The NS3 Protein of Bluetongue Virus Exhibits Viroporin-like Properties*

Ziying Han and Ronald N. Harty‡

From the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6049

Bluetongue virus (BTV) is a member of the Orbivirus genus within the Reoviridae family of segmented double-stranded RNA viruses. BTV remains an agriculturally important veterinary pathogen transmitted primarily to sheep by biting midges. BTV encodes seven structural (VP1–7) and four non-structural (NS1, NS2, NS3, and NS3A) proteins. NS3 is a 229-amino acid protein encoded by the viral S10 RNA segment, and NS3A is a 216-amino acid protein in which synthesis is initiated from an in-frame downstream AUG codon within the Ser-10 RNA segment (1, 2). Both proteins have been shown to be N-linked glycosylated and associated with lipid membranes in infected cells (2–5).

Hyatt et al. (3, 6, 7) were the first to provide evidence that NS3/NS3A may be involved in the late stages of BTV assembly and that NS3/NS3A were required for efficient release of virus-like particles from BTV-infected cells. For example, electron microscopic studies suggested that NS3 was associated with areas of plasma membrane perturbation (3). Similar findings have been reported for the NS3 protein encoded by the closely related African horse sickness virus (5, 8, 9). More recently, an elegant study by Beaton et al. (10) demonstrated that NS3 of BTV facilitates virus release by interacting with specific host-trafficking proteins at the plasma membrane. The authors suggest that NS3 functions as a bridge, linking progeny virions with cellular export machinery to promote virus release (10). Thus, NS3 appears to play a key role in virus assembly and release.

We were initially intrigued by the presence of conserved PXXY and PSAP motifs within NS3 that are identical in sequence to late budding domains (L-domains) that we and others have identified in matrix proteins of RNA viruses (11). These L-domains have been shown to interact with specific host proteins, and the resultant virus-host interactions are thought to facilitate virus budding by an, as yet, undetermined mechanism. Results from our initial experiments indicated that the PXXY and PSAP motifs of NS3 did not appear to function as typical L-domains, in that these motifs did not promote the release of NS3-containing virus-like particles from mammalian cells. Interestingly, we did find that cells expressing NS3 were permeable to the translational inhibitor hygromycin B in a dose-dependent manner. This finding suggested that NS3 may be capable of permeabilizing or destabilizing lipid membranes; a property shared by a group of viral proteins termed viroporins (12). We went on to demonstrate that NS3 possesses additional characteristics commonly associated with viroporins. For example, NS3 was able to form homo-oligomers when expressed in mammalian cells. Furthermore, like other viroporins, NS3 is a transmembrane protein containing two transmembrane domains (TM1 and TM2). Mutations introduced into TM1 to disrupt the hydrophobic nature of this region abolished the ability of NS3 to permeabilize the plasma membrane. In contrast, the deletion of amino acids comprising TM2 did not result in disruption of NS3-induced membrane permeability. Interestingly, enhanced permeability to hyg-B correlated with the ability of NS3 proteins to target predominantly to the Golgi apparatus. In sum, our results are consistent with a possible role for NS3 as a viroporin that may facilitate the release of virus particles and contribute to the pathogenesis of BTV infections.

MATERIALS AND METHODS

Cells and Antisera—293T or COS-1 cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen) and 1 x penicillin-streptomycin (Invitrogen). The antisera used included anti-hemagglutinin (HA) mAb (Roche Applied Science), anti-c-Myc mAb 9E10 to detect c-Myc-tagged VP40 (Uni-
Viroporin-like Activity of NS3

RESULTS

NS3 Expression Permeabilizes Cells to Hygromycin B—We wanted to determine whether NS3-WT was capable of destabilizing and/or permeabilizing cell membranes by using a hyg-B permeabilization assay. Hyg-B is a general inhibitor of translation, and it is normally impermeable to mammalian cells when applied for a short period of time at low concentrations. However, hyg-B will enter mammalian cells in which the plasma membrane has been permeabilized. This assay has been used previously by others to identify proteins that can form pores in lipid membranes (15–24).

