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CRM1 Mediates Nuclear Export of Nonstructural Protein 2 from Parvovirus Minute Virus of Mice

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The nonstructural protein 2 (NS2) from parvovirus minute virus of mice (MVMp) is a 25-kDa polypeptide which localizes preferentially to the cytoplasm and associates with cellular proteins in cytoplasm. These lines of evidence suggest that NS2 is positively exported from the nucleus to cytoplasm and functions in cytoplasm. We report here that nuclear export of NS2 is inhibited by leptomycin B (LMB), a drug that specifically blocks nuclear export signal (NES)–chromosomal region maintenance 1 (CRM1) interactions. CRM1 binds specifically to the 81- to 106-amino-acid (aa) region of NS2, and the region of NS2 actually functions as a NES. Interestingly, this region appears to be distinct from a typical NES sequence, which consists of leucine-rich sequences. These results indicate that NS2 protein is continuously exported from the nucleus by a CRM1-dependent mechanism and suggest that CRM1 also exports to distinct type of NESs. © 1999 Academic Press

Autonomous parvoviruses have linear, single-stranded DNA genomes of approximately 5 kb with palindromic hairpin ends. They have been found associated with tumor material and display oncosuppressive activity (1). The closely related parvoviruses express two nonstructural proteins (NSs), NS1 and NS2, during their life cycles. These proteins have a common N terminus but show a difference in size and subcellular distribution. NS1, and 83 kDa protein, is accumulated in the cell nucleus and is involved in viral DNA replication and modulation of viral and cellular promoters. NS2 is present in different isoforms that have a molecular mass in the range of 25 kDa generated by alternative splicing events (2, 3).

Mutants of MVMp with splice acceptor or termination mutations in NS2 replicate very poorly in murine cells (4). In murine cells, deletion or truncation of NS2 affects efficient capsid protein assembly and causes a decrease in translation of viral mRNA, and the mutant viruses replicate to very low levels in mice (5–8). In MVMp, a phosphorylated form of NS2 is found preferentially in the cytoplasm (9), and it also associates with the 14-3-3 proteins which are connected to signal transduction (10). Thus, NS2 is mainly found and interacts with cellular proteins in cytoplasm. However, the functions of NS2 in cytoplasm are still poorly understood.

Recently, CRM1 was found to be a receptor for various NES sequences, including viral proteins, such as HIV-1-Rev and HTLV-1-Rex. The CRM1 belongs to the importin β family, the members of which act as carriers to transport proteins between the cytoplasm and the nucleus (11–17). HIV-1-Rev and HTLV-1-Rex bind their respective RNA response elements, the Rev response element (RRE), and the Rex response element, and subsequently export singly spliced and genomic viral RNA to cytoplasm. Export of these proteins depends on CRM1, and LMB has been shown to block the export by CRM1. While MVMp NS2 protein is mainly found in cytoplasm whether it is exported by active mechanisms is not yet known. In this study, we show evidence that the nuclear export of NS2 is mediated by a CRM1 dependent-manner.

MATERIALS AND METHODS

Plasmid constructions. The CRM1 expression vector (pcDNA3/HA-tagging CRM1) was made by incorporating the HA epitope at N-terminus of CRM1, which was cloned into the *Bam*HI site of

Abbreviations used: aa, amino acid; bp, base pair; kDa, kilodalton; NES, nuclear export signal; LMB, leptomycin B; HA, hemagglutinin epitope; His_6, six consecutive histidine residues; PBS, phosphate buffered saline; FCS, fetal calf serum; HIV-1, human immunodeficiency virus-1; HTLV-1, human T cell leukemia virus-1.

