL–**KRRKK**

Nuclear localization of LANA2 may be required for p53 inhibition activity

To determine the functional relevance of the NLS motif of LANA2, we tested the anti-apoptotic activity of a cytoplasmic LANA2 mutant after activation of p53 in both U2OS and HeLa cells. Cells were transfected with EGFP, EGFP-LANA2 or the cytoplasmic EGFP-LANA2 mutant 1 plasmids, and visualized under a fluorescence microscope to determine the efficiency of transfection. Both EGFP-LANA2 and EGFP-LANA2 mutants were expressed at similar levels, as was also observed by Western blot analysis (Figure 4A). Cells were then treated with doxorubicin and 48 h after transfection the percentage of apoptotic cells was determined. As shown in Figure 4(B), the apoptosis induced by p53 activation measured by FACS analysis was partially inhibited by LANA2, as described previously [7]. However, this apoptosis was not equally abrogated when the mutant 1, defective for nuclear localization, was expressed. Similar results were obtained after a TUNEL assay was performed (Figure 4C). Fluorescence indicative of apoptosis was clearly detected in cells transfected with both EGFP, as well as with the cytoplasmic mutant of LANA2. In contrast, expression of

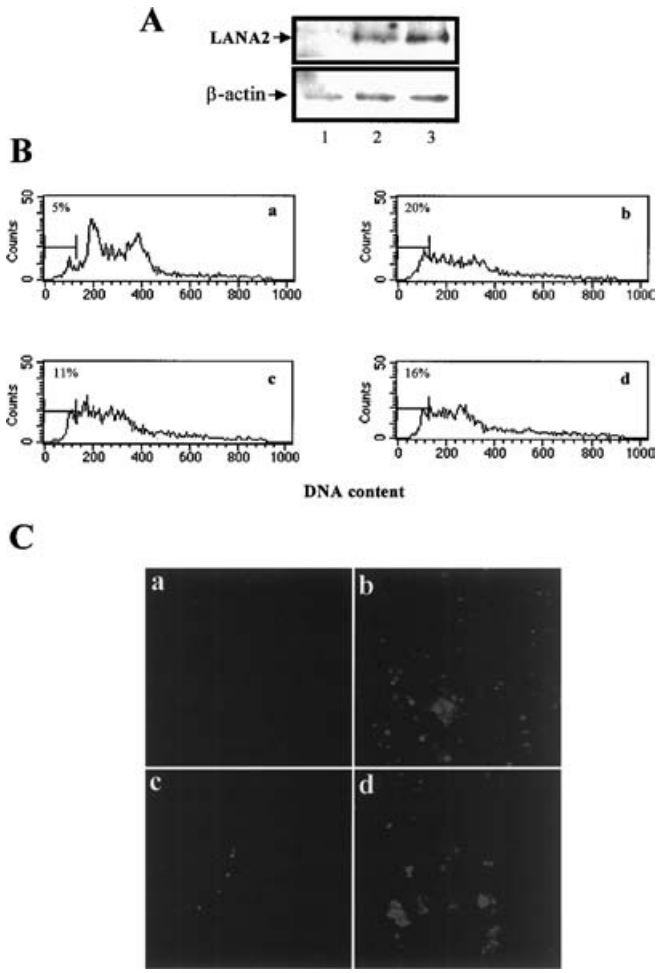

Figure 4 LANA2 requires a functional NLS to inhibit the apoptosis induced by p53

(**A**) HeLa cells were transfected with EGFP (lane 1), EGFP-LANA2 (lane 2) or EGFP-LANA2 mutant 1 (lane 3) and protein expression was determined by Western blot analysis using monoclonal antibodies against LANA2 or β-actin 24 h after transfection. Similar protein levels of both EGFP-LANA2 and EGFP-LANA2 mutant were observed. (**B**) U2OS cells were transfected with EGFP (panels a and b), EGFP-LANA2 (panel c) or EGFP-LANA2 mutant 1 (panel d). Post-transfection (18 h), cells were treated with doxorubicin (0.4 μM) (panels b, c and d), and the cells were processed for DNA content analysis 30 h after treatment. Numbers indicate the percentage of cells in the sub-G1 phase of the cell cycle. (**C**) HeLa cells were transfected with EGFP (panels a and b), EGFP-LANA2 (panel c) or EGFP-LANA2 mutant 4 (panel d) and treated with doxorubicin as described in (**A**). A TUNEL assay was performed 30 h post-treatment, and fields representative of independent assays are shown.

EGFP-LANA2 partially inhibited induction of apoptosis by doxorubicin. These data highlight the importance of the NLS sequence in the functionality of the viral protein.

DISCUSSION

The transport of macromolecules across the nuclear envelope is mediated by nuclear pore complexes, which are supramolecular assemblies that are integral parts of the nuclear envelope [17–19]. Proteins with a molecular mass of up to 40 kDa are considered to be able to diffuse freely through the nuclear pore complex. In contrast, proteins with a molecular mass of more than 40 kDa have to be translocated through the pore channel [8–10]. LANA2 is an 80 kDa protein encoded by the KSHV gene ORFK10.5, expressed in KSHV-infected haematopoietic tissues, which demonstrates a finely speckled nuclear pattern exclusive of nucleolar zones in BCBL cells, similar to the subnuclear localization of LANA1 (ORF73). It is therefore likely that LANA2 contains defined sequences that ensure efficient nuclear localization of this viral protein.

