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Review

Viral Subversion of Nucleocytoplasmic Trafficking

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Abstract

Trafficking of proteins and RNA into and out of the nucleus occurs through the nuclear pore complex (NPC). Because of its critical function in many cellular processes, the NPC and transport factors are common targets of several viruses that disrupt key constituents of the machinery to facilitate viral replication. Many viruses such as poliovirus and severe acute respiratory syndrome (SARS) virus inhibit protein import into the nucleus, whereas viruses such as influenza A virus target and disrupt host mRNA nuclear export. Current evidence indicates that these viruses may employ such strategies to avert the host immune response. Conversely, many viruses co-opt nucleocytoplasmic trafficking to facilitate transport of viral RNAs. As viral proteins interact with key regulators of the host nuclear transport machinery, viruses have served as invaluable tools of discovery that led to the identification of novel constituents of nuclear transport pathways. This review explores the importance of nucleocytoplasmic trafficking to viral pathogenesis as these studies revealed new antiviral therapeutic strategies and exposed previously unknown cellular mechanisms. Further understanding of nuclear transport pathways will determine whether such therapeutics will be useful treatments for important human pathogens.

Keywords mRNA export, nuclear import, nuclear pore complex, nuclear transport, nucleocytoplasmic trafficking, virus

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The nucleus is a double membrane-bound organelle in eukaryotic cells, which contains the cell's genetic material. The inner lipid bilayer delineates the nuclear contents and the outer layer is continuous with the endoplasmic reticulum (ER). This physical segregation provides a layer of regulation that allows for selection of molecules to be transported into and out of the nucleus, which is critical for proper gene expression and cell survival. Trafficking of material between the nucleus and cytoplasm occurs through the nuclear pore complex (NPC), which is the gateway between these compartments (1).

The NPC is a large, multisubunit complex of approximately 100 MDa in vertebrates that consists of about 30 different proteins, termed nucleoporins (Nups) (1–3). Structurally, the NPC is made up of an eightfold symmetrical framework that surrounds a central transport channel that spans the inner and outer membranes of the nuclear envelope (1). Nups in this channel have unfolded domains containing FG repeats, which are docking sites for transport receptors (1). Peripheral Nups interact with the central core, form rings at the nuclear and cytoplasmic sides of the NPC and also bind Nups embedded in the nuclear envelope. In the nuclear side, Nups form filaments that connect to generate the nuclear basket. At the cytoplasmic side, the Nup filaments protrude into the cytoplasm. The composition of Nups at the NPC was thought to be universal. However, distinct combinations of Nups have been found in NPCs of different cell types, indicating that some NPCs may have distinct functions depending on their composition (reviewed in 4).

NPCs allow free passage of ions and small molecules, but molecules larger than approximately 20–40 kDa are

actively transported through the NPC by the mobile nuclear transport machinery (5–10). Distinct nuclear transport pathways regulate the movement of these macromolecules into and out of the nucleus. For example, proteins with nuclear functions can be imported via the NPC by various pathways depending on their nuclear localization signals, whereas nuclear RNAs are actively exported after transcription via different mechanisms to be translated or to function in the cytoplasm. Proper gene expression is critical for the cell to adapt to its constantly changing needs (11). Therefore, efficient transport through the NPC must be a selective and highly regulated process. Partly because of the high level of regulation, viruses often exploit nucleocytoplasmic transport pathways in order to facilitate viral replication and evade the host antiviral response. In this review, we focus on discoveries that shed light onto virus–host interactions that occur at the level of nucleocytoplasmic trafficking. In addition, we highlight recent efforts to develop new antiviral therapeutic strategies that target host nuclear transport pathways that are crucial for viral propagation and that are revealing novel cellular processes.

Nuclear Transport of Proteins

Most facilitated transport of proteins through the NPC requires soluble transport receptors and a transport signal. Proteins targeted into or out of the nucleus interact with transport receptors via a short amino acid motif, named nuclear localization signal (NLS) or nuclear export signal (NES), respectively (1). Soluble transport receptors termed karyopherins (also known as importins, exportins, transportins and snurportin) are multidomain transport factors that bind to cargo through its cargo-binding domain. Karyopherin α (Kapα) recognizes so-called classical NLS motifs, whereas karyopherin β (Kapβ) interacts with non-classical NLS sequences such as the PY-NLS motif, which is found in structurally disordered regions of proteins and contains invariant proline and tyrosine residues in the Kapβ recognition sequence (1,12). Karyopherins also contain an NPC-binding domain(s) and an N-terminal-binding domain for the small GTPase Ran, which interacts with the karyopherin to regulate the association and dissociation of the transport receptor–cargo complex. Ran acts a molecular switch and alternates between GDP- and GTP-bound states. In its GTP form, interaction of Ran with import complexes results in their dissociation. On the other hand, formation of export complexes requires Ran in its GTP-bound state (1). Conversion between the GDP- and GTP-bound forms of Ran is regulated by the cytoplasmic GTPase-activating protein (RanGAP) and the guanine nucleotide exchange factor (RanGEF), which is located at the nucleoplasmic side of the NPC (1,5,13). Thus, RanGDP is found in the cytoplasm, whereas RanGTP is primarily nuclear. The asymmetric distribution of these regulatory factors creates a Ran gradient across the nuclear envelope that is fundamental for the directionality of nucleocytoplasmic transport. During import, karyopherins facilitate the transport of cargo across the NPC. Once in the nucleus, RanGTP stimulates the dissociation of the karyopherin–cargo complex. For export, karyopherin–cargo complex formation is stimulated by RanGTP and results in the translocation of the complex through the NPC. In the cytoplasm, hydrolysis of RanGTP to its GDP state results in the dissociation of the karyopherin–cargo complex (1). Here, we discuss how viruses disrupt these protein transport pathways to manipulate cellular processes and inhibit host antiviral mechanisms to facilitate viral replication (Figure 1).