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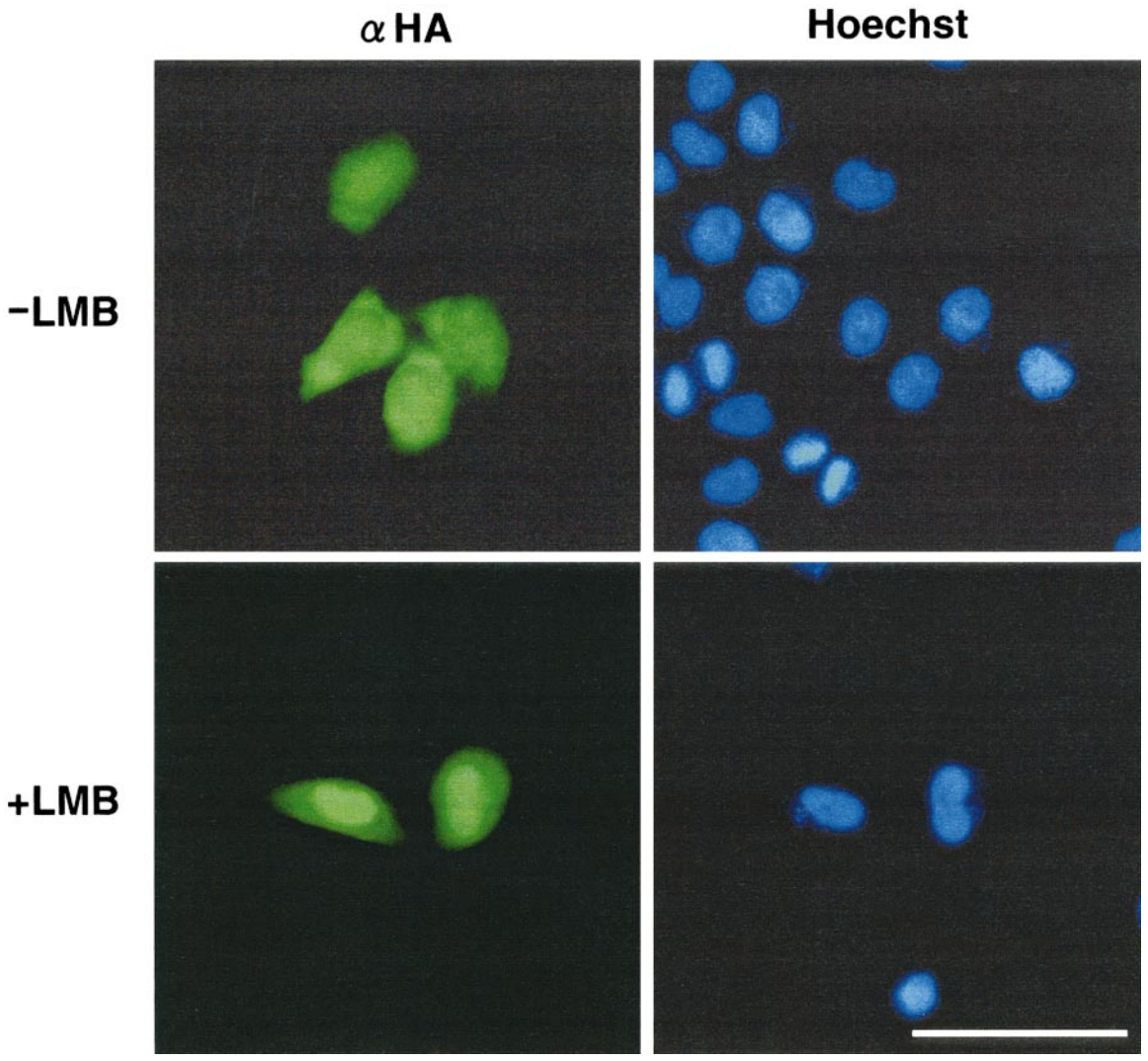


FIG. 1. Nuclear export of NS2 by a CRM1-dependent manner. HeLa cells were transiently transfected with plasmids encoding a HA-tagging form of the wild type-NS2. At 48 h after transfection, cells were treated with or without LMB (5 ng/ml) for 3 h, then fixed and stained with anti-HA antibody and Hoechst 33342. Bar, 25 μm.

pcDNA3 (Invitrogen). The CRM1 gene containing the entire coding region was amplified from a human kidney cDNA library by the PCR technique with a set of primers 5′-ACAGGATCCCTATGCCAGC-AATTATGACAA-3′ (CRM1F), 5′-ACAGGATCCACAAAAATGGGC-ATGAAG-3′ (CRM1R), each end contains *Bam*HI restriction sites. The PCR product was digested with *Bam*HI, and inserted into the *Bam*HI site of the pcDNA3/HA. The integrity of the plasmid sequence was ascertained by sequencing according to the manufacturer's instructions (Applied Biosystem Co. Ltd.).