Virophorin-like Activity of NS3

FIG. 1. Hygromycin B permeability assay. A, COS-1 cells were transfected with NS3-WT for 24 h. The cells were then radiolabeled with [35S]Met-Cys and incubated in the presence of the indicated concentrations of hyg-B for 3 h (lanes 2–6). Cell extracts were harvested, and proteins were immunoprecipitated using anti-HA antiserum and analyzed by SDS-PAGE. Mock-transfected cells served as a negative control (m, lane 1). The percent of NS3 protein present in each sample as determined by phosphorimaging analysis is indicated. The amount of NS3 present in the untreated sample (lane 2) was set at 100%. The positions of NS3, NS3-G (N-linked glycosylated form), and NS3A (truncated form) are indicated. B, COS-1 cells were mock-transfected (m, lanes 1 and 4) or transfected with either NS3-WT (lanes 2 and 3) or Ebola virus VP40 (lanes 5 and 6). Transfected cells were either left untreated (−, lanes 2 and 5), or treated as described above with 1.0 mM hyg-B (+, lanes 3 and 6). The positions of NS3, NS3-G, and VP40 are indicated.

Vuorinen of Pennsylvania Cell Center), and horseradish peroxidase-linked anti-rat and anti-mouse secondary antibodies (Santa Cruz Biotechnology, Inc.).

Plasmids—A plasmid encoding the NS3 gene from bluetongue virus (serotype 1) was generated at the Institute for Animal Health (Surrey, UK) and was kindly provided by A. Wade-Evans (1). The NS3 open reading frame was amplified by PCR and inserted into vector pCAGGS using EcoRI and XhoI restriction endonucleases. During the PCR amplification step, a HA epitope tag (YPYDVPDYA) was joined in-frame at the C terminus of NS3 to generate NS3-WT. Mutations were introduced into the NS3 gene by PCR and site-directed mutagenesis to generate plasmids encoding NS3 mutants numbers 6 (A131E/L132E/L133E) and 7 (L124E/L125E/L126E/L128E/A131E/L132E/L133E). Deletion mutants of NS3 including NS3-ΔTM1 (amino acids 115–148) and NS3-ΔTM2 (amino acids 156–181). All mutant NS3 proteins maintain the HA epitope tag in-frame at their C termini. An expression plasmid for Ebola virus (Zaire) VP40 (pVP40-WT) has been described previously (13, 14). All mutant NS3 proteins maintain the HA epitope tag and are expressed at similar levels by Western blot analysis using anti-HA antiserum and analyzed by SDS-PAGE. Mock-transfected cells served as a negative control (m, lane 1). The percent of NS3 protein present in each sample as determined by phosphorimaging analysis is indicated. The amount of NS3 present in the untreated sample (lane 2) was set at 100%. The positions of NS3, NS3-G (N-linked glycosylated form), and NS3A (truncated form) are indicated. B, COS-1 cells were mock-transfected (m, lanes 1 and 4) or transfected with either NS3-WT (lanes 2 and 3) or Ebola virus VP40 (lanes 5 and 6). Transfected cells were either left untreated (−, lanes 2 and 5), or treated as described above with 1.0 mM hyg-B (+, lanes 3 and 6). The positions of NS3, NS3-G, and VP40 are indicated.

To demonstrate further that the observed permeability to hyg-B was due to the expression of NS3, cells expressing either NS3 or the membrane-associated VP40 protein of Ebola virus were included in this assay (Fig. 1B). As observed in Fig. 1A, a significant decrease in the level of NS3 protein was observed in cells incubated with 1.0 mM hyg-B compared with those incubated in the absence of hyg-B (Fig. 1B, compare lanes 2 and 3). However, no detectable difference in the level of Ebola virus VP40 was observed under the same experimental conditions. These results suggest that there is specificity to the observed shutoff of protein synthesis and that expression of NS3-WT affects the permeability of the cell to hyg-B.