Viral Disruption of Nucleocytoplasmic Transport of Proteins

Poliovirus and human rhinovirus disrupt nucleocytoplasmic trafficking of proteins by viral-mediated proteolytic cleavage of specific Nups

Poliovirus (PV) and human rhinovirus (HRV) are positive-stranded RNA viruses from the Picornaviridae family that replicate entirely in the cytoplasm of the host cell. Infection of mammalian cells with PV and HRV results in the cytoplasmic mislocalization of cellular proteins bearing a classical NLS, which requires the Kapα/Kapβ1 import pathway (14,15). In addition, cytoplasmic relocalization of M9 NLS-containing host proteins, which requires the Kapβ2/transportin1 nuclear import pathway, was also observed during PV and HRV infection (14,15). These and other studies revealed that infection with PV and HRV resulted in the proteolytic degradation of several nucleoporins, including Nup62, Nup98 and Nup153,

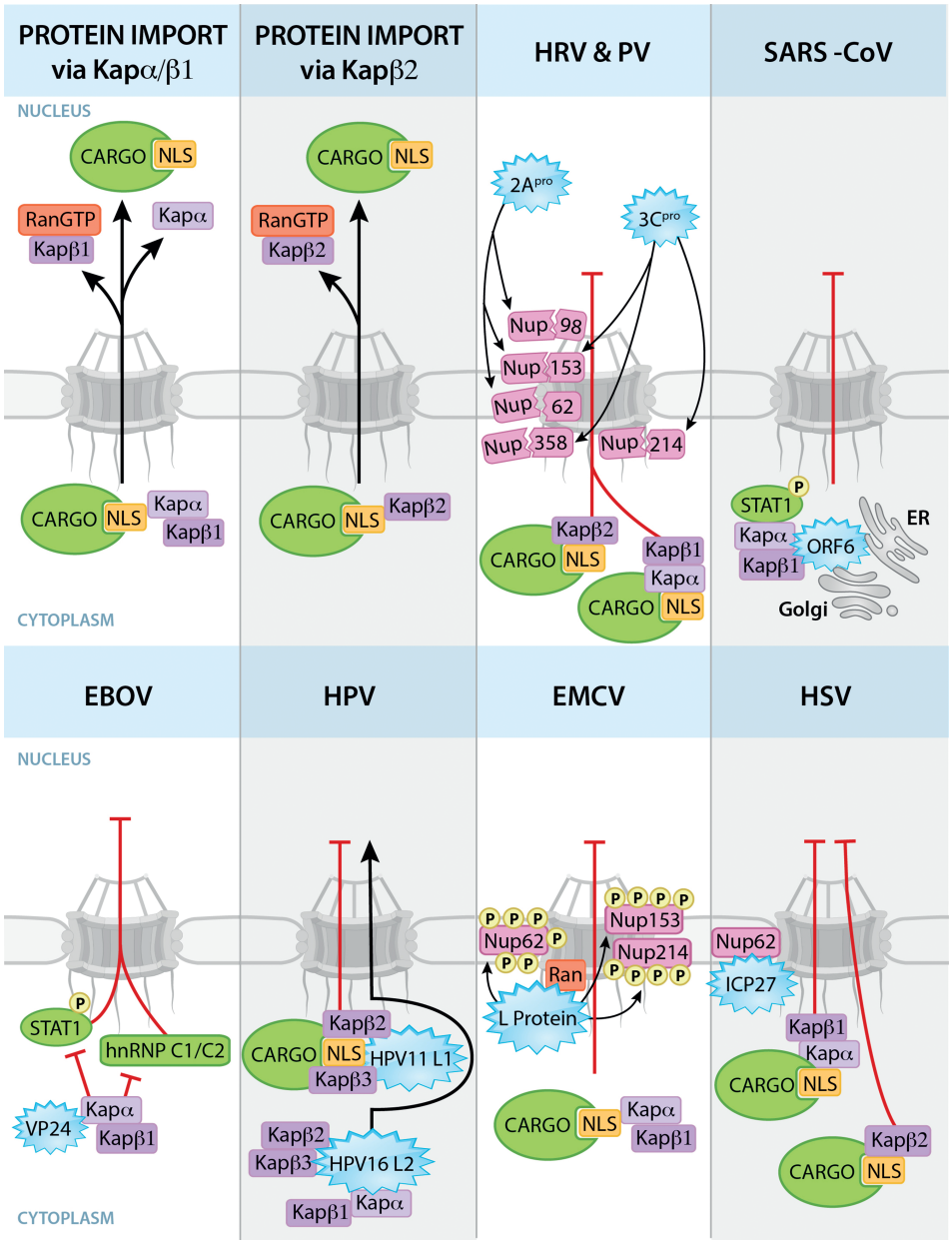

Figure 1: Viral strategies to disrupt nucleocytoplasmic trafficking of proteins. Nuclear import pathways mediated by Kapα/Kapβ1 or Kapβ2 are shown. Kapα and Kapβ2 bind proteins or cargos with specific NLSs. Kapβ1 and β2 translocate the import complexes through the NPC via interactions with Nups. The import complexes are dissociated by RanGTP at the nucleoplasmic side of the NPC. Viral proteins (blue starbursts) interact with the depicted host factors to disrupt nuclear transport pathways. $2A^{pro}$ and $3C^{pro}$ of HRV and PV degrade Nups and block nuclear import of proteins via the Kapα/β1 and Kapβ2 pathways. SARS-CoV ORF6 protein effectively disrupts nuclear import of phosphorylated STAT1 by tethering PY-STAT1–Kapα/Kapβ complex to ER/Golgi membranes. Alternatively, EBOV-VP24 binds Kapα preventing its interaction with phosphorylated STAT1 and hnRNP C1/C2, which accumulate in the cytoplasm. In HPV, while HPV11 L1 binds Kapβ2/β3 and disrupts cargo import, the viral HPV16 L2 protein gets imported into nucleus by binding to Kapβ2, Kapβ3 and Kapα/Kapβ1 complex. To inhibit protein import, L protein of EMCV hyperphosphorylates Nups and binds Ran. ICP27 protein of HSV interacts with Nup62 and blocks nuclear import of proteins via Kapα/Kapβ1 and Kapβ2 pathways. Disruption of nuclear import of proteins by other viruses is discussed in the text.

and possibly other factors, by the viral-expressed 2A protease ($2A^{pro}$) (14–20). In fact, inhibiting the PV $2A^{pro}$ suppressed the relocalization of cellular proteins, demonstrating that this effect is $2A^{pro}$-dependent (17). An in-depth analysis of different rhinovirus species revealed that $2A^{pro}$ targeted Nup62, Nup98 and Nup153 for proteolysis, albeit the rate and sites of cleavage were different (20). Moreover, the HRV 3C protease ($3C^{pro}$) and its precursor form, 3CD, also target nucleoporins such as Nup153, Nup214 and Nup358 for degradation, leading to disruption of NPC permeability and nucleocytoplasmic trafficking and mislocalization of nuclear proteins (21). Thus, both PV and HRV target nucleoporins to alter the composition of the NPC and trafficking of proteins into and out of the nucleus, which may function as a mechanism to inhibit host antiviral defense pathways by disrupting the translocation of important cellular proteins involved in immunity.