The aim of the present work was to characterize the residues of LANA2 responsible for its nuclear localization. We initially fused either the wild-type protein or the LANA2 deletion constructs to the heterologous EGFP and determined the subcellular localization of the respective fusions in living cells. The full-length EGFP-LANA2 fusion protein localized efficiently into the nucleus, as described previously [7]. Transfection of the deletion constructs revealed that only the fragment comprising nucleotides 1066–1321 was able to transport EGFP to the nucleus. In addition, expression of this fragment showed a punctate distribution, similar to the typical speckled pattern observed after LANA2 staining [7]. This finding allowed us to pinpoint this region as the sequence required to direct LANA2 to these specific intranuclear structures (Figure 1B). Inspection of this fragment revealed the presence of a putative bipartite NLS that maps to amino acids 367–384. However, the spacing of the critical basic residues in the LANA2 NLS is not analogous to that seen in the classical bipartite NLS, where the clusters of basic residues are separated by 10 to 12 amino acids. Double amino acid substitutions demonstrated that the two basic residues located in the first cluster of LANA2 NLS at amino acid positions 367 and 368, or the basic residues located in the first stretch of basic residues inside the second cluster of the NLS, positions 375 and 376, were functionally important for nuclear import activity. Other basic residues required are those located at positions 371, 378, 380 or 381, showing a NLS constituted by a high number of functionally relevant basic residues distributed in very close clusters. Comparison of these results with the classical bipartite NLS shows that LANA2 NLS is a domain for nuclear import with different requirements in the number and distribution of the basic amino acid residues.

LANA2 is predominantly localized to the nucleus of the cells, and this is consistent with its proposed nuclear roles in transcriptional regulation [6] and for cell survival in response to DNA damage [7]. In agreement with this, our results indicate that LANA2 requires a functional NLS to inhibit the apoptosis induced by p53. Since a defect in LANA2 nuclear translocation may impair its biological function, the cellular trafficking of LANA2 must be tightly regulated.

We are very grateful to Dr Yuan Chang and Dr Patrick Moore for supplying the full-length and deletion constructs of LANA2 pcDNA plasmids. We also thank Dr Manuel Collado for careful reading of the manuscript. This work was supported by a grant from the Universidad Complutense de Madrid (PR78/02-11043) and from Ministerio de Ciencia y Tecnologia de España (BIO2002-02417). C. R. is an investigator of the Ramon y Cajal Program.

REFERENCES

1 Moore, P. S. and Chang, Y. (1995) Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. N. Engl. J. Med. **332**, 1182–1185
2 Cesarman, E. and Knowles, D. M. (1999) The role of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in lymphoproliferative diseases. Semin. Cancer Biol. **9**, 165–174
3 Soulier, J., Grollet, L., Oksenhendler, E., Cacoub, P., Cazals-Hatem, D., Babinet, P., d'Agay, M. F., Clauvel, J. P., Raphael, M., Degos, L., et al. (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood **86**, 1276–1280

4 Moore, P. S., Boshoff, C., Weiss, R. A. and Chang, Y. (1996) Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. Science **274**, 1739–1744
5 Ganem, D. (1997) KSHV and Kaposi's sarcoma: the end of the beginning? Cell **91**, 157–160
6 Lubyova, B. and Pitha, P. M. (2000) Characterization of a novel human herpesvirus 8-encoded protein, vIRF3, that shows homology to viral and cellular interferon regulatory factors. J. Virol. **74**, 8194–8201
7 Rivas, C., Thlick, A. E., Parravicini, C., Moore, P. S. and Chang, Y. (2001) Kaposi's sarcoma-associated herpesvirus LANA2 is a B-cell-specific latent viral protein that inhibits p53. J. Virol. **75**, 429–438
8 Adam, S. A. (1999) Transport pathways of macromolecules between the nucleus and the cytoplasm. Curr. Opin. Cell Biol. **11**, 402–406
9 Görlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. Annu. Rev. Cell Dev. Biol. **15**, 607–660
10 Nakielny, S. and Dreyfuss, G. (1999) Transport of proteins and RNAs in and out of the nucleus. Cell **99**, 677–690
11 Dingwall, C. and Laskey, R. A. (1991) Nuclear targeting sequences–a consensus?. Trends Biochem. Sci.**16**, 478–481
12 Boulikas, T. (1993) Nuclear localization signals (NLS). Crit. Rev. Eukaryot. Gene Expr. **3**, 193–227
13 Corbett, A. H. and Silver, P. A. (1997) Nucleocytoplasmic transport of macromolecules. Mol. Biol. Rev. **61**, 193–211
14 Pemberton, L. F., Blobel, G. and Rosenblum, J. S. (1998) Transport routes through the nuclear pore complex. Curr. Opin. Cell Biol. **10**, 392–399
15 Stauber, R., Gaitanaris, G. A. and Pavlakis, G. N. (1995) Analysis of trafficking of Rev and transdominant Rev proteins in living cells using green fluorescent protein fusions: transdominant Rev blocks the export of Rev from the nucleus to the cytoplasm. Virology **213**, 439–449
16 Miller, M., Park, M. K. and Hanover, J. A. (1991) Nuclear pore complex: structure, function, and regulation. Physiol. Rev. **71**, 904–949
17 Stoffler, D., Fahrenkrog, B. and Aebi, U. (1999) The nuclear pore complex: from molecular architecture to functional domains. Curr. Opin. Cell Biol. **11**, 391–401
18 Bayliss, R., Corbett, A. H. and Stewart, M. (2000) The molecular mechanism of transport of macromolecules through nuclear pore complexes. Traffic **1**, 448–456
19 Vasu, S. K. and Forbes, D. J. (2001) Nuclear pores and nuclear assembly. Curr. Opin. Cell Biol. **13**, 363–375

Received 6 December 2002/16 May 2003; accepted 27 May 2003
Published as BJ Immediate Publication 27 May 2003, DOI 10.1042/BJ20021890