Crosstalk between nucleocytoplasmic trafficking and the innate immune response to viral infection

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The nuclear pore complex is the sole gateway connecting the nucleoplasm and cytoplasm. In humans, the nuclear pore complex is one of the largest multiprotein assemblies in the cell, with a molecular mass of ∼110 MDa and consisting of 8 to 64 copies of about 34 different nuclear pore proteins, termed nucleoporins, for a total of 1000 subunits per pore. Trafficking events across the nuclear pore are mediated by nuclear transport receptors and are highly regulated. The nuclear pore complex is also used by several RNA viruses and almost all DNA viruses to access the host cell nucleoplasm for replication. Viruses hijack the nuclear pore complex, and nuclear transport receptors, to access the nucleoplasm where they replicate. In addition, the nuclear pore complex is used by the cell innate immune system, a network of signal transduction pathways that coordinates the first response to foreign invaders, including viruses and other pathogens. Several branches of this response depend on dynamic signaling events that involve the nuclear translocation of downstream signal transducers. Mounting evidence has shown that these signaling cascades, especially those steps that involve nucleocytoplasmic trafficking events, are targeted by viruses so that they can evade the innate immune system. This review summarizes how nuclear pore proteins and nuclear transport receptors contribute to the innate immune response and highlights how viruses manipulate this cellular machinery to favor infection. A comprehensive understanding of nuclear pore proteins in antiviral innate immunity will likely contribute to the development of new antiviral therapeutic strategies.

A defining feature of all eukaryotic cells is the nuclear envelope, which encloses the cell's genetic material and separates the nucleoplasm, where RNA is synthesized and processed, from the cytoplasm, where mRNA is translated into proteins (1–3). The nuclear envelope is contiguous with the endoplasmic reticulum (ER), and it contains two membranes, the outer and inner nuclear membranes, which are separated by a luminal space that is contiguous with the lumen of the ER. Within the nuclear envelope, thousands of macromolecular channels are embedded, termed the nuclear pore complexes (4–6), which mediate the nucleocytoplasmic trafficking of macromolecules needed for a number of cellular processes, such as DNA replication, transcription, translation, and antiviral innate immunity (7). Mutations and gene fusions of nucleoporins (Nups) cause many diverse human diseases including autoimmune diseases (RanBP2/Nup358) and increased susceptibility to viral infections (translocated promoter region [TPR], Nup153, and RanBP2/Nup358) (8, 9). Despite this, the exact molecular mechanisms by which these mutations contribute to these various pathologies remain mysterious. In this review, we discuss recent advances that reveal how nuclear pore proteins contribute to antiviral innate immunity and highlight how viruses manipulate this cellular machinery to evade the innate immune response and favor viral infection. Finally, we briefly review recent progress that has been made in developing novel antiviral therapeutics that target nucleocytoplasmic transport.

Overview of the cellular nuclear transport machinery

The nuclear pore complex is one of the largest protein complexes in the cell, with an estimated molecular mass of 50 MDa in yeast and 110 to 125 MDa in metazoans and an outer diameter of 80 to 120 nm and an inner diameter of ∼40 nm (10, 11). Each nuclear pore complex is composed of multiple copies (ranging from 8 to 64) of about 34 different nuclear pore proteins, known as Nups (labeled Nup followed by their predicted molecular weight), most of which are conserved among different organisms (8, 12–16). Structurally, the nuclear pore complex consists of an inner ring, which resides in the center of the pore, cytoplasmic, and nuclear rings, which are similar to each other and attach to either side of the inner ring, cytoplasmic filaments, which project from the cytoplasmic ring, and a nuclear basket, which is attached to the nuclear ring (8, 15–19) (Fig. 1*A*). Surrounding each nuclear pore complex is a highly curved section of the nuclear envelope where the outer nuclear membrane is fused with the inner nuclear membrane (19). Across this membrane, and within the lumen of the nuclear envelope, a circular scaffold known as the luminal ring surrounds the nuclear pore complex (20–22).

The central channel of the pore is lined with Nups that contain phenylalanine–glycine repeats (FG-Nups). These repeats interact with one another to form a meshwork, which