Viral antagonism of the host immune response by blocking STAT nuclear import

The severe acute respiratory syndrome coronavirus (SARS-CoV) is a positive-stranded RNA virus responsible for the potentially deadly human disease SARS. SARS-CoV may disrupt specific cellular pathways to suppress host immune responses, resulting in disease spreading. Host invasion by pathogens elicits a number of immune responses to facilitate pathogen clearance. During infection, activated signal transducer and activator of transcription 1 (STAT1) is imported into the nucleus to bind to interferon-stimulated response elements (ISRE) found on the promoter region of interferon (IFN)-inducible genes (22). During SARS-CoV infection, the viral open reading frame 6 (ORF6) protein blocks STAT1 nuclear translocation without affecting its phosphorylation (23). To prevent nuclear import of STAT1, SARS-CoV ORF6 binds and tethers the nuclear import factors Kapα2 and Kapβ1 to the ER/Golgi membrane, effectively blocking STAT1 transport into the nucleus (24) and the C-terminus of ORF6 is essential for its import block activity (25). Thus, SARS-CoV blocks nucleocytoplasmic trafficking of host immune signaling proteins, which is an effect that likely contributes to viral infection.

Ebola virus (EBOV) is a negative-stranded RNA virus that primarily replicates in the cytoplasm of the host cell and is responsible for hemorrhagic fever in humans. The EBOV VP24 viral protein blocks the nuclear translocation of tyrosine-phosphorylated STAT1 (PY-STAT1) to inhibit host IFN-α/β and IFN-γ signaling (26). Truncation mutants of the VP24 protein revealed that amino acids 26–50 are important for its ability to block IFN-β expression (26). Mechanistically, VP24 specifically binds to the PY-STAT1 nuclear transport receptor Kapα1 and disrupts the formation of the Kapα-PY-STAT1 transport complex, preventing PY-STAT1 nuclear translocation (27). Importantly, the VP24 viral protein of the mouse-adapted Zaire and Reston EBOV species also interacted with Kapα1 and disrupted PY-STAT1 nuclear import (28). Further characterization of VP24 from Zaire, mouse-adapted Zaire and Reston EBOV revealed that VP24 also interacts with karyopherins α5 and α6 and inhibits the binding of karyopherin to PY-STAT1 (28). In addition, EBOV VP24 interaction with Kapα1 has also been shown to disrupt nuclear import of hnRNP C1/C2, which interacts with the same region of Kapα1 as PY-STAT1 (29). Redistribution of hnRNP C1/C2 to the cytoplasm may facilitate EBOV replication similar to hnRNP C cytoplasmic relocalization by PV, which has been shown to stabilize PV RNA to promote viral replication (30).