Role of Transmembrane Regions TM1 and TM2 of NS3 in Permeabilizing Mammalian Cells—Because expression of NS3-WT rendered cells permeable to hyg-B, we sought to identify the region(s) of NS3 that was important for this function. We reasoned that the two transmembrane regions identified previously in NS3 would be important; thus, NS3-ΔTM1 and NS3-ΔTM2 deletion mutants were constructed (Fig. 2A) and tested in this hyg-B permeability assay (Fig. 2B). As described above, expression of NS3-WT allowed the entry of hyg-B into...
cells, resulting in a significant inhibition of protein synthesis, as judged by a decrease in NS3, NS3G, and NS3A protein expression (Fig. 2B, compare lanes 2 and 3). Interestingly, an identical concentration of hyg-B was unable to inhibit protein synthesis in cells expressing NS3-ΔTM1 (Fig. 2B, compare lanes 4 and 5). Unlike the results for NS3-WT, identical amounts of NS3-ΔTM1 were detected in cells incubated in the absence or presence of 1.0 mM hyg-B (Fig. 2B, lanes 6 and 7). These results were reproducible and indicated that amino acids 118–148 comprising TM1 of NS3 are critical for permeabilization of cells to hyg-B. In contrast to the results obtained with NS3-ΔTM1, hyg-B was able to enter and inhibit protein synthesis in cells expressing NS3-ΔTM2 (Fig. 2B, compare lanes 6 and 7). Thus, unlike TM1, amino acids 156–181 comprising TM2 of NS3 are not essential for permeability to hyg-B. Levels of NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 detected by immunoprecipitation in the presence or absence of hyg-B were quantitated by phosphorimaging analysis (Fig. 2C). Mock-transfected cells served as a negative control (Fig. 2B, lane 1).

Intracellular Localization of NS3-WT, ΔTM1, and ΔTM2 by Immunofluorescence—Because NS3-ΔTM1 was lacking the proximal transmembrane region, it was likely that the inability of NS3-ΔTM1 to allow entry of hyg-B into cells (Fig. 2) would correlate with a change in intracellular localization. Indirect immunofluorescence was used to determine the intracellular localization patterns for NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 in transfected cells (Fig. 3A). COS-1 cells were transfected with the appropriate plasmid, and the cells were fixed and stained at 24 h post-transfection. As expected, NS3-WT was found to be enriched in the Golgi apparatus and was present in smaller amounts on the plasma membrane (Fig. 3A). The localization pattern for NS3-ΔTM1 was altered significantly compared with that of NS3-WT (Fig. 3A). NS3-ΔTM1 exhibited a more diffuse staining pattern throughout the cytoplasm with no obvious enrichment at either the Golgi apparatus or the plasma membrane (Fig. 3A). Interestingly, the localization pattern of NS3-ΔTM2 (Fig. 3A) more closely resembled that of NS3-WT than that of NS3-ΔTM1. This result is consistent with our finding that NS3-WT and NS3-ΔTM2 function similarly in the hyg-B permeability assay (Fig. 2).

We next examined whether NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 co-localized with cellular actin and PDI (ER marker protein) (Fig. 3, B and C). NS3-WT and NS3-ΔTM2 were found to co-localize in part with membrane-bound actin filaments, whereas NS3-ΔTM1 did not exhibit co-localization with actin (Fig. 3B). The majority of NS3-ΔTM1 appeared to co-localize with PDI in the ER (Fig. 3C). Although NS3-WT and NS3-ΔTM2 also displayed some ER staining, both proteins were clearly evident in the Golgi as well (Fig. 3C). Together, these findings indicate that NS3-ΔTM1 is lacking a Golgi targeting signal and that Golgi and membrane targeting appear to correlate with membrane permeabilizing activity.