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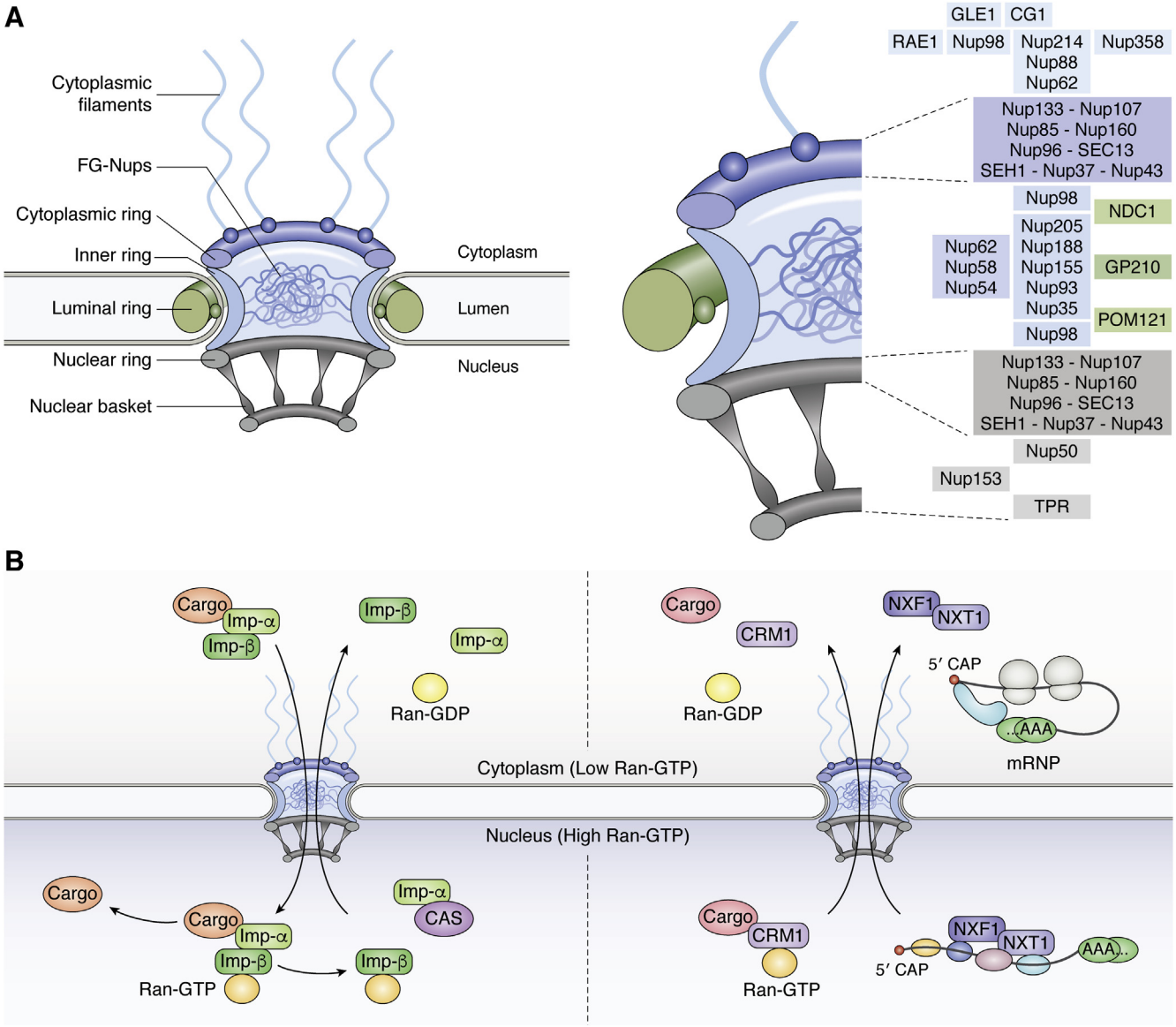

Figure 1. Schematic representation of the nuclear pore complex and the nuclear import and export cycles. *A*, the nuclear pore complex is embedded into the nuclear envelope and composed of nucleoporins (Nups) that are structurally arranged into the inner ring, cytoplasmic and nuclear rings, cytoplasmic filaments, and nuclear basket. Within the lumen of the nuclear envelope, a circular scaffold known as the luminal ring surrounds the nuclear pore complex. The central channel of the pore is lined with Nups that contain phenylalanine–glycine repeats (FG-Nups). *B*, the movement of macromolecules and complexes across the nuclear envelope is facilitated by nuclear transport receptors. In the canonical nuclear import pathway, a cargo is recognized by nuclear import receptors importin-α (imp-α) and importin-β (imp-β) and is ferried across the pore. Once in the nucleus, the binding of Ran-GTP to importin-β causes the disassembly of the import complex and releases the cargo. Importin-β bound to Ran-GTP is transported back to the cytoplasm, whereas importin-α is recycled by CAS protein (also known as exportin2). GTP hydrolysis of Ran releases importin-β for the next round of import. For nuclear export, a cargo with nuclear export signal is usually bound by CRM1 (also known as exportin1). After the export complex enters the cytoplasm, Ran-GTP is hydrolyzed to Ran-GDP, and this promotes dissociation of the complex. The export of mRNAs is different from that of proteins because mRNAs are bound by many proteins in the form of messenger ribonucleoprotein (mRNP) complexes. mRNA export requires the nuclear transport receptor NXF1/NXT1 (also known as TAP/p15). Following the completion of export, the mRNP undergoes remodeling events where the transport receptors and many bound proteins are removed, whereas other protein factors such as ribosomes join. CAS, cellular apoptosis susceptibility; CRM1, chromosomal maintenance 1; imp-α, importin-α; imp-β, importin-β; mRNP, messenger ribonucleoprotein; NXF1, nuclear RNA export factor 1; NXT1, nuclear transport factor 2-like export factor 1.

