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Virus Research 95 (2003) 3–12
Virus Research
www.elsevier.com/locate/virusres

Trafficking of viral genomic RNA into and out of the nucleus: influenza, Thogoto and Borna disease viruses

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Abstract

Most RNA viruses that lack a DNA phase replicate in the cytoplasm. However, several negative-stranded RNA viruses such as influenza, Thogoto, and Borna disease viruses replicate their RNAs in the nucleus, taking advantage of the host cell's nuclear machinery. A challenge faced by these viruses is the trafficking of viral components into and out of the nucleus through the nuclear membrane. The genomic RNAs of these viruses associate with proteins to form large complexes called viral ribonucleoproteins (vRNPs), which exceed the size limit for passive diffusion through the nuclear pore complex (NPC). To insure efficient transport across the nuclear membrane, these viruses use nuclear import and export signals exposed on the vRNPs. These signals recruit the cellular import and export complexes, which are responsible for the translocation of the vRNPs through the NPC. The ability to control the direction of vRNP trafficking throughout the viral life cycle is critical. Various mechanisms, ranging from simple post-translational modification to complex, sequential masking-and-exposure of localization signals, are used to insure the proper movement of the vRNPs.

Keywords: Viral ribonucleoproteins; Nucleocytoplasmic transport; Influenza disease virus; Thogoto disease virus; Borna disease virus

1. Introduction

RNA viruses can be separated into three major classes based on the organization of their genomes: (i) single-stranded RNA of negative polarity, (ii) single-stranded RNA of positive polarity, and (iii) double-stranded RNA. Most negative-stranded RNA viruses replicate their genome in the cytoplasm. However, several of them have a nuclear phase, relying on some of the unique features of this compartment to complete their replication cycle. For example, influenza, Thogoto and Borna disease viruses (BDVs), which are the primary focus of this article, depend on the nuclear RNA splicing machinery to produce several of their transcripts. In the positive-stranded RNA virus class, only the retroviruses replicate in the nucleus. Among them, only the lentiviruses such as human immunodeficiency virus (HIV) rely on the active import of their genomes to reach the nucleus. However, the genome of lentiviruses enters the nucleus as a double-stranded DNA molecule after the genomic RNA has been reverse-transcribed. Thus, the genome trafficking of these viruses will not be described here in detail. Finally, no double-stranded RNA virus has been found to replicate its RNAs within the nucleus.

1.1. The nuclear pore complex

The cell nucleus is separated from the cytoplasm by a double membrane. Viruses that replicate their genome in the host cell nucleus have evolved strategies for moving viral components across this membrane barrier twice: first to reach the nucleus after uncoating, and then back to the cytoplasm after RNA replication has occurred. Trafficking through this membrane occurs only at specific locations, the nuclear pore complexes (NPCs) and it is tightly controlled. The NPC is a large complex of 125 MDa spanning both membranes with extensions into the cytoplasm and into the nucleus. Over 100 different polypeptides (nucleoporins) comprise this aqueous channel (for review, see Allen et al., 2000; Bodoor et al., 1999; Stoffler et al. 1999) and the size limit for passive diffusion through the NPC is believed to be 9 nm (Bonner et al., 1975; Paine et al., 1975).

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doi:10.1016/S0168-1702(03)00159-X

1.2. Viral ribonucleoproteins (vRNPs)

Viral RNAs are always tightly associated with viral proteins, forming ribonucleoprotein (RNP) complexes. In the case of influenza virus, the vRNP is believed to be 10–20 nm wide (Compans et al., 1972). Recently, the structure of an influenza virus mini-RNP was partially revealed by electron microscopy (Martin-Benito et al., 2001). The vRNA, coated by nucleoproteins (NPs) (1 NP for each 24 nucleotides (Compans et al., 1972; Ortega et al., 2000)), forms a loop. The trimeric polymerase complex (PB1–PB2–PA) binds to the partially complementary ends of the vRNA, giving rise to a complex panhandle structure (Martin-Benito et al., 2001).