Mutations in TM1 of NS3 Affect Permeability of Hyg-B—Because the TM1 region of NS3 is important for Golgi targeting and membrane permeabilization, we sought to identify sequences within this region responsible for these phenotypes. Toward this end, mutations were introduced into the TM1 region of NS3 to generate mutant 6 (A131E/L132E/L133E) and mutant 7 (L124E/L125E/I126E/L132E/L133E) (Fig. 4A). Mutants 6 and 7, along with NS3-WT as a positive control and mock- or VP40-transfected cells as negative controls, were employed in the hyg-B permeability assay (Fig. 4B). Once again, protein expression as judged by the level of NS3-WT was reduced significantly in cells incubated in the presence of 1.0 mM hyg-B, whereas expression of Ebola virus VP40 remained unchanged in the absence or presence of 1.0 mM hyg-B (Fig. 4B). In contrast to cells expressing NS3-WT, cells expressing NS3 mutants 6 and 7 remained impermeable to identical amounts of hyg-B (Fig. 4B).

Intracellular Localization of NS3-WT and Mutants 6 and 7 by Immunofluorescence—We next sought to determine whether the inability of mutants 6 and 7 to permeabilize cells to hyg-B

---

**Fig. 2. Role of transmembrane regions of NS3 for viroporin-like activity.** A, diagram of NS3-WT, NS3-ΔTM1, and NS3-ΔTM2. B, COS-1 cells were either mock-transfected (Mock, lane 1) or transfected with NS3-WT (lanes 2 and 3), NS3-ΔTM1 (lanes 4 and 5), or NS3-ΔTM2 (lanes 6 and 7) for 24 h. The cells were pretreated with or without 1.0 mM hygromycin B and then radiolabeled with [35S]Met-Cys and incubated in the absence (−) or presence (+) of 1.0 mM hyg-B for 3 h. The cell extracts were harvested, and NS3 proteins were immunoprecipitated and analyzed by SDS-PAGE. C, NS3 proteins detected by immunoprecipitation in the absence (black bars), or presence (white bars) of 1.0 mM hyg-B (HB) were quantitated by phosphorimaging analysis.
correlated with an inability to localize to the Golgi. Cells were transfected with plasmids expressing NS3-WT, mutant 6, or mutant 7, and localization of these proteins was determined by indirect immunofluorescence (Fig. 5). NS3-WT localized to the Golgi apparatus and to the plasma membrane in transfected cells (Fig. 5). In contrast, neither mutant 6 nor mutant 7 were found to localize to the Golgi apparatus or to the plasma membrane (Fig. 5). Both mutants displayed an overall diffuse cytoplasmic staining pattern with little to no co-localization with actin (Fig. 5A), but rather a pronounced co-localization with PDI in the ER (Fig. 5B). Thus, these findings are consistent with the TM1 region being important for Golgi targeting and membrane permeabilization. Specifically, amino acids ALL at positions 131–133 appear to be important, and it is likely that the overall hydrophobic nature of TM1 is crucial for viroporin-like activity.

**Fig. 3.** Localization of NS3-WT, NS3ΔTM1, and NS3ΔTM2. COS-1 cells were transfected with NS3-WT, NS3ΔTM1, and NS3ΔTM2, fixed at 24 h after transfection in 4.0% paraformaldehyde for 10 min, and then permeabilized with 0.2% Triton X-100 for 10 min. For ER staining, the cells were incubated with anti-PDI mouse mAb and anti-HA rat mAb in 3% BSA/PBS at 37 °C for 30 min. Subsequently, the cells were incubated with secondary anti-mouse IgG-R and anti-rat IgG conjugated with chicken Alexa Fluor 488 in 3% BSA/PBS at 37 °C for 30 min. For actin staining, the cells were incubated with anti-HA mouse mAb in 3% BSA/PBS at 37 °C for 30 min and then with fluorescein isothiocyanate-labeled anti-mouse IgG and rhodamine-labeled phalloidin in 3% BSA/PBS at 37 °C for 30 min. The cells were mounted in Prolong anti-fade solution and analyzed by confocal microscopy. NS3 protein is shown in green, and PDI or actin is shown in red.