To construct NS2 expression vectors, the NS2 gene containing the entire coding region was amplified from viral infected cells by the RT-PCR technique. MVMp virus, obtained from the ATCC, was passaged in A9 cells and stocks were prepared for subsequent experiments. A9 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO-BRL) supplemented with 10% fetal calf serum (FCS). The confluent cultured cells were trypsinized, washed with PBS, and suspended in DMEM (without FCS) containing viral inoculum at a theoretical m.o.i. of 2 to 3. After incubation at 37°C for 1 h, cells were washed to remove the inoculum, resuspended in DMEM with 5% FCS, and plated at 4×10^6 cells per 10-cm-diameter dish. The infected cells were harvested at days 2 postinfection, and the total RNA was prepared for RT-PCR templates. First-strand cDNA was synthesized by standard techniques using 5 μg of total RNA and oligo-dT primer. The wild type-NS2 gene containing the coding region was amplified by the PCR technique with a set of primers, 5′-ACAGGATCCCCATGGCTGGAAATGCTTACTC-3′ (NS2F) and 5′-ACAGGATCCAAGTGATTTCAGGCCTGTAAA-3′ (NS2R), each end contains *Bam*HI restriction sites. The PCR product was digested with *Bam*HI, and inserted into the *Bam*HI site of the pcDNA3/HA, pcDNA3.1/His A (Invitrogen) and pGEX-5X-1 (Pharmacia). To express various forms of GST-NS2 fusion proteins were prepared by the PCR technique with a set of primers, each end contains *Bam*HI and *Sal*I restriction sites and inserted into the *Bam*HI and *Sal*I sites of the pGEX-5X-1. To observe subcellular localization of the 81-106 aa region of NS2 when the cells were treated with LMB or not, we constructed the expression vector as green fluorescent protein (GFP)-fusion proteins. The GFP-fusion vector of the NS2 (81-106 aa) was constructed from pGEX-5X-1-NS2 (81-106 aa) by digesting with *Bam*HI and *Sal*I fragment which inserted into the *Bgl*II and *Sal*I sites of the pEGFP-C2 (Clontech). As a positive control to the functional NES, the GFP-fusion vector of HIV-1-Rev-NES was constructed by fused to the carboxyl terminus of GFP for synthetic

oligonucleotides specifying the amino acid position 75-84 (LPPLER-LTLD) in HIV-1-Rev. The integrity of the plasmids sequence were ascertained by sequencing according to the manufacturer's instructions described above.

Immunofluorescence and microscopic analysis. To detect the subcellular localization of NS2, HeLa cells were grown to subconfluency on Lab-Tec Chamber (NUNC) in DMEM with 10% FCS. Cells were transfected with 100 ng of pcDNA3/HA/NS2 expression vector using the polycation Superfect (Qiagen) according to the manufacturer's protocol. After 48 h, cells were incubated for 3 h with the same medium containing 5 ng/ml of LMB (LMB was kindly provided by Dr. Minoru Yoshida of the University of Tokyo). LMB-treated or -untreated cells were fixed at room temperature with 3% paraformaldehyde for 30 min and then permeabilized with cold 2% Triton X-100 in PBS for 5 min. After blocking with 3% BSA and 0.1% Triton X-100 in PBS, the cells were incubated with anti-HA antibody (12CA5 mAb, Boehringer Mannheim) for 1 h at 37°C and were stained with Hoechst 33342 (Boehringer Mannheim) and Cy2-conjugated anti-mouse IgG antibody (Amarsham) for 1 h at room temperature. Immunofluorescence was observed under a fluorescence microscope (Leica, FLUOVERT-FU).

Immunoprecipitation and Western blot analysis. For coimmunoprecipitations, 1×10^5 of 293T cells per 6 cm-diameter dish were cotransfected with 1 μg of the pcDNA3/HA/CRM1 and pcDNA3.1/His A/NS2 expression vectors as described above. After 36 h, cells were lysed in 1 ml of RIPA buffer (20 mM HEPES (pH 8.0), 150 mM NaCl, 1% Triton X-100, 1 mM DTT, 1 mM EDTA, 0.1 mM Na_3VO_4, 5 mM NaF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF) for 30 min on ice, and cell debrises were removed by centrifugation at 14,000 $\times$ g for 15 min. The lysates were first cleared with protein G beads (Pharmacia) for 30 min, followed by incubation with anti-His_6 antibody (Boehringer Mannheim), anti-HA antibody (3F10 rAb, Boehringer Mannheim) or pre-immune mouse IgG for 30 min on ice. Finally, the antibody complexes were captured with protein G beads for 2 h. The immunoprecipitates were washed four times with RIPA buffer. They were fractionated by 10% SDS-PAGE and transferred to Immobilon P transfer membrane (PVDF) (Millipore). Western blot was probed with anti-HA antibody in TBS-T buffer (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.05% Tween 20) containing 0.5% skim milk (Difco) for 1 h at room temperature after blocking with TBS-T containing 5% skim milk for 1 h. Rat antibodies were detected with horseradish peroxidase (HRP)-linked goat antibodies to rat IgG (Jackson ImmunoResearch Lab, Inc.) by enhanced chemiluminescence (Amersham).