1.3. Nucleocytoplasmic trafficking

Since vRNPs appear to exceed the size limit for passive import into the nucleus, they must be actively translocated. In general, intercompartmental transport of macromolecules within cells requires that such cargo molecules contain transport signals. Cargo molecules possessing import or export signals are recognized by the members of a large and diverse family of proteins called karyopherins. Importins such as karyopherin alpha and beta recruit cargoes for transport into the nucleus, and exportins such as Crm1 (chromosome maintenance region 1) bind cargoes directed to the cytoplasm. A small GTP-binding protein with GTPase activity, Ran, has been proposed to control the directionality of the transport (Görlich et al., 1996; Izaurralde et al., 1997; Nachury and Weis, 1999). The proposed mechanism involves a nucleocytoplasmic gradient of the GTP-bound form of Ran (high RanGTP/RanGDP ratio in the nucleus, low ratio in the cytoplasm). This gradient is maintained by the presence of the Ran nucleotide exchange factor RCC1 in the nucleus (triggering the exchange of GDP for GTP) and the RanGAP (Ran GTPase activating protein), which is preferentially located in the cytoplasm. Ran, in its GTP-associated form, binds to the exportin–cargo complex and mediates its translocation to the cytoplasm. The GTP hydrolysis in the cytoplasm results in the release of the cargo (for review, see Görlich and Kutay, 1999; see also Fig. 1).

1.4. Transport signals

Active translocation through the nuclear membrane is a process specific to cargoes containing transport signals: nuclear import signal (NLS) and/or nuclear export signal (NES). Classical NLSs contain either a short stretch of basic lysine residues, like that of the SV40 large T-antigen NLS (PKKKRKV), or two clusters of basic residues separated by a spacer of variable length, as found in nucleoplasmin (KRxxxxxxxxxxKKKK). Proteins carrying these signals recruit molecules of the karyopherin alpha family, which, after binding to karyopherin beta, form an import-competent trimeric complex that moves into the nucleus in a RanGDP-dependent manner. Other types of NLSs, possibly paired with different cellular partners, have been described for several cellular and viral proteins (for review, see Chook and Blobel, 2001). NES-containing cargoes that use the Crm1 export pathway, such as HIV Rev, bear leucine-rich export signals (LxxLxxLxL). However, the Crm1-dependent cytoplasmic translocation is only one of the export pathways, and different types of NESs have been described (see Görlich and Kutay, 1999; Weis, 2002).

This review will focus on the transport of the vRNPs of influenza, Thogoto and BDVs into and out of the nucleus, on the proteins and signals involved in this movement, and the transport process, including its regulation.

2. Influenza virus

2.1. Import

Influenza virus, the prototype of the Orthomyxoviruses family, is an enveloped virus whose genome is composed of eight single-stranded RNA segments of negative polarity, encoding 11 proteins. During entry into cells, the virus first binds to sialic acid residues on cellular membrane glycoproteins through its receptor, hemagglutinin (HA), and then it is endocytosed. Acidification of the endocytic vesicle results in a conformational change of HA, exposing a short peptide responsible for the fusion between the viral and the endosomal membranes (for review, see Skehel and Wiley, 2000). Another acidic pH induced change is required before the vRNPs are released into the cytoplasm and targeted to the nucleus. A small proton channel in the viral membrane, M2, facilitates the acidification of the viral core (Pinto et al., 1992; Sugrue and Hay, 1991), resulting in an irreversible change in M1 and its subsequent dissociation from the vRNPs (Bui et al., 1996). This acidic pH mediated release of M1 is necessary for vRNP nuclear import (Bui et al., 1996; Whittaker et al., 1996).

2.2. NLSs on influenza virus NP

Although all the proteins of the vRNP complex (NP and the polymerases PB1, PB2 and PA) carry NLSs (Akkina et al., 1987; Jones et al., 1986; Nieto et al., 1994), the NP was shown to be sufficient to mediate the nuclear import of viral RNAs (O'Neill et al., 1995). A more detailed analysis revealed the presence of three