**Fig. 4.** Permeability assays for NS3 mutants in TM1 region. Diagram and hyg-B permeability assays for TM1 mutants. A, diagram and amino acid sequence of the TM1 region of NS3-WT, NS3 mutant 6 (A131E/L132E/L133E), and NS3 mutant 7 (L124E/L125E/I126E/A131E/L132E/L133E). B, hyg-B permeability assay. Cells were transfected with the indicated plasmid and incubated in the presence (+) or absence (−) of 1.0 mM hyg-B as indicated in the legend to Fig. 2. The radiolabeled NS3 proteins were immunoprecipitated and analyzed by SDS-PAGE.
Oligomerization of NS3—Virtually all of the viroporins identified to date possess the ability to oligomerize and form aggregates in mammalian cells. Oligomerization of these viral proteins is thought to be critical for the formation and expansion of the hydrophilic pore in lipid bilayers. We sought to determine whether NS3 was able to oligomerize in transfected cells. Human 293T cells were mock-transfected or transfected with NS3-WT, NS3-H9004 TM1, or NS3-H9004 TM2, and cell extracts were harvested at 24 h post-transfection. The cell extracts were either heated at 95–100 °C in Laemmli sample buffer containing β-mercaptoethanol or not incubated with β-mercaptoethanol and not heated at 95–100 °C before analysis by SDS-PAGE and Western blot (Fig. 6). A protein species migrating at ~100–110 kDa (possibly indicative of tetramers) was detected by Western blot only in cell extracts that were not boiled and received no β-mercaptoethanol (Fig. 6, lanes 3, 5, and 7). This high molecular weight form of NS3 was not present under normal reducing conditions (Fig. 6, lanes 4, 6, and 8). The monomeric species of NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 were detected under both reducing and nonreducing conditions (Fig. 6, lanes 3–8). Detection of this high molecular weight species of NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 suggests that these proteins may contain disulfide linkages or may exist in an SDS-resistant form because of their hydrophobic nature. These results also suggest that the TM1 and TM2 regions are not required for homo-oligomerization of NS3. It should be noted that gel filtration analysis was also employed to demonstrate that NS3-WT, NS3-ΔTM1, and NS3-ΔTM2 were able to homo-oligomerize (data not shown). Taken together, these data clearly indicate that NS3 can oligomerize in mammalian cells and that TM1 and TM2 regions are not essential for oligomerization of NS3.

DISCUSSION

The mechanism by which reoviruses, such as bluetongue virus, are released from infected cells is not completely understood. Accumulating evidence indicates that the NS3 protein plays an important role in this late stage of virus replication. The NS3 protein of BTV has recently been postulated to function as a bridging molecule between the VP2 outer capsid protein of BTV and the host annexin II-p11 complex to facilitate virus release (10). Thus, specific virus-host interactions are likely to be important for the efficient release of mature reovirions.

Because the NS3 protein is thought to function in virus release, the conservation of the PPXY and PSAP motifs within NS3 was intriguing. The PPXY and PSAP motifs resemble functional L-domains conserved in viral matrix proteins (Gag, VP40, and M) of enveloped RNA viruses (retro-, filo-, and rhabdoviruses) that drive the budding process. The L-domain motifs allow Gag, VP40, and M proteins to bud from cells alone in the form of virus-like particles by mediating interactions with host proteins (11). Although BTV is a nonenveloped virus, these proline-rich motifs may mediate interactions with host proteins to facilitate virus release. However, we found that the PPXY or PSAP motifs of NS3 did not appear to function as

FIG. 5. Localization of NS3-WT, mutant 6, and mutant 7 by indirect immunofluorescence. COS-1 cells were transfected with the indicated plasmids and then fixed and permeabilized at 24 h after transfection. The cells were incubated with the appropriate antisera to detect NS3 (green) and PDI (red) and also with rhodamine-labeled phalloidin for actin staining.