Other globally important human pathogens inhibit host immune signaling by disrupting nuclear transport of proteins. For example, measles virus nucleocapsid (N) protein inhibits host IFN signaling pathways by preventing import of active STAT to the nucleus without affecting Jak and STAT phosphorylation or STAT degradation (31). In addition, rotavirus antagonizes IFN signaling pathways via inhibition of transcription of host antiviral factors by blocking nuclear import, but not activation, of STAT1, STAT2 and NF-κB (32). In sum, blockage of nuclear import of immune signaling factors by associating with and disrupting soluble transport factors represents an effective viral strategy to inhibit the host antiviral response.

Encephalomyocarditis virus alters nucleocytoplasmic trafficking as a tactic to achieve IFN suppression and viral replication

Encephalomyocarditis virus (EMCV) is a positive-stranded RNA virus from the Picornaviridae family that manipulates nucleocytoplasmic transport to propagate in the cytoplasm of infected cells and suppress the host

immune response. The EMCV leader (L) protein, a non-enzymatic viral component, disrupts nuclear transport of proteins by binding and suppressing the activity of Ran-GTPase, thus altering the Ran gradient that is essential for the regulation of nucleocytoplasmic transport (33,34). In addition, EMCV infection results in Nup62, Nup153 and Nup214 hyperphosphorylation in an L protein-dependent manner, which may alter the integrity of and/or transport receptor–cargo complex binding to the NPC (34). The hyperphosphorylation of Nups and disruption of the RanGTP gradient represent an alternative mechanism by which EMCV effectively interferes with nucleocytoplasmic trafficking compared to the proteolytic activity associated with other picornaviruses discussed above (34). Similarly, infection with mengovirus, a positive-stranded RNA picornavirus, results in the hyperphosphorylation of Nup62 in an L protein-dependent manner, which may also alter host nuclear import and export pathways (35). Moreover, EMCV infection results in increased NPC permeability and enhanced relocalization of nuclear proteins to the cytoplasm of infected cells (36). These effects on nucleocytoplasmic transport were shown to require the zinc finger motif and phosphorylation of the threonine 47 (Thr47) residue of the EMCV leader protein (36). Thus, EMCV utilizes several different mechanisms to alter nuclear transport of host cell factors to block host IFN response and promote viral replication.

Theiler's murine encephalomyelitis virus disrupts IRF3 function by altering its subcellular localization

Theiler's murine encephalomyelitis virus (TMEV) is a single-stranded RNA virus known to disrupt nucleocytoplasmic trafficking of cellular factors to interfere with the expression of host antiviral mechanisms (37,38). The TMEV leader (L) protein promotes the redistribution of host proteins, such as the polypyrimidine tract-binding (PTB) protein and IFN regulatory factor 3 (IRF3) (37). By interfering with IRF3 subcellular localization, TMEV may prevent transcription of host genes, such as IFNs, involved in antiviral response (37). In addition, TMEV infection results in Nup98 hyperphosphorylation (38). Thus, to effectively replicate in the host cell, TMEV alters the cellular distribution of host defense factors via disruption of nucleocytoplasmic trafficking.

Venezuelan equine encephalitis virus and nuclear pores

Venezuelan equine encephalitis virus (VEEV) is a positive-stranded RNA virus that has the ability to cause fatal disease in humans and equine species. Subcellular localization studies revealed that a fraction of the VEEV capsid protein localizes to the nuclear envelope, where it may regulate nucleocytoplasmic trafficking (39). In fact, the capsid protein blocks several nuclear transport pathways without affecting the diffusion of small proteins through the NPC (39). This block is mediated by a complex formed with VEEV capsid protein, the nuclear export receptor CRM1 and karyopherin/importin α/β (40). This complex blocks nuclear transport of cargos, which accumulate in the central channel of the NPC (40). However, the physiological consequences of such a blockage require further examination.

Human papillomavirus inhibits Kap β2- and Kap β3-mediated nuclear import

Human papillomavirus (HPV) is a DNA virus known to be involved in various human diseases ranging from skin warts and some cardiovascular diseases to cervical and other cancers. HPV types 11 (HPV11) and 16 (HPV16) have been shown to associate with host nuclear import receptors (41,42). The HPV11 L1 major capsid protein binds to karyopherins β2 and β3 and disrupts nuclear import through these pathways (41). This inhibitory function was also observed in the L1 capsid protein of high-risk HPV16 (41). Although HPV11 L1 capsid protein blocks nuclear protein import, the functional consequences of such a block are unknown. Similarly, the HPV16 L2 minor capsid protein directly interacts with the nuclear import receptors Kapβ2 and Kapβ3, and also forms a complex with the Kapα2/Kapβ1 heterodimer by interacting with Kapα2 (42). By binding different soluble import receptors, HPV16 L2 protein efficiently translocates to the nucleus of infected cells to facilitate assembly of HPV virions (42).