Glutathione S-transferase (GST) pull-down assay. GST-NS2 fusion proteins and GST were expressed in *E. coli* strain BL21 and purified with gluthatione-Sepharose beads according to the manufacturer's protocol (Pharmacia). Amounts of GST-fusion proteins were estimated by Coomassie Brilliant Blue staining. For pull-down assay, the CRM1 protein was labeled in the TNT-coupled reticulocyte lysate system (Promega) with T7 RNA polymerase and ^{35}S-methionine/cysteine. Translation product was pre-cleaned with GST bound to glutathione-Sepharose beads, then was incubated with GST-fusion proteins-bound glutathione-Sepharose beads at 4°C for 2 h in 500 μl of buffer A (20 mM Tris-HCl (pH 8.0), 120 mM NaCl, 0.05% Tween 20, 1 mM DTT, 1 mM EDTA, 10% glycerol, 0.1 mM Na_3VO_4, 5 mM NaF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF). The complexes were washed four times with buffer A, and pull-down complexes were analyzed by 10% SDS/PAGE and an image analyzer (BAS 2000; Fujix). The binding efficiency between NS2 and CRM1 in the presence or absence of RanGTP was performed by using pull-down assay, based on methodology described by Tang *et al.* (18). The recombinant G19V Ran (a mutant form of Ran that lacks GTPase activity and hence remains constitutively GTP-bound) was expressed and purified as described previously (19). GST-wild type-NS2 fusion protein was incubated with 5 μl of ^{35}S-labeled CRM1 in 100 μl of buffer B (20 mM Tris-HCl (pH 8.0), 120 mM NaCl, 5 mM $MgCl_2$, 10 mM GTP, 0.05% Tween 20, 1 mM DTT, 5% glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF) in the presence or absence of 0.3 μg/μl of G19V Ran at room temperature for 1 h. The beads-bound proteins were analyzed by 10% SDS/PAGE and an image analyzer.

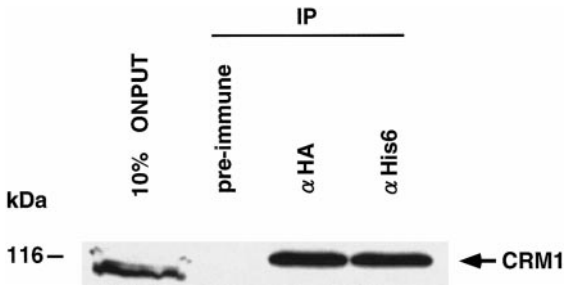


FIG. 2. CRM1 can interact with NS2 *in vivo.* 293T cells were cotransfected with pcDNA3/HA/CRM1 and pcDNA3/His A/NS2. Whole cell extracts were prepared and immunoprecipitated (IP) with pre-immune, anti-His_6 or anti-HA antibody. The immunoprecipitates were then analyzed by immunoblotting with anti-HA antibody.

RESULTS

Leptomycin B distrupts the cytoplasmic localization of NS2. We considered the possibility that the cytoplasmic localization of NS2 in interphase is ensured by the CRM1-mediated nuclear export system. To test this possibility, we used LMB, a specific inhibitor of the CRM1-dependent nuclear export, that is shown to inhibit interactions of the CRM1 with NES. Expression vector of NS2 protein was constructed by incorporating the HA epitope at N-terminus of NS2, and HeLa cells were transfected by using the polycation Superfect according to the manufacturer's protocol. As shown in Fig. 1, a HA-tagging form of NS2 was accumulated in the nucleus when cells were treated with LMB. In contrast, there was no change in distribution of NS2 without LMB. These data suggest that nuclear export of NS2 is mediated by CRM1-dependent manner.

NS2 interacts with CRM1 in vivo. We next investigated whether NS2 could interact with CRM1 *in vivo* by using coimmunoprecipitation experiments. Expression vectors were constructed by incorporating the HA epitope at N-terminus of CRM1 and the His_6 epitope at N-terminus of NS2, and 293T cells were cotransfected as described above. A mouse monoclonal antibody specific for the His_6 epitope was utilized for a coimmunoprecipitation. Western blot analysis of this immunoprecipitate with anti-HA antibody confirmed their identity as CRM1 (Fig. 2). This shows that NS2 is indeed able to interact with CRM1 *in vivo.*

CRM1 binds specifically to the 81- to 106-aa region of NS2. It has been reported that NES-containing proteins interact with CRM1, and interaction between CRM1 and NESs is essential for nuclear export of NES-containing proteins (11, 12, 15). To determine a