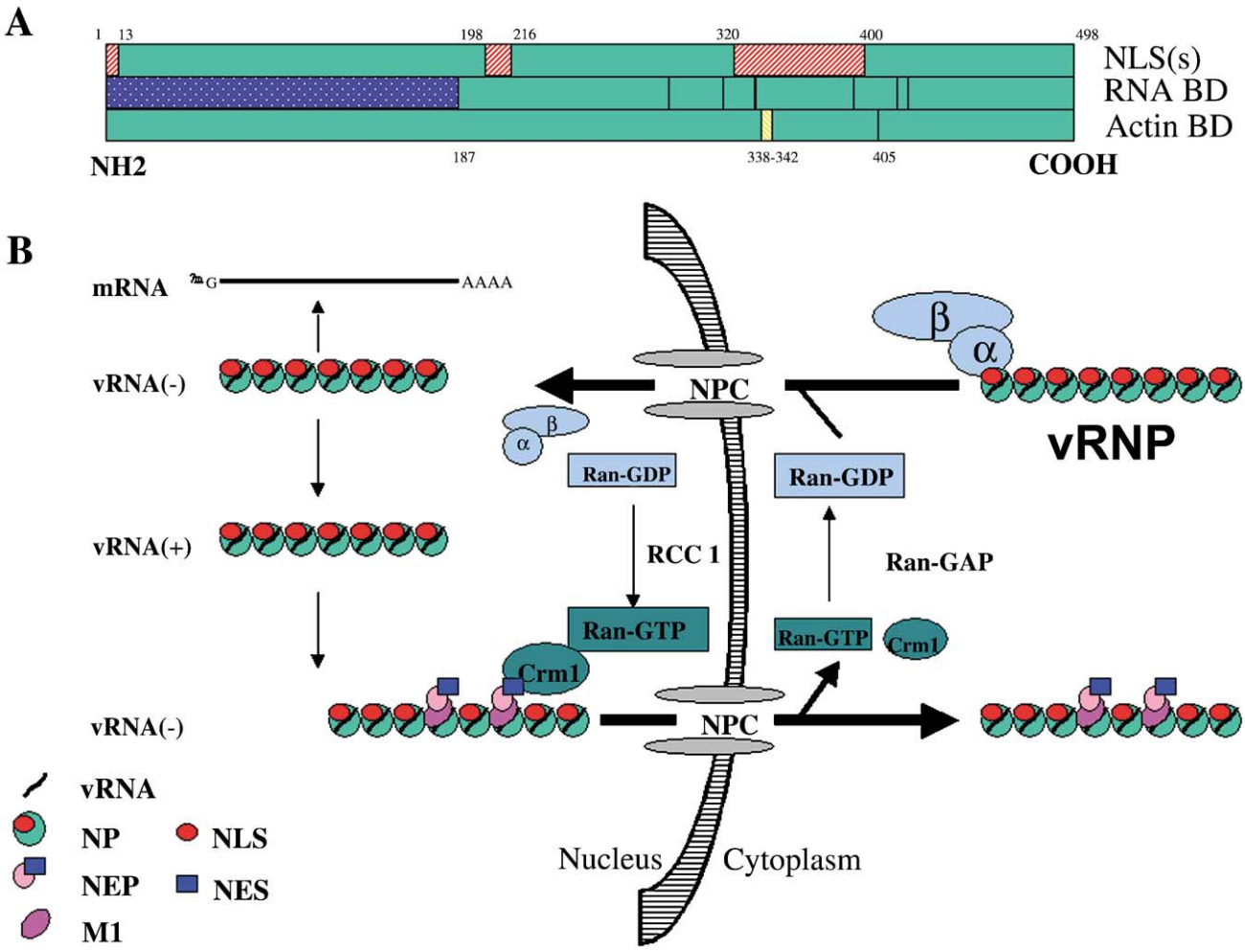

Fig. 1. Nucleocytoplasmic trafficking of influenza A virus vRNPs. (A) Schematic representation of the influenza A virus NP. The upper part represents the three nuclear localization signals (NLSs) in hatched red: the unconventional NLS (amino acid 1–13); the bipartite NLS (198–216); and the putative NLS whose location has not been precisely determined yet (320–400). The middle section shows the main RNA-BD and other discrete amino acids are also involved in RNA-binding (R267, R314, W330, A332, W386, F412, R416) (Elton et al., 1999). The bottom part shows the actin-binding site in yellow (338–342 and 405). (B) The incoming vRNP interacts with karyopherin alpha through the unconventional NLS on NP (represented by the red circle on NP). Recruitment of karyopherin beta allows the RanGDP-mediated nuclear translocation of the vRNP into the nucleus. vRNA is transcribed into mRNA and replicated into vRNA. Synthesis of the late viral genes M1 and NEP terminates RNA replication. NEP interacts with the vRNP most likely through M1 and recruits Crm1. NEP NES (blue square) triggers vRNP export in a Crm1/RanGTP-dependent manner. The viral polymerases associated with the vRNPs are not included here.

NLSs on the NP of influenza A viruses (see Fig. 1A; Bullido et al., 2000; Neumann et al., 1997; Wang et al., 1997): an unconventional NLS is at the very N-terminus. This sequence (M_1ASQGTKRSYEQM_{13}) alone is sufficient to trigger the translocation to the nucleus of the cytoplasmic measles P protein (Wang et al., 1997). A second NLS resides in the central part of NP (K_{198}Rxxxxxxxxxxxxx$RKxR_{216}$). This bipartite signal appears to be weaker than the unconventional N-terminal NLS (Weber et al., 1998). Finally, deletion of these two signals still results in partial nuclear localization of the NP, suggesting the presence of a third NLS proposed to be located between amino acids 320 and 400 (Bullido et al., 2000; Wang et al., 1997; Fig. 1A).