Herpes simplex virus inhibits nuclear import of proteins via interaction with the NPC

The important human pathogen herpes simplex virus (HSV) is a DNA virus that interferes with host nuclear transport pathways. The HSV-1 ICP27 protein blocks nuclear import of proteins via the Kapα/β1 and Kapβ2

(transportin) nuclear import pathways by associating to the NPC through direct interactions with Nup62 (43). Although HSV-1 blocks host nuclear trafficking via ICP27, HSV-1 can still export its viral mRNAs by directly interacting with the nuclear export factor Aly/Ref, followed by recruitment of the mRNA export factor NXF1/TAP (44), as will be discussed below. These functions of ICP27 may ensure the translation of viral mRNAs while the expression and trafficking of host proteins is drastically affected.

In sum, both RNA and DNA viruses have developed varied strategies to regulate the NPC and nuclear transport of proteins to promote an environment more favorable to viral replication. To respond to these challenges, the host cell may secrete antiviral cytokines, such as IFNs. In fact, studies have shown that IFNs upregulate the expression of certain nucleoporins (16,45), which illustrates a host strategy to restore balance to nucleocytoplasmic trafficking.

Nuclear Export of mRNA

Most DNA and even a few RNA viruses replicate within the nucleus of a host cell, and these viruses utilize nuclear export pathways to express their genes (46). In fact, studies of viral mechanisms to export viral RNA revealed two proteins that are important nuclear export factors of host RNAs, CRM1 (chromosome region maintenance 1, also known as exportin 1, XPO1) and NXF1 (nuclear export factor 1, also known as TIP-associated protein, TAP) (47–49). These proteins are exploited by several viruses to promote viral mRNA export and/or inhibit host mRNA trafficking in order to prevent a proper host immune response (Figure 2).

Before being exported from the nucleus, mRNAs must be properly processed into a mature mRNA by undergoing capping, splicing and polyadenylation (50,51). After processing, mRNAs are loaded with mRNA export factors, which recruit export receptors to the mRNAs. The transcription–export (TREX) complex coordinates nuclear export of mRNAs with transcription and processing (52–54). This complex is comprised of the multisubunit THO complex that functions in transcription elongation (53, 55–57) and the export factors UAP56 (also known as HEL) and REF (also known as ALY). UAP56 is an ATP-dependent RNA helicase that is also involved in splicing and it is required for assembly of the TREX complex. In the ATP-bound form, UAP56 binds REF, stimulating the association of REF with mRNA (58–60). Subsequently, REF interacts with the mRNA export receptor heterodimer NXF1-NXT1 (also known as TAP-p15), which mediates nuclear export of the mRNAs by binding phenylalanine–glycine (FG) repeat domains on Nups at the NPC (49, 61–63). FG-containing Nups, such as Nup98, mediate translocation of the mRNP (messenger ribonucleoprotein) complex through the NPC (63,64). Another mRNA export factor involved in this process is Rae1 (also known as Mrnp41), a nucleocytoplasmic shuttling protein that interacts with mRNA, NXF1 and Nup98 (65–73). Rae1 may recruit NXF1 to Nup98 (67). A more detailed discussion on Rae1 is found in another review (74). Upon reaching the cytoplasmic side of the NPC, the mRNP is released by the ATPase activity of Dbp5, which is stimulated by Gle1 and IP6 (75–78).

While NXF1 is the main mRNA export receptor in humans, CRM1 is an important receptor required for export of proteins with a leucine-rich NES and is also known to export certain classes of RNA, including subsets of mRNAs (79–81). Nuclear export of RNA via CRM1 requires an adaptor protein bound to cargo RNA that interacts with CRM1 via an NES (82).

Viruses Are Tools for Discovery of Host Pathways

The identification of CRM1 as a nuclear export receptor highlights the use of viruses as important tools for discovery. CRM1 was originally identified as a result of investigating the mechanism by which complex retroviruses such as human immunodeficiency virus 1 (HIV-1) were able to subvert host mechanisms that prevent export of unspliced or incompletely spliced RNA transcripts (47,48). HIV-1 encodes the Rev protein that interacts with these viral RNAs via the Rev response element (RRE) (83–85). This interaction exposes the NES of Rev, which binds CRM1 and promotes export of viral RNAs from the nucleus via the NPC (47).

NXF1 was also discovered through the study of viral interactions with host proteins. Simple retroviruses such as Mason-Pfizer monkey virus (MPMV) do not encode