FIG. 6. Oligomerization of NS3-WT, NS3ΔTM1, and NS3ΔTM2. Human 293T cells were transfected with the indicated plasmids, and the cells were lysed at 24 h post-transfection in 1 × PBS by three freeze/thaw cycles. The lysates were centrifuged at 13,000 × g for 2 min, and the samples were analyzed by SDS-PAGE under reducing (+) or nonreducing (−) conditions. NS3 proteins were detected by Western blot using anti-HA mAb and enhanced chemiluminescence. Mock-transfected cells served as a negative control (Mock, lanes 1 and 2).
Viroporin-like Activity of NS3

Viroporins typically contain at least one stretch of hydrophobic amino acids capable of forming a hydrophilic pore in lipid membranes through which low molecular weight compounds (e.g. hyg-B) may pass. These pores are often formed as a result of oligomerization of the protein. Indeed, expression of NS3 directly destabilized the plasma membrane. A similar correlation has been identified for the coxsackievirus 2B viroporin protein (24). The mechanism by which these Golgi-targeted proteins effect permeabilization of the plasma membrane remains to be determined. In addition, the ability of NS3 to permeabilize the Golgi membrane also remains to be determined.

Viroporins are classified as small hydrophobic viral proteins that can permeabilize and destabilize lipid membranes (12). Viroporins typically contain at least one stretch of hydrophobic amino acids capable of forming a hydrophilic pore in lipid membranes through which low molecular weight compounds (e.g. hyg-B) and ions (e.g. calcium) may pass. These pores are often formed as a result of oligomerization of the protein. Indeed, our findings clearly indicate that NS3 can oligomerize to form homo-oligomers readily in transfected cells (Fig. 5). Furthermore, disruption of the transmembrane regions of NS3 revealed that amino acids 118–148 of TM1, but not amino acids 156–181 of TM2, were critical for viroporin-like activity and possible pore formation. Mutations that disrupted the hydrophobic nature of TM1 and specifically amino acids 151–153 (ALL) abolished both Golgi targeting and the ability of NS3 to render cells permeable to hyg-B.

NS3 of BTV joins a growing list of viral proteins identified as having viroporin-like activity. Viroporins identified thus far include: influenza A virus M2, poliovirus 2BC and 3AB, rotavirus NSP4, HIV-1 gp41, togavirus 6K, human respiratory syncytial virus SH, hepatitis A virus 3A, 2B, and 2BC, hepatitis C virus E1, coxsackievirus 2B, avian reovirus p10, and NS proteins of Japanese encephalitis virus (15–18, 20–23, 25–38). Viroporins have been implicated in playing roles in pathogenesis, cytotoxicity, and virus fusion and release. For example, the NS proteins of Japanese encephalitis virus have been postulated recently to contribute to viral pathogenesis and cytopathic effects in infected cells because of viroporin-like activity (15).

A more in-depth structural and functional analysis of BTV NS3 is needed to understand further the potential viroporin-like activity in BTV-infected cells. Results from these future studies will provide us with a better understanding of the precise role of NS3 in virus release and pathogenesis.

Acknowledgments—We thank Dr. A. Wade-Evans for generously providing reagents. We also thank S. Hanna for constructing the original NS3-WT plasmid.