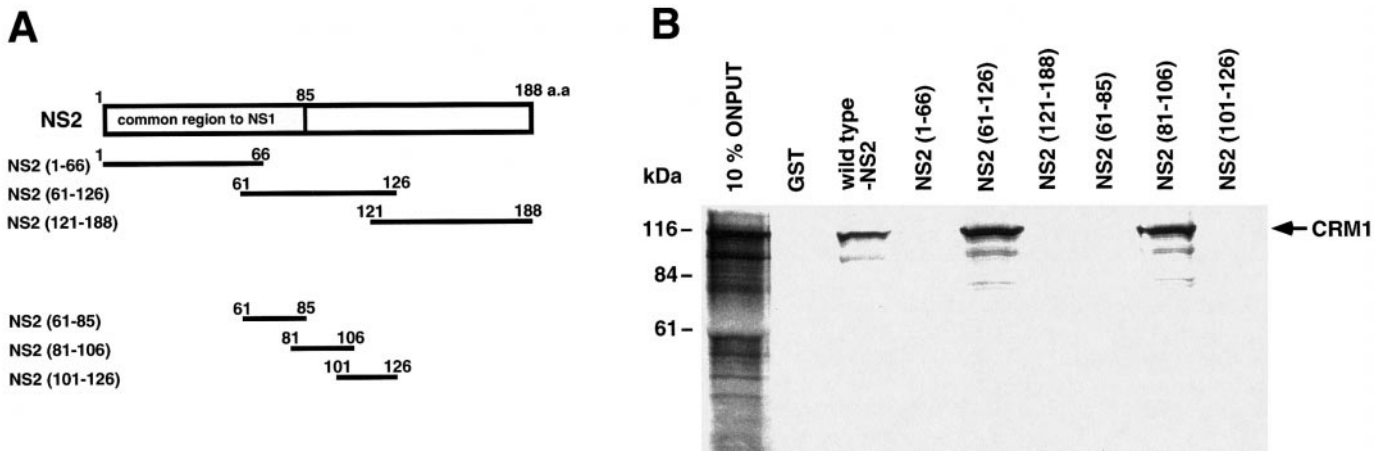


FIG. 3. Interaction between CRM1 and NS2 *in vitro.* (A) Schematic presentation of NS2 subfragments used for pull-down assay. Stippled box of N-terminus (1-85 aa) was indicated that the region is common to NS1 and NS2. (B) GST pull-down assay of wild type-NS2 and deletion mutants as GST fusion proteins using a ^{35}S-labeled CRM1. Positions of molecular mass markers are indicated.

binding region of NS2 with CRM1, we expressed as GST fusion proteins of NS2, and tested for the ability to bind to CRM1 translated *in vitro* by using a GST pull-down assay. Full-length and several deletion mutants of NS2 were used for a binding assay (Fig. 3A). As shown in Fig. 3B, *in vitro* translated product of CRM1 was capable of binding the containing 81-106 aa region of NS2. These results indicate that CRM1 binds specifically to the 81-106 aa region of NS2. However, the possibility that the specific interaction between CRM1 and NS2 could be mediated by a factor provided from reticulocyte lysate, cannot be formally excluded.

The formation of NS2-CRM1 complexes is blocked by LMB, and CRM1 binds efficiently to the NS2 in the presence of RanGTP. Recent biochemical studies on the physiological function of LMB indicated that LMB abolished association of CRM1 with NES by binding directly to CRM1, thereby inhibited nuclear export of NES-containing proteins (11–13, 15). Next, we investigated whether the interaction between NS2 and CRM1 is blocked by LMB. As shown Fig. 4A, addition of LMB to 100 ng concentration completely blocked the formation of NS2-CRM1 complexes.

It is well known that CRM1 binds efficiently to NES-containing proteins in the presence of RanGTP. Specifically, while Fornerod *et al.* (11) have reported that the CRM1-NES interaction is dependent on the presence of RanGTP, and indeed involves the formation of an obligate NES/CRM1/RanGTP ternary complex, Fukuda *et al.* (12) and Ossareh-Nazari *et al.* (15) have reported a readily detectable CRM1-NES interaction in the absence of any added RanGTP. Therefore, we compared the binding efficiency between NS2 and CRM1 in the presence or absence of RanGTP. As shown in Fig. 4B, the