The first localization sequence described in NP was found to promote nuclear accumulation in *Xenopus* oocytes (Davey et al., 1985). It is noteworthy that this same sequence, when tested in mammalian cells, triggered the opposite effect: cytoplasmic accumulation (Weber et al., 1998). Which of the three NLSs described above actually is/are used by the vRNP to target itself to the nucleus remains to be determined. The NP of influenza B and C viruses has no homology to the influenza A virus NP in the region corresponding to the first two NLSs. The 60 N-terminal amino acids of influenza B virus NP have been deleted without any consequences for NP localization (Stevens and Barclay, 1998). This suggests that the sequences conferring

nuclear import activity to the NP of influenza B virus may lie elsewhere in the protein.

2.3. Nuclear import of vRNP

Interaction of the vRNP with the import machinery occurs via the N-terminus of NP. In a yeast-two-hybrid assay, using NP as bait, O'Neill et al. (1995) identified a factor homologous to ySRP-1, termed NPI-1 for NP interactor 1, which was later called karyopherin alpha (Radu et al., 1995). The binding sites to karyopherin alpha 1 (S_3xGTKRSYxxM$_{13}$) and alpha 3 (T_6KRSxxxM$_{13}$) were further defined and carefully mapped by alanine scanning to the N-terminal part of NP, which contains the unconventional NLS (Wang et al., 1997). Taking advantage of an import assay in digitonin permeabilized cells, O'Neill et al. (1995) defined the minimal components, cellular and viral, for nuclear import of vRNP. Four cellular proteins were required: karyopherin alpha 1 and beta, Ran and P_{10}. Karyopherin alpha 1 is thought to interact with the vRNP, recruiting karyopherin beta into a trimeric complex that docks at the NPC. Nuclear translocation requires Ran and P_{10} in the presence of energy provided by an ATP-generating system (Fig. 1B). The work described above clearly establishes NP as the main factor in vRNP import. Still, the vRNA nuclear translocation remains poorly understood: which NLS(s) is (are) actually used by the vRNPs? What are the amino acids on karyopherin alpha that bind to the vRNP? These are only a few of the questions to be answered in the future.

2.4. Export

2.4.1. Nuclear export of vRNP

2.4.1.1. RNP formation.

Incoming and newly synthesized vRNAs serve as templates for mRNA and complementary (+)cRNA synthesis, the latter being amplified into vRNAs, which associate with several viral proteins. Export of the newly synthesized vRNPs through the nuclear membrane before reaching the cytoplasm is a key step during the viral life cycle. During vRNA replication, vRNPs are tightly associated with the chromatin and the nuclear matrix (Bui et al., 2000; Bukrinskaya et al., 1979). vRNP export from the nucleus was found to start with the expression of a late viral gene (see below) encoding the matrix protein. M1 (Martin and Helenius, 1991; Whittaker et al., 1996). It was, therefore, proposed that M1 could displace the vRNP from the nuclear matrix (Zhirnov and Klenk, 1997). Indeed, association with M1 leads to termination of viral RNA replication (Baudin et al., 2001; Nasser et al., 1996) and promotes NP-RNA core formation (Huang et al., 2001). Electron microscopy and glycerol gradient centrifugation experiments suggested that NP monomers organize into a helical structure similar to vRNP purified from virus, only in the presence of both vRNA and M1 (Huang et al., 2001).

M1 binding to the vRNPs appears to rely on RNA–protein and protein–protein interactions. M1 interaction with naked RNA was mapped to two distinct regions within the protein: one containing a short basic palindrome that serves as an NLS for the M1 (R_{101}KLKR_{105}), and the second containing a zinc finger domain (C_{148}xCxxx/xHxH$_{162}$) (Eister et al., 1997; Wakefield and Brownlee, 1989; Ye et al., 1987, 1999). The basic RNA-binding domain (BD) was found to be most important for vRNP binding (Eister et al., 1997; Ye et al., 1999) and export (Liu and Ye, 2002). However, deletion of these two RNA-BDs did not completely abolish the M1–RNP interaction, suggesting the presence of a protein–protein interaction as well. It is not yet clear which domain of M1 binds to the protein component of the vRNP; Ye et al. (1999) suggested that the 76 N-terminal residues of M1 were important but a recent report implicated only the C-terminal part of M1 (164–252) (Baudin et al., 2001).

2.4.1.2. vRNP targeting to the NPC via the NEP (nuclear export protein).

The next step in export is the targeting of these newly formed vRNPs to the NPC and translocation into the cytoplasm. Early experiments using heterokaryons (Whittaker et al., 1996) suggested that M1 is responsible for exporting vRNPs and for preventing their re-entry into the nucleus. Although these results were confirmed by others (Huang et al., 2001), they did not explain how the vRNPs recruit and interact with the cellular export machinery. It was proposed that another viral protein plays a role in this step (Elton et al., 2001; Neumann et al., 2000; O'Neill et al., 1998).