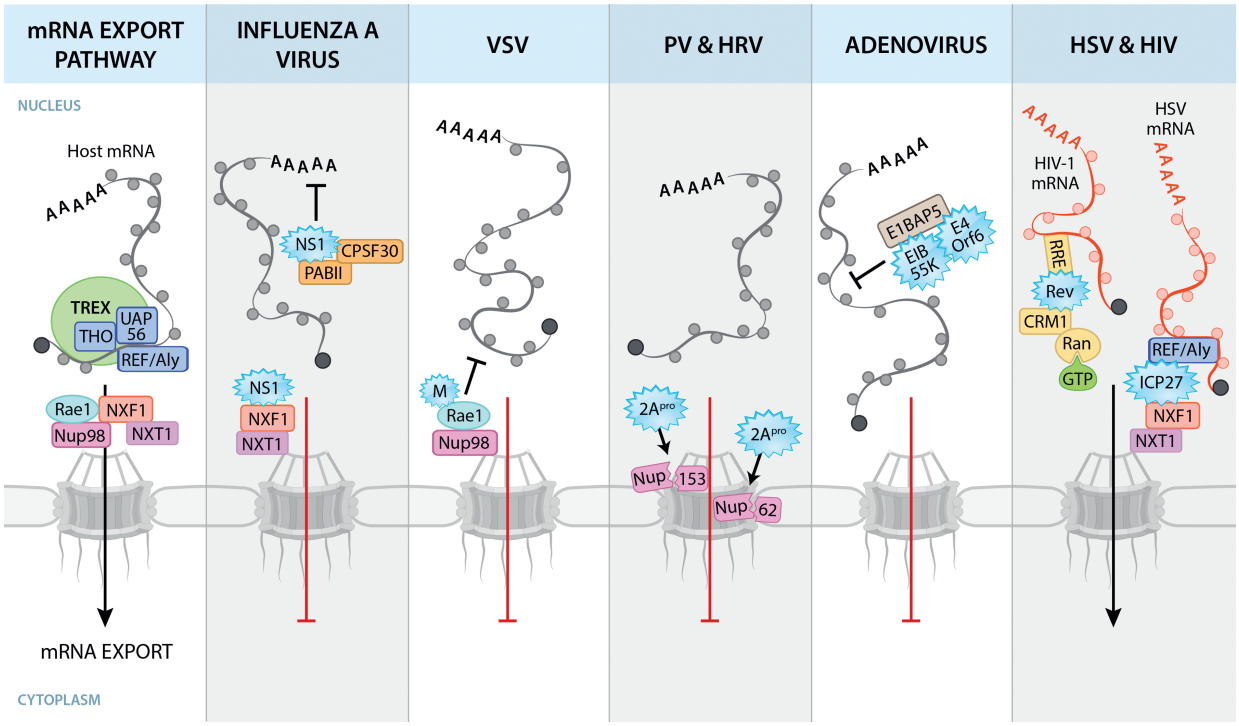

Figure 2: Viral disruption of host mRNA nuclear export pathways. Host mRNA export is coordinated by the TREX complex, which consists of THO, UAP56 and REF/Aly. The association of REF with mRNA recruits the mRNA export receptor heterodimer NXF1-NXT1, which mediates export of mRNAs by interacting with Nups at the NPC. Circles surrounding mRNAs depict RNA-binding proteins. Viral proteins (depicted as blue starbursts) disrupt mRNA nuclear export by interacting with host factors. IAV NS1 binds and disrupts factors involved in cellular mRNA processing and export. VSV M protein interacts with Rae1 and Nup98, resulting in mRNA nuclear export block. $2A^{pro}$ of PV and HRV cleaves Nups to disrupt NPC architecture. AdV E1B 55K and E4orf6 proteins disrupt NXF1-mediated host mRNA export by binding to E1B-AP5. Other viruses, such as herpesviruses and HIV, utilize cellular transport pathways to promote viral mRNA export. The herpesvirus protein ICP27 facilitates preferential export of viral mRNAs through interaction with REF/Aly and NXF1. The HIV-1 Rev protein facilitates nuclear export of unspliced or partially spliced viral mRNAs through the Rev-responsive element (RRE), an RNA signature on these viral mRNAs. Rev-bound viral RNA binds CRM1 and RanGTP and is translocated through the NPC.

a Rev protein. Thus, nuclear export of unspliced RNAs from this virus occurs via a constitutive transport element (CTE), a *cis*-acting stem loop that contains the RNA sequence that interacts with NXF1/TAP to facilitate export (49,86). Importantly, microinjection of excess CTE-containing RNA into Xenopus oocytes was shown to block export of cellular mRNAs, while export of CRM1-dependent molecules was unaffected. This established NXF1/TAP as the major mRNA export receptor in eukaryotes (63).

Recently, a novel mechanism for export of large mRNPs was discovered that was functionally similar to the budding of herpesvirus virions from the nucleus of host cells (87–89). This process is independent of NPC activity but dependent upon type A lamins, which along with type B lamins make up a fibrous lattice that forms the nuclear lamina associated with the inner nuclear membrane (90). The authors noted the presence of poly(A) RNA within large foci that appeared to invaginate and bud through the inner and outer nuclear membranes and proposed that herpesviruses may have hijacked this endogenous eukaryotic mechanism to facilitate budding of large viral particles that are too large to pass through the NPC (88). The similarity between these two processes highlights yet another way in which the study of viruses can lead to

important discoveries that increase our understanding of eukaryotic cellular mechanisms.

Viral Inhibition of Host mRNA Export

RNA viruses promote viral replication via disruption of mRNA export pathways

In addition to exploiting host mRNA nuclear export pathways, viruses also block export of host mRNAs. The expression of mRNAs encoding cellular defense proteins is critical for the host to mount a proper immune response to invading pathogens. Therefore, mRNA export pathways are an enticing target for viruses to block host expression of antiviral genes. Similar to most viral infections, influenza A virus (IAV) replication in vertebrate cells is recognized by the innate immune system. Upon recognition, the innate immune system triggers signal transduction pathways that lead to production of type I IFNs, which are antiviral cytokines that induce the expression of mRNAs encoding antiviral factors (91), including nucleoporins (16,45). IAV has evolved several mechanisms to inhibit this response, mainly through non-structural protein 1 (NS1), a multifunctional protein with activities in both the nucleus and cytoplasm (91,92).