REFERENCES

1. Wade-Evans, A. M. (1990) Nucleic Acids Res. 18, 4920
2. Wu, X., Chen, S. Y., Iwata, H., Companys, R. W., and Roy, P. (1992) J. Virol. 66, 7104–7112
3. Hyatt, A. D., Gauld, A. R., Coupar, B., and Eaton, B. T. (1991) J. Gen. Virol. 72, 2263–2267
4. Bansal, O. P., Stokes, A., Bansal, A., Bishop, D., and Roy, P. (1998) J. Virol. 72, 3362–3369
5. van Staden, V., Smit, C. C., Stoiltz, M. A., Maree, F. F., and Huismans, H. (1998) Arch. Virol. Suppl. 14, 251–258
6. Hyatt, A. D., Zhao, Y., and Roy, P. (1993) Virology 193, 592–603
7. Hyatt, A. D., Eaton, B. T., and Brookes, S. M. (1989) Virology 173, 21–34
8. van Staden, V., Stoiltz, M. A., and Huismans, H. (1995) Arch. Virol. 140, 289–306
9. van Nierkerk, M., Smit, C. C., Fick, W. C., van Staden, V., and Huismans, H. (2001) Virology 279, 495–508
10. Beaton, A. R., Rodriguez, J., Reddy, Y. K., and Roy, P. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 13154–13159
11. Freed, E. O. (2002) J. Virol. 76, 4679–4687
12. Gonzalez, M. E., and Carrasco, L. (2003) FEBS Lett. 552, 28–34
13. Licitra, J. M., Simpson-Holley, M., Wright, N. T., Han, Z., Paragas, J., and Harty, R. N. (2003) J. Virol. 77, 1812–1819
14. Han, Z., Boshra, H., Sanzey, J. O., Zwers, S. H., Paragas, J., and Harty, R. N. (2003) J. Virol. 77, 1793–1800
15. Chang, Y. S., Liao, C. L., Tsao, C. H., Chen, M. C., Liu, C. I., Chen, L. K., and Lin, Y. L. (1999) J. Virol. 73, 6257–6264
16. Aldabe, R., Baro, A., and Carrasco, L. (1996) J. Biol. Chem. 271, 23134–23137
17. Arroyo, J., Boceta, M., Gonzalez, M. E., Michel, M., and Carrasco, L. (1995) J. Virol. 69, 4095–4102
18. Alonso, M. A., and Carrasco, L. (1982) Eur. J. Biochem. 127, 567–569
19. Doedens, J. R., and Kirkegaard, K. (1995) EMBO J. 14, 894–907
20. Lama, J., and Carrasco, L. (1995) FEBS Lett. 367, 5–11
21. Lama, J., and Carrasco, L. (1996) J. Gen. Virol. 77, 2109–2119
22. Perez, M., Garcia-Barreno, B., Meler, J. A., Carrasco, L., and Guinea, R. (1997) Virology 235, 342–351
23. van Kuppevelt, F. J., Melchers, W. J., Kirkegaard, K., and Doedens, J. R. (1997) Virology 227, 111–118
24. de Jong, A. S., Wessels, E., Dijkman, H. B., Galama, J. M., Melchers, W. J., Wijzens, P. H., and van Kuppevelt, F. J. (2003) J. Biol. Chem. 278, 1012–1021
25. Lama, J., and Carrasco, L. (1992) Biochem. Biophys. Res. Commun. 188, 972–981
26. Telfer, A. E., Scaria, A., Hermintosh, T. W., Ryeer, J. S., Wold, L. J., and Wold, W. S. (1996) J. Virol. 70, 2296–2306
27. Tian, P., Ball, J. M., Zeng, C. Q., and Estes, M. K. (1996) J. Virol. 70, 6973–6981
28. Ruiz, M. C., Abad, M. J., Charlpillanne, A., Cohen, J., and Michelangeli, F. (1997) J. Gen. Virol. 78, 2883–2893
29. Cocchione, A. R., Dettori, S., Chionne, P., Kundoli, I. A., Amoroso, P., Guadagnino, V., Greco, M., and Rapicetta, M. (1998) Res. Virol. 99, 209–218
30. Chavez, A., Busquets, M. A., Pujol, M., Alinsa, M. A., and Caijal, Y. (1998) Analyst 123, 2251–2256
31. Jecht, M., Gauss-Muller, V., and Kusov, Y. V. (1998) J. Virol. 72, 8013–8020
32. Pisani, G., Divizia, M., Pana, A., and Morace, G. (1995) Virology Res. 36, 299–309
33. Bonadini, S., Sanz, G. F., Martin, G., and Sanz, M. A. (1994) Leukemia 8, 1599–1600
34. Grdina, R., Trkola, A., Purtmacher, M., Klima, A., Steinid, F., Palese, P., and Katinger, H. (1994) J. Virol. 68, 4031–4034
35. Brewen, E. P., Bellamy, A. B., and Taylor, J. A. (2000) J. Gen. Virol. 81, 1955–1959
36. Bodelon, G., Labrada, L., Martinez-Costas, J., and Benavente, J. (2002) J. Biol. Chem. 277, 17769–17796
37. Aguirre, A., Baro, A., Carrasco, L., and Nieva, J. L. (2002) J. Biol. Chem. 277, 40434–40441
38. Tosteson, M. T., Hibert, M. L., and Fielde, B. N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10549–10552