The first suggested candidate was NS2 (non-structural protein 2)/NEP. NS2 binds to the C-terminal portion of M1 (Ward et al., 1995; Yasuda et al., 1993) and interacts with Crm1 and several nucleoporins in a yeast-two-hybrid assay (Neumann et al., 2000; O'Neill et al., 1998; Yasuda et al., 1993). This protein bears leucine-rich NES, which can functionally replace the HIV Rev NES (O'Neill et al., 1998; Paragas et al., 2001). Use of virus-like particles demonstrated that the deletion of the NS2 or alteration of its NES could inhibit vRNP export (Neumann et al., 2000; Paragas et al., 2001). Since the NS2 is a structural protein (Lamb et al., 1978; Richardson and Akkina, 1991), it possesses a functional NES (O'Neill et al., 1998; Paragas et al., 2001), and is required for vRNP export (Neumann et al., 2000; O'Neill et al., 1998), it was suggested that it should be renamed NEP for nuclear export protein instead of non-structural protein 2. Interestingly, the NES of influenza

C virus NEP differs slightly from the influenza A and B virus NEP-NES (I_{12}LLRMSKMQL$_{21}$ and I_{10}EWRMKKMAI$_{19}$, respectively). It is composed of two leucine-rich segments (L_{95}WLPMKSLSL$_{105}$ and M_{122}KHQILTRLKL$_{132}$), both required for the functionality of the NES (Paragas et al., 2001).

Another viral protein, NP, which is well recognized for its import functions, was recently proposed to recruit the cellular export machinery as well (Elton et al., 2001). In this report, the authors suggest that NP of influenza A virus can bind to Crm1 and is exported to the cytoplasm in a Crm1-dependent manner. However, an NP NES and a site that bind Crm1 have not been identified. Still, it is possible that influenza virus RNPs use redundant export pathways or that several vRNP components cooperate to enhance the efficiency of recruiting the export machinery.

2.4.1.3. vRNP translocation to the cytoplasm.

Crm1 involvement in influenza virus vRNP export was further confirmed by the use of leptomycin B (LMB), a drug that specifically targets the Crm1 pathway (Elton et al., 2001; Ma et al., 2001; Watanabe et al., 2001). Using LMB-treated cells, Ma et al. (2001) defined a possible new step in the export pathway, in which the vRNPs interact with nuclear lamins before being transferred to the NPC. A discrepancy between these studies and the previous findings was that, while NP and vRNPs were retained in the nucleus following LMB treatment, the drug did not inhibit translocation of M1 or NEP to the cytoplasm. These results question the association of M1 and NEP with the vRNPs and the involvement of these proteins in the export process. One possible explanation is that export requires only small amounts of M1 and NEP. Another possibility is that these two proteins associate only briefly with the vRNPs. The final component required for vRNP translocation is the small GTPase protein Ran (reviewed in Görlich and Kutay, 1999). The vRNP must form a tripartite complex with Crm1 and RanGTP in order to dock to the NPC (Fig. 1B). Once in the cytoplasm, vRNPs are prevented from re-entry by several mechanisms discussed below, involving NP and M1.

2.4.2. Control of vRNP export

vRNP trafficking must be tightly controlled in order to prevent premature export, which would curtail replication of viral RNA. Also, nuclear re-entry following export must not occur. At least three control mechanisms appear to be operating.

2.4.2.1. Late expression of the viral proteins involved in export.

Three temporal phases of viral transcription have been described (Inglis and Mahy, 1979). Transcription of the M segment, which encodes the M1 protein needed for vRNP export, takes place during the third (late) phase, which starts 3–3.5 h post-infection (Hatada et al., 1990; Inglis and Mahy, 1979). This temporal pattern insures that a full round of vRNA amplification occurs before M1 and NEP promotes the export of the vRNPs.

2.4.2.2. Phosphorylation of M1, NEP and NP.

Phosphorylation appears to control the export activity of the three influenza virus proteins M1, NEP and NP. H7, a broad-spectrum kinase inhibitor (Hidaka et al., 1984), was shown to block the export of vRNPs by preventing M1 expression (Bui et al., 2000). This inhibition could not be rescued by the addition of unphosphorylated M1 in trans, suggesting a role of M1 phosphorylation in vRNP nuclear export (Bui et al., 2000). Reinhardt and Wolff (2000) proposed that M1, through its interaction with the cellular receptor of activated C kinase (RACK1), could be phosphorylated by protein kinase C. Similarly, Pleschka et al. (2001) reported that efficient vRNP export requires a functional MAPKinase pathway. They observed that the inhibition of MAPK pathway decreased the ability of an NEP–Rev fusion protein to export a viral-like RNA, containing a reporter gene and a Rev response element. Although this study was not able to show a direct phosphorylation of NEP by a kinase of the MAPK pathway, other investigators reported that NEP could be phosphorylated (Richardson and Akkina, 1991). Finally, it was reported that broad-spectrum kinase modulators could affect the localization of NP (Bui et al., 1996). The latter study has focused on NP and not on vRNP trafficking. Therefore, the role of NP phosphorylation in vRNP export remains to be determined.