In the nucleus, NS1 inhibits mRNA processing and export (74). The steps of mRNA processing and export are closely linked, as some proteins that interact with mRNAs remain bound throughout both processes, whereas others are exchanged for factors specific for each step (93). NS1 inhibits pre-mRNA splicing by binding to the U6 snRNA component of the spliceosome and/or through interactions with the putative splicing protein NS1-BP (94,95). NS1 further disrupts host mRNA processing by binding to the 30-kDa subunit of the cleavage and polyadenylation specificity factor (CPSF30) and the poly(A)-binding protein II (PABII), which are involved in binding the polyadenylation signal and in the elongation of the poly(A) chain of mRNAs, respectively (96,97). The interaction of NS1 with these proteins inhibits 3′-end processing of host mRNAs and contributes to inhibition of host gene expression. However, production of viral transcripts is unaffected by NS1-mediated disruption of mRNA processing because poly(A) tail synthesis on viral mRNAs is carried out by the viral polymerase complex (98,99). Additionally, NS1 may facilitate splicing of the viral M1 mRNA segment by interacting with the host mRNA-binding proteins, NS1-BP (NS1-binding protein) and hnRNP K (100). This permits efficient processing of viral mRNA transcripts before nuclear export occurs. In addition to disrupting host mRNA processing, IAV further disrupts expression of host antiviral genes via NS1 interactions with the host mRNA export machinery. NS1 interacts with the mRNA export factors NXF1-NXT1, which form a complex with Rae1 and E1B-AP5, preventing nuclear export of poly(A) RNA (101). Altogether, these studies show that NS1 of IAV employs several mechanisms to inhibit the connected and highly regulated processes of mRNA processing and export. Interestingly, mRNAs encoding antiviral factors are retained in the nucleus owing to NS1-mediated inhibition of mRNA nuclear export (102), indicating that disruption of these pathways likely represents a viral strategy to promote viral replication and avoid the host immune response.

Certain RNA viruses that replicate in the cytoplasm also inhibit host mRNA nuclear export. Vesiculoviruses, such as vesicular stomatitis virus (VSV), are negative-stranded RNA viruses that prevent proper mRNA export through the action of the VSV matrix (M) protein (45, 69, 102–106), resulting in inhibition of host gene expression. This effect decreases competition of VSV mRNAs with host mRNAs for use of the translational machinery. Similar to IAV infection, blockage of mRNA export by VSV also prevents expression of mRNAs that encode antiviral factors (102). The M protein contains NLSs that allow its import into the nucleus, where it exerts its inhibitory function on mRNA export (104,107). Once inside the nucleus, M protein interacts with the mRNA export factor Rae1, which is in complex with Nup98 (69,105). This prevents export of bulk poly(A) mRNAs during VSV infection. It has been reported that the interaction of M protein with Rae1 and Nup98 inhibits host transcription (108). However, high levels of polyadenylated RNA are retained inside the nucleus in the presence of M protein indicating complete mRNA synthesis, as shown by various methods including nucleocytoplasmic fractionation followed by microarray analysis and real-time reverse transcriptase polymerase chain reaction (102), oligo-dT *in situ* hybridization (45,69,105,106) and mRNA nuclear export assays in Xenopus oocytes (103–106). These results indicate that M protein utilizes post-transcriptional

mechanisms to inhibit gene expression. Transcriptional studies are necessary to investigate whether M protein regulates expression of certain subsets, rather than the general population, of mRNAs. This is possible, as Nup98 has been shown to regulate transcription of subsets of genes (109,110).

The inhibition of bulk poly(A) mRNA export by M protein may occur via several mechanisms. The VSV M–Rae1–Nup98 complex may inhibit the export of a subset of mRNAs that include major regulators of gene expression, thereby indirectly triggering a shutoff of host gene expression. Another possibility is that Rae1–Nup98 may facilitate export of bulk NXF1-mediated mRNA export. Therefore, M protein inhibition of Rae1–Nup98 would lead to retention of the majority of mRNAs in the nucleus. Interestingly, M protein-mediated block of mRNA export can be antagonized by IFN, which upregulates the expression of Nup98, Nup96 and Rae1 (16,45). Genome-wide studies that identify mRNAs that are directly targeted by the Rae1–Nup98 complex at early stages of infection as well as additional biochemical studies on the interaction of M protein with the mRNA export machinery will further reveal the mechanism of action of VSV M protein.

Interestingly, both Rae1 and Nup98 function during mitosis to regulate spindle assembly (111,112). VSV M protein interaction with this complex inhibited mitotic progression and triggered substantial cell death (113). This has several implications for VSV, which is an oncolytic virus that is currently being developed as a cancer therapeutic (114–116). As tumor cells have an increased mitotic index, VSV-mediated mitotic cell death likely contributes to its oncolytic activity.