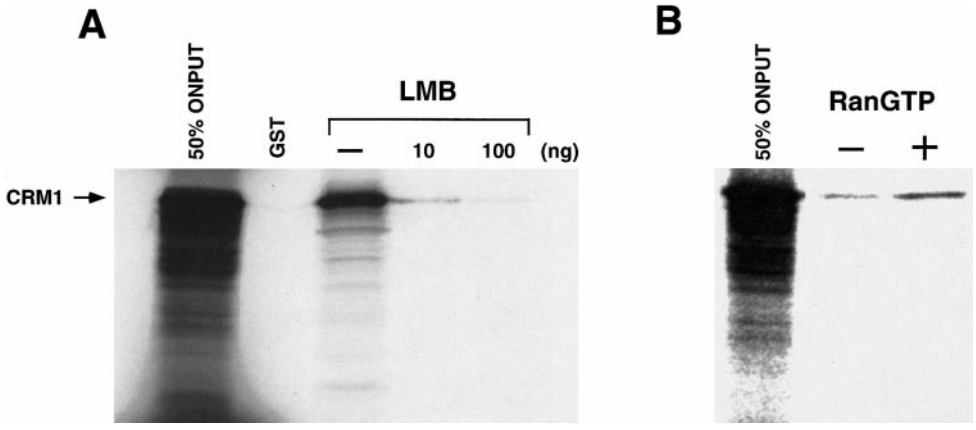
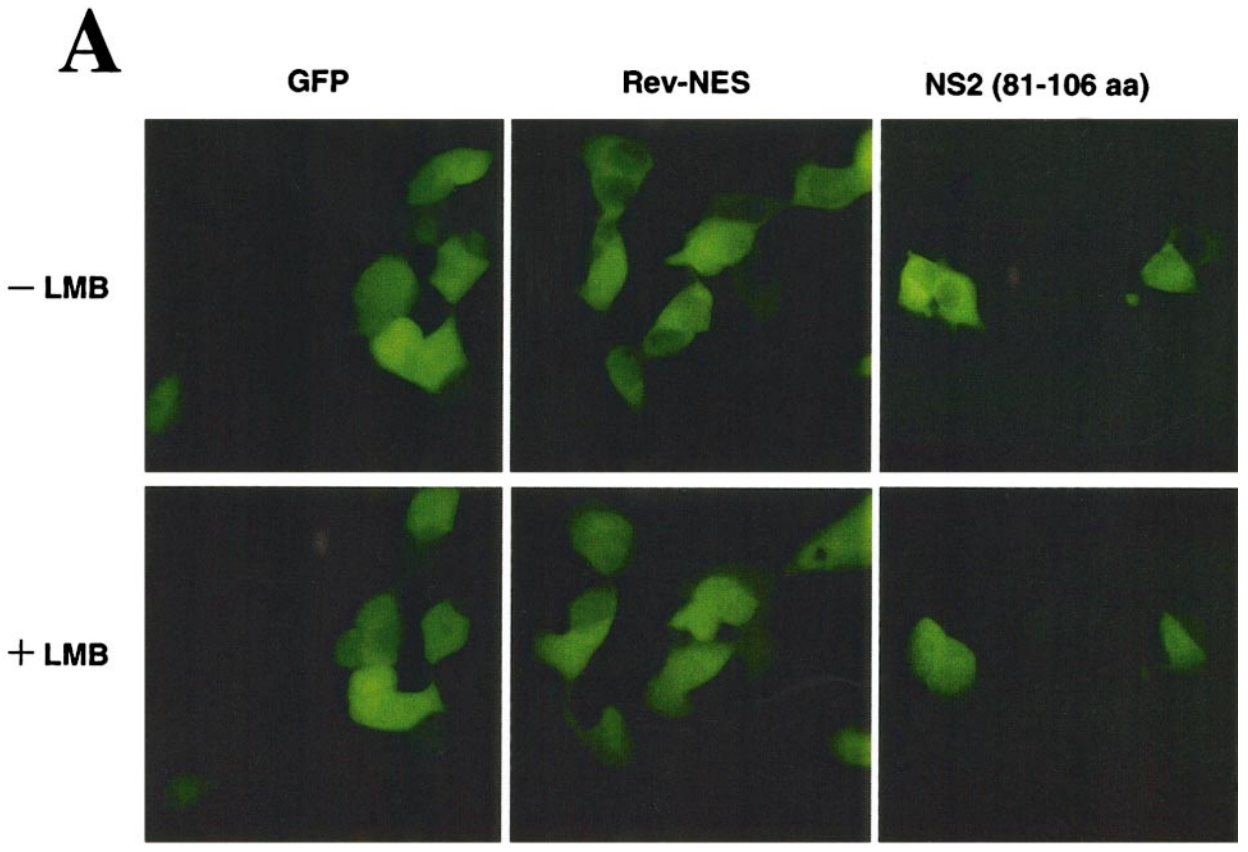


FIG. 4. Effect of RanGTP and LMB to the binding efficiency between NS2 and CRM1. (A) ^{35}S-labeled CRM1 translated *in vitro* was incubated alone or with 10 ng, 100 ng of LMB in buffer A containing GST-wild type-NS2 bound glutathione-Sepharose beads. (B) GST-wild type-NS2 was incubated with ^{35}S-labeled CRM1 translated in vitro in buffer B in the absence or presence of RanGTP. Pull-down complexes were analyzed as described under Materials and Methods.