2.4.2.3. Cytoplasmic retention.

M1 and NP are able to control the cytoplasmic retention of recently exported vRNP (Digard et al., 1999; Whittaker et al., 1996). In heterokaryon experiments, M1 was shown to prevent the nuclear re-entry of the exported vRNPs (Whittaker et al., 1996). A temperature-sensitive (ts) mutant ts51 (Ritchey and Palese, 1977), whose M1 is hyperphosphorylated (Whittaker et al., 1995) and retained in the nucleus under non-permissive conditions (Rey and Nayak, 1992), was used in heterokaryon experiments and its vRNPs were able to enter the nucleus under non-permissive conditions confirming the role of M1 in retaining the vRNPs in the cytoplasm (Bui et al., 1996; Whittaker et al., 1996). A possible role for NP in cytoplasmic retention of vRNP comes from the finding that the C-terminal part of NP bears an actin-binding site (Fig. 1A; Digard et al., 1999). Moreover, actin–NP-RNA was shown to form a stable complex, suggesting a new mechanism for sequestering vRNP in the cytoplasm (Digard et al., 1999).

3. Thogoto virus

3.1. Import

Another member of the Orthomyxoviridae family is Thogoto virus (TV), the prototype of the *Thogotovirus* genus. Like influenza virus, its genome is composed of several single-stranded RNA molecules (six) of negative sense. Viral NP, when expressed alone, can translocate to, and accumulate in, the nucleus (Weber et al., 1998). TV-NP seems to lack an influenza A virus NP-like unconventional NLS (Weber et al., 1998). Furthermore, TV-NP does not appear to bind karyopherin alpha (Weber et al., 1998). TV-NP does bear a bipartite NLS similar to nucleoplasmin and influenza A virus NP (K_{179}RxxxxxxxxxKxKK$_{193}$). Although this signal was sufficient to drive the import of the cytoplasmic protein MxA, its deletion from TV-NP did not render it incapable of translocating to the nucleus, suggesting that TV-NP carries one or more additional NLS(s). Together these data suggest that the TV-NP NLSs and the cellular import machinery used for NP transport might be atypical.Several studies implicate the TV-NP protein in the nuclear import of vRNA; vRNPs, purified from Thogoto virions, were shown to accumulate in the nucleus following microinjection into the cytoplasm (Kochs and Haller, 1999b). Subsequently, Gomez-Puertas et al. (2000) found a similar result when they transfected in vitro-reconstituted vRNP, made mostly of TV-NP and synthetic vRNA. Supplementary evidence came from a study of MxA, a human interferon-inducible protein, which is a member of the dynamin superfamily of high-molecular weight GTPases. MxA was found to inhibit the replication of several viruses including TV (for review, see Haller and Kochs, 2002). In fact, the C-terminal part of MxA, in its GTP form, binds to vRNPs through an interaction with TV-NP (Kochs and Haller, 1999a), preventing the nuclear import of the vRNPs (Kochs and Haller, 1999b). These data suggest that TV-NP is a major player in vRNP import.

3.2. Export

Like influenza viral RNPs, TV RNPs must also reach the cytoplasm after vRNA amplification had occurred in the nucleus. Virtually nothing is known about the export pathway(s) used by the vRNPs, including the identity of the viral protein(s) involved. TV does not encode a protein similar to the influenza virus NEP. Therefore, it is possible that domains within the M protein (Kochs et al., 2000) and/or the NP protein control this export activity.

4. Borna disease virus

4.1. Import

BDV is the prototype of the Bornaviridae family of the order *Mononegavirales*. This enveloped virus contains one genomic single-stranded RNA molecule of negative polarity. An interesting feature of BDV is that, although its genomic organization is very similar to that of other *Mononegavirales*, it differs from other members of the family in being replicated in the nucleus rather than the cytoplasm of the cells it infects (for review, see Tomonaga et al., 2002). After entry into the cell, the vRNP reaches the nucleus, where replication and transcription take place.