As discussed above, picornaviruses are RNA viruses that replicate within the host cell cytoplasm and regulate nucleocytoplasmic trafficking. Many picornaviruses, including the important human pathogens PV and HRV, inhibit nucleocytoplasmic trafficking of host proteins and mRNAs to promote viral protein synthesis and disrupt host expression of antiviral factors. As discussed above, PV and HRV infection results in the mislocalization or degradation of several important nuclear export factors such as Nup62, Nup98 and Nup153 (14–16, 19, 20). Cleavage and subsequent degradation or mislocalization of these proteins is mediated by the viral $2A^{pro}$, which leads to changes in NPC architecture that affects both host protein and mRNA transport (14–17, 19, 20). Overall, RNA viruses employ a multitude of strategies to inhibit nucleocytoplasmic trafficking, which suppresses the host innate immune response and enhances viral replication.

Disruption of host gene expression by DNA viruses facilitates export of viral RNA

Not only RNA viruses disrupt nucleocytoplasmic trafficking of mRNA. Many DNA viruses selectively inhibit host mRNA export, while ensuring that viral mRNAs are efficiently exported after transcription. Adenoviruses (AdVs) are double-stranded DNA viruses that infect many vertebrate species, including humans. Two adenoviral protein products, E1B-55K and Ad E4 open reading frame 6 (E4orf6), have been shown to mediate the degradation of cellular proteins that may have a deleterious effect on viral propagation (117). The interaction of E1B-55K and E4orf6 with host proteins results in the formation of a complex with E3 ubiquitin ligase activity that may contribute to inhibition of host mRNA export and promotion of late viral mRNA export from the nucleus (118,119). In one model, it was proposed that the E1B-55K and E4orf6 ubiquitin ligase activity promotes the degradation of an as of yet unidentified cellular protein involved in host mRNA export (118). Another possibility is that E1B-55K disrupts NXF1-mediated host mRNA export by binding to E1B-AP5, a member of the RNP family that interacts with NXF1 (63). While it is clear that AdV is able to regulate cellular mRNA export to favor export of late AdV mRNAs, more studies are needed to establish how AdV infection promotes nuclear accumulation of host mRNAs.

As discussed above, herpesviruses are experts at hijacking host cell functions to ensure viral replication. These viruses replicate within the nucleus and therefore must export viral transcripts to the cytoplasm for protein synthesis. One of the proteins encoded by the α-herpesvirus HSV-1 is ICP27, which disrupts host mRNA processing (120,121) while allowing the export of intronless viral transcripts (122–124). ICP27 binds directly to the RNA export factor ALY/REF and NXF1, which recruits viral mRNAs to export receptors for preferential transport into the cytoplasm (122–124). Interestingly, other related herpesviruses do not inhibit host mRNA processing during infection, but do encode an ICP27-like protein that

favors viral mRNA export. These viruses include human cytomegalovirus (hCMV), Kaposi's sarcoma-associated herpesvirus (KSHV), Epstein–Barr virus (EBV) and varicella-zoster virus (VZV) (125–129).

Nucleocytoplasmic Transport: A New Frontier in Antiviral Therapy

These studies show that viruses dedicate many resources to the disruption of nucleocytoplasmic trafficking and the host can antagonize these effects. These findings attest to the importance of these pathways in proviral and antiviral mechanisms. As such, nuclear import and export pathways are enticing targets for the development of novel antiviral therapeutics. Recently, screens for small-molecule inhibitors of the NS1 protein of IAV revealed both unique virus/host interactions and potentially useful antiviral strategies (130,131). In one study, a high-throughput screen was performed to identify small molecules that could antagonize NS1-mediated inhibition of host gene expression. One of the compounds identified interfered with infection of several viruses by inducing the expression of REDD1 (also known as DDIT4), an inhibitor of the mTORC1 pathway that is required for IAV infection (130,132). Overexpression of REDD1 resisted IAV infection while REDD1 knockout cells were more permissive to viral replication, demonstrating a role for REDD1 as a host defense factor. Interestingly, a second compound identified in the same screen uncovered a new link between pyrimidine biosynthesis pathways and mRNA nuclear export. This compound, a quinoline carboxylic acid, directly inhibited the host enzyme dihydroorotate dehydrogenase (DHODH), which is required for *de novo* pyrimidine biosynthesis but not for synthesis of pyrimidines via the salvage pathway. Thus, pyrimidine synthesis is not totally shut down and cytotoxicity does not occur upon compound treatment. The inhibition of DHODH led to an increase in NXF1 expression and subsequent relief of mRNA export block mediated by both IAV NS1 and VSV M proteins (102). The compound inhibited viral replication owing to the reduction of pyrimidine pools that viruses need to execute robust viral transcription and the release of host mRNA export block, which resulted in expression of antiviral factors (102,133). Simultaneously, host cells maintained homeostasis by utilizing the salvage pathway or pyrimidines derived from the partially inhibited *de novo* biosynthesis pathway (102,133).

Another strategy to inhibit virus replication is to target interactions between viral proteins and their nuclear transport receptors to prevent their import into the nucleus to promote viral replication. This was recently shown to be the case for the NS5 protein of dengue virus (DENV2) in a study in which the small-molecule Ivermectin was able to inhibit NS5 interaction with Kapα/β, resulting in inhibition of nuclear import and infection (134).

Together, these studies show that the use of high-throughput screening techniques to discover small molecules that alter host or viral gene expression has broad implications for the development of antiviral agents. Furthermore, the study of these compounds may reveal new interactions and regulatory functions of nuclear transport pathways, much like the use of viruses as tools of discovery.

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