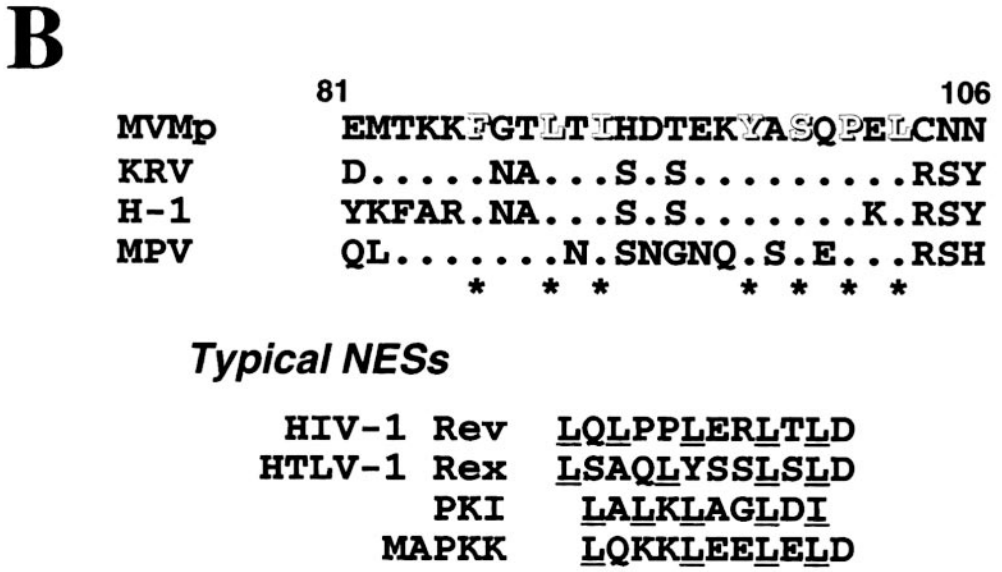


FIG. 5. The 81-106 aa region of NS2 can functions as a NES. (A) Expression vector of GFP and GFP-fusion proteins were transfected into 293T cells. At 16 h after transfection, GFP fluorescence was recovered under the fluorescence microscope. Then, same cells were treated with LMB (10 ng/ml) for 1 h and GFP fluorescence was recovered. The LMB treatment on nuclear export in this study was controlled by analysis of the GFP and GFP-Rev-NES. (B) Alignment of the translated MVMp NS2 (81-106 aa) sequence with those of major rodent parvoviruses or the consensus motif of the hydrophobic residue-rich NESs. Parvoviruses; parvovirus H-1 (H-1), mouse parvovirus (MPV), and Kilham rat virus (KRV). Shading indicated that the sequence is identical between MVMp and the other virus. Comparison of a typical NES sequences of HIV-1 Rev, HTLV-1 Rex, PKI and MAPKK. Leucines and chemically related amino acids involved in NES function are underlined.

efficient binding of NS2 to CRM1 increased in the addition of RanGTP than in its absence. These data indicate that NS2 also has the same binding activities as those of previously known NESs; the efficient binding of NS2 to CRM1 was not only inhibited by LMB but also increased by the addition of RanGTP.

The 81- to 106-aa region of NS2 actually functions as a distinct type of NES. To test whether the 81-106 aa region of NS2 functions as an NES, we constructed the GFP-fusion proteins which were transfected into 293T cells, observing subcellular localization of GFP-fusion proteins when cells were treated with LMB. As shown in Fig. 5A, the cytoplasmic localization of GFP-NS2 (81-106 aa) was distributed evenly throughout the cell by LMB treatment. We compared the 81-106 aa sequence with other rodent parvoviruses of NS2 or the consensus motif of leucine-rich NESs (Fig. 5B). Indeed, a minimal binding region of MVMp NS2 with CRM1 lies within the amino acid sequence that is at least conserved in rodent parvoviruses. Interestingly, the 81-106 aa region of NS2 appears to be a distinct type of NES sequence, differ significantly from typical NESs in the organization of the hydrophobic residues, especially amino acid sequences rich in leucine. These results strongly demonstrate that the 81- to 106-aa region of NS2 actually functions as an LMB-sensitive NES, and distinct type of NESs may bind to CRM1, which may also be sensitive to LMB.

DISCUSSION

NS2 protein of autonomous parvoviruses localizes mainly in the cytoplasm, in spite of the low molecular weight (approximately 25 kDa), is not distributed evenly throughout the cell. This may provide the possibility that the NS2 is positively exported from the nucleus to cytoplasm. We hypothesized that NS2 was exported by a CRM1-mediated mechanism and would be inhibited by LMB. As shown in Figs. 1 and 3, the nuclear export of NS2 was inhibited by LMB, and CRM1 binds specifically to the 81-106 aa region of NS2. Moreover, the binding efficiency between NS2 and CRM1 increased by the presence of RanGTP, and was inhibited by the addition of LMB (Fig. 4).

Recently, it was found that nuclear export of distinct type NES-containing Rev-like proteins of the equine infectious anemia virus (EIAV) and the feline immunodeficiency virus (FIV) were inhibited by LMB (20). In this study, we show evidence that NS2 also binds to CRM1 through the region of a distinct type of NES sequence, and nuclear export of the region is inhibited by LMB. These results indicate that the region of NS2 can functions potentially as an LMB-sensitive NES, and demonstrate that nuclear export of proteins by CRM1-dependent manner may be not restricted to typical NES-containing proteins.