One major component of the vRNP, the N protein, has been shown to possess nuclear localization properties (Kobayashi et al., 1998). Of the three stretches of basic amino acids that could serve as NLS (Boulikas, 1993), only the most N-terminal (P_3KRRLVDDA$_{11}$) is necessary and sufficient to promote the nuclear translocation of N.

More recently, NLSs were found in two other viral components: the P protein and P_{10}. The P protein contains two unusual, proline-rich NLSs; one at its N-terminus (P_{29}RPRKIPR$_{36}$) and the other at the C-terminus (P_{181}PRIYPQLPSAPT$_{193}$) (Shoya et al., 1998). Both NLSs appear to work independently. In P_{10}, the nuclear import activity lies in an unusual NLS at its N-terminus (R_6LTLLELVRRLNGN$_{19}$), which is also the binding site for karyopherin alpha (Wolff et al., 2002).

All these studies have focused on the transport signals required for the individual proteins. However, the minimal set of components (viral and cellular) for vRNP import remains to be determined. It is believed that N, the main component of the vRNP, is responsible for the vRNA import. However, since the P protein can interact with itself, N and P_{10} (Malik et al., 2000, 1999; Schwemmle et al., 1999), P and P_{10} may also play necessary or supportive roles in the nuclear import of vRNP (Fig. 2).

4.2. Export

Several features of the N protein point to a role for it in vRNP export from the nucleus. First, N bears a canonical leucine-rich NES (L_{128}TE*L*E*I*SS*I*FSHCC$_{141}$) with export activity in a Crm1-dependent manner (Kobayashi et al., 2001). Second, the mRNA encoding the N protein is translated at two different start sites, leading to a full-length N protein (p_{40}N) and a shorter form (p_{38}N), which lacks the N-terminal NLS but can be imported into the nucleus as part of a complex with P or p_{40}N (Kobayashi et al., 1998).

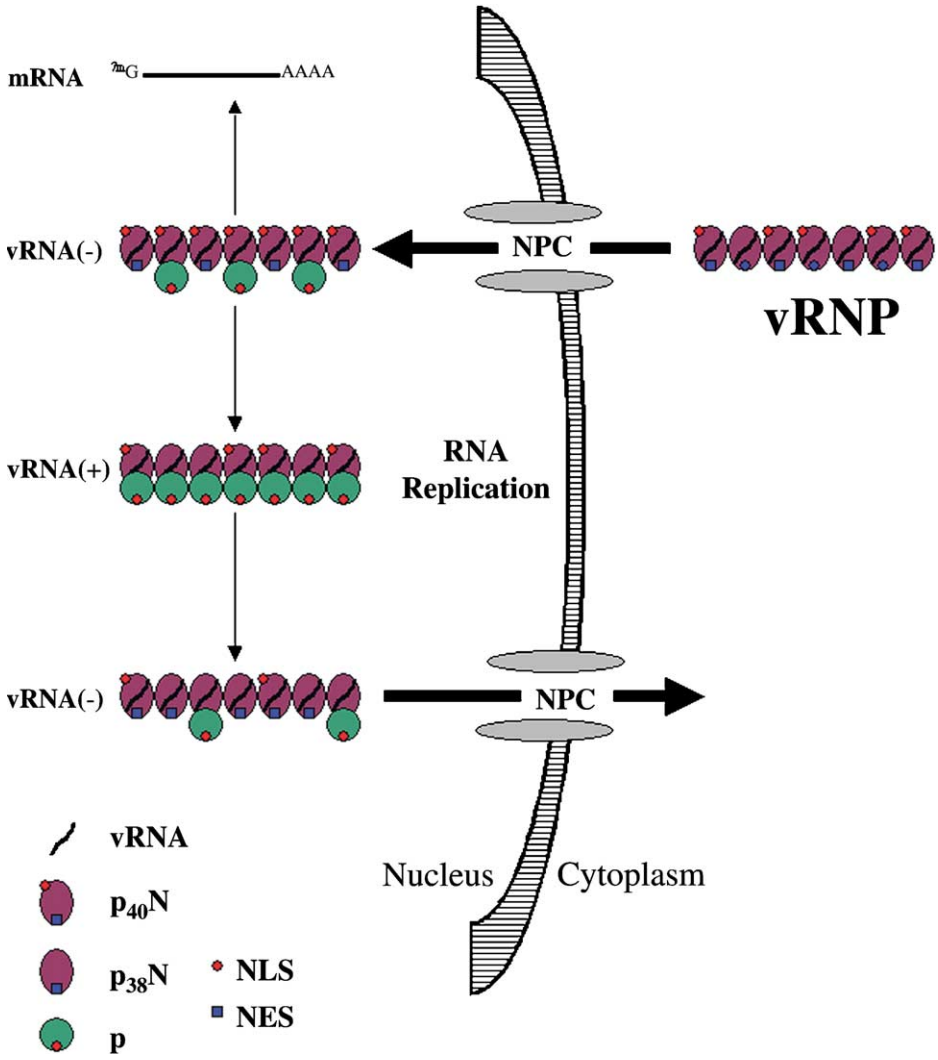

Fig. 2. Nucleocytoplasmic trafficking of BDV vRNPs. $p_{40}N$ and $p_{38}N$ bind to vRNA and are the main protein components of the vRNP. An NLS (red circle) in $p_{40}N$ is thought to promote the nuclear translocation of the vRNP. It is not yet clear if the P protein, via its NLSs, plays a role in vRNP import. Within the nucleus, the vRNA is amplified and newly synthesized vRNPs containing $p_{40}N$, $p_{38}N$ and P are assembled. The P protein, interacting with the $p_{40}N$ and the $p_{38}N$ most likely masks their NES during RNA replication (blue square). This complex gains the ability to be exported by the virtue of the NES present both in $p_{40}N$ and $p_{38}N$. How the import-competent vRNP differs from these freshly made vRNPs, which are exported through the NPC, remains to be determined. The P and P_{10} proteins have not been clearly shown to be part of the incoming vRNP and have therefore been omitted from this diagram (adapted from Kobayashi et al. (2001)).

The P protein appears to act as an export inhibitor during vRNA amplification. Support for this notion comes from the observation that P can inhibit the export activity of $p_{38}N$ by binding to a site that overlaps with the NES of N (Kobayashi et al., 2001). Upon completion of RNA replication, vRNPs leave the nucleus. Kobayashi et al. (2001) proposed a model to explain the switch triggering the export of vRNPs. The balance between the level of P, $p_{40}N$ and $p_{38}N$ would dictate the direction of the vRNP movement. As replication progresses, the accumulation of N would overcome the P block of vRNP export. Also $p_{38}N$, lacking the NLS, when incorporated into newly synthesized vRNPs, would increase the relative amount of NESs compared to NLSs, triggering the export of vRNPs (Fig. 2).

However, this model fails to account for differences between the exported and the imported vRNPs. Therefore, it is difficult to explain how the exported vRNP becomes import-competent during the next replication cycle. Finally, a possible role of M, by analogy with influenza virus, has not been explored yet.

5. Human immunodeficiency virus

The HIV genome is imported into the nucleus, not as a vRNP but as a cDNA molecule, after reverse transcription of the viral RNA in the cell cytoplasm. Since this review focuses on viruses whose genomes enter and leave the nucleus as RNP, the trafficking of

the HIV genome falls beyond the scope of this review. Nonetheless, a brief discussion about intracellular trafficking of this virus is warranted.

The imported HIV cDNA molecule is associated with several viral and cellular proteins to form the pre-integration complex (PIC). This complex includes the viral double-stranded DNA genome, the reverse transcriptase (RT), the integrase, the MA, the Vpr protein and several cellular proteins. All the viral components except the RT have been shown to play a role in the PIC import. The contribution of each such protein seems to vary, depending on the type of the infected cell and its state of differentiation (for review, see Cullen, 2001; Sherman and Greene, 2002).

The export of the HIV genome relies on a small 13 kDa protein, Rev. The genome is an intron-containing mRNA and must, therefore, avoid the cellular checkpoints that insure the nuclear retention of incompletely spliced mRNA. Rev, through its arginine-rich domain (amino acids 38–49), binds specifically to a 240 bp portion of the unspliced vRNA that has extensive secondary structure, called RRE (for Rev responsive element). Rev also contains a leucine-rich NES (amino acids 71–82) and mediates the vRNA export through the Crm1 pathway (for review, see Hope, 1999; Yi et al., 2002). Clearly, important lessons have been learnt from studying the intra-cellular trafficking of the components of the HIV.

6. Conclusion

The crossing of nuclear membranes by RNA genomes is a key event for RNA viruses with a life cycle that includes a nuclear phase. As we come to understand the nucleocytoplasmic trafficking mechanisms of cellular proteins and the structure of the NPC, we see that the translocation of the vRNP cannot happen by simple diffusion. These active transport processes harness signals on viral proteins as well as cellular adapter molecules. A major effort has been undertaken to identify the viral proteins involved in the movement of vRNPs, and to characterize the key functional domains within these trafficking molecules. Yet very few studies have focused on the vRNP as a whole, and too often the precise contribution of each protein—sometimes of each signal within one protein—to the transport of the vRNP complex remains unknown or is controversial. As our understanding of these processes increase, the intracellular trafficking of viral components represents a useful set of targets for developing powerful antiviral compounds.

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