NS2 protein of MVM is required for capsid assembly in murine cells (6). Moreover, the expression of all viral proteins is reduced when the murine cells are infected with the NS2-null of parvovirus H-1, and the experiments with reporter-gene constructs suggested that a sequence present in the 3′-untranslated region of all viral mRNAs might render them susceptible to translational modulation by NS2 (21). Similar widespread defects in viral protein accumulation were not apparent in the MVMp NS2 mutants studied previously (7, 8). Although NS2 is such a multifunctional protein, the question now arises: What is the role of NS2 exported in a CRM1-dependent manner? It was shown that several viral and cellular proteins, such as HIV-1-Rev, HTLV-1-Rex, Protein Kinase Inhibitor Protein (PKI), and mitogen-activated protein kinase kinase (MAPKK) were exported to the cytoplasm by CRM1 (22–24). Therefore, one might expect that NS2 mediates the export of unspliced viral mRNA and plays a significant role in virus production. Further research clarifying the molecular links between NS2 export and viral products should provide interesting insights into parvovirus pathogenicity.

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REFERENCES

1. Rommelaere, J., and Cornelis, J. J. (1991) *J. Virol. Methods* **33,** 233–251.
2. Cotmore, S. F., and Tattersall, P. (1995) *Semin. Virol.* **6,** 271–281.
3. Vanacker, J. M., and Rommelaere, J. (1995) *Semin. Virol.* **6,** 291–297.
4. Cater, J. E., and Pintel, D. J. (1992) *J. Gen. Virol.* **73,** 1839–1843.
5. Brownstein, D. G., Smith, A. L., Johnson, E. A., Pintel, D. J., Naeger, L. K., and Tattersall, P. (1992) *J. Virol.* **66,** 3118–3224.
6. Cotmore, S. F., D'Abramo, A. M., Jr., Carbonell, L. F., Bratton, J., and Tattersall, P. (1997) *Virology* **231,** 267–280.
7. Naeger, L. K., Cater, J., and Pintel, D. J. (1990) *J. Virol.* **64,** 6166–6175.
8. Naeger, L. K., Salome, N., and Pintel, D. J. (1993) *J. Virol.* **67,** 1034–1043.
9. Cotmore, S. F., and Tattersall, P. (1990) *Virology* **177,** 477–487.
10. Brockhaus, K., Plaza, S., Pintel, D. J., Rommelaere, J., and Salome, N. (1996) *J. Virol.* **70,** 7527–7534.
11. Fornerod, M., Ohno, M., Yoshida, M., and Mattaj, I. W. (1997) *Cell* **19,** 1051–1060.

12. Fukuda, M., Asano, S., Nakamura, T., Adachi, M., Yoshida, M., Yanagida, M., and Nishida, E. (1997) *Nature* **20,** 308–311.
13. Kudo, N., Khochbin, S., Nishi, K., Kitano, K., Yanagida, M., Yoshida, M., and Horinouchi, S. (1997) *J. Biol. Chem*. **21,** 29742–29751.
14. Neville, M., Stutz, F., Lee, L., Davis, L., and Rosbash, M. (1997) *Curr. Biol.* **7,** 767–775.
15. Ossareh-Nazari, B., Bachelerie, F., and Dargemont, C. (1997) *Science* **278,** 141–144.
16. Stade, K., Ford, C. S., Guthrie, C., and Weis, K. (1997) *Cell* **90,** 1041–1050.
17. Ullman, K. S., Powers, M. A., and Forbes, D. J. (1997) *Cell* **90,** 967–970.
18. Yang, J., Bardes, E. G., Moore, J. D., Brennan, J., Powers, M. A., and Kornbluth, S. (1998) *Genes Dev*. **12,** 2131–2143.
19. Yokoya, F., Imamoto, N., Tachibana, T., and Yoneda, Y. (1999) *Mol. Biol. Cell.* **10,** 1119–1131.
20. Otero, G. C., Harris, M. E., Donello, J. E., and Hope, T. J. (1998) *J. Virol*. **72,** 7593–7597.
21. Li, X., and Rhode, S. L., III (1993) *Virology* **194,** 10–19.
22. Fischer, U., Huber, J., Boelens, W. C., Mattaj, I. W., and Luhrmann, R. (1995) *Cell* **90,** 1051–1060.
23. Wen, W., Meinkoth, J. L., Tsien, R. Y., and Taylor, S. S. (1995) *Cell* **82,** 463–473.
24. Fukuda, M., Gotoh, I., Gotoh, Y., and Nishida, E. (1996) *J. Biol. Chem*. **271,** 20024–20028.