appears to phase separate from the bulk solution and thus acts as a permeability barrier (23). The meshwork prevents the movement of macromolecules and complexes that are larger than ∼30 to 40 kDa (*e.g.*, proteins, RNAs, and viruses). To cross the pore, these macromolecules need to be ferried by nuclear transport receptors (also known as importins, exportins, transportins, or karyopherins) (24–27).

In general, the formation and disassembly of nuclear transport receptors with their macromolecular cargos are regulated by the small Ras-like GTPase Ran, which cycles between GDP-bound and GTP-bound states. The conversion from GDP- to GTP-bound state is promoted by the guanine nuclear exchange factor regulator of chromosome condensation 1, which resides in the nucleus, whereas the hydrolysis of

GTP to GDP is catalyzed by Ran's intrinsic GTPase activity that is stimulated by Ran GTPase–activating protein 1, which is tightly associated with the cytoplasmic filament protein RanBP2/Nup358. As a result of these two locally restricted reactions, the ratio of Ran-GTP/Ran-GDP is ∼200-fold higher in the nucleus than in the cytoplasm (28, 29). In addition to this "Ran-GTP gradient," overall Ran concentration is kept relatively high in the nucleus and low in the cytoplasm because of the activity of nuclear transport factor 2 (NTF2), which associates with Ran-GDP in the cytosol, then ferries it into the nucleus where upon GTP hydrolysis releases Ran (30–36). As such, the nucleoplasm acts as a sink for Ran, and this is catalyzed by the energy released by the GTP hydrolysis reaction.

GTP hydrolysis drives all Ran-dependent import and export. Proteins that are imported contain nuclear localization signals (often referred to as NLSs), which are recognized by specialized sets of import receptors in the cytoplasm. Importin-α (also known as karyopherin-α) binds to canonical NLSs, which consist of one or more clusters of basic amino acids (37–40). Simultaneously, importin-α binds to importin-β (also known as karyopherin-β) (41, 42), which interacts with FG repeats and can ferry associated proteins across the nuclear pore (43, 44). Once inside the nucleus, Ran-GTP binds to importin-β and causes the disassembly of the cargo–importin-α/β complex (43, 45). As such, the nucleoplasm becomes a sink for nuclear import substrate proteins. The importin-β–Ran-GTP complex can then diffuse back to the cytoplasm, whereas importin-α is recycled back by cellular apoptosis susceptibility protein (also known as exportin2) (46). Subsequent GTP hydrolysis, stimulated by Ran GTPase–activating protein 1, releases importin-β for the next round of import (Fig. 1*B*). In some cases, proteins may contain noncanonical NLSs. For proteins that contain proline–tyrosine NLSs, such as heterogenous nuclear ribonucleoproteins (hnRNPs), they are imported by transportin-1 (also known as importin-β2) (47–49). Proteins can also be directly recognized by importin-β, and examples of these are ribosomal proteins, the HIV proteins Rev and Tat (40, 50–52).

For the nuclear export of protein substrates, these typically contain nuclear export signals (often referred to as NESs), which are usually leucine-rich sequences and recognized by the major exportin, chromosomal maintenance 1 (CRM1, also known as exportin1/XPO1) bound to Ran-GTP (53, 54). CRM1 interacts with FG repeats and thus ferries its cargoes across the pore. After this complex enters the cytoplasm, GTP hydrolysis of Ran promotes the dissociation of the export complex and the release of cargo (Fig. 1*B*). As such, the cytosol acts as a sink for these protein cargoes. Cargo-free CRM1 is then free to diffuse back across the pore into the nucleoplasm. For the export of most noncoding RNAs and some proteins, CRM1 does not bind to these cargos directly but is instead recruited to the RNAs by adapter proteins such as PHAX (phosphorylated adapter RNA export protein) (55–59).

The export of mRNAs is different from that of proteins and most noncoding RNAs because mRNAs are associated with a dynamic repertoire of proteins in the form of large messenger ribonucleoprotein (mRNP) complexes (60, 61). Most mRNAs do not rely on CRM1 and Ran for export but instead require the nuclear transport receptor heterodimer nuclear RNA export factor 1 (NXF1)/NTF2-like export factor 1 (NXT1), which is structurally related to NTF2 (62–64). NXF1/NXT1 (also known as TAP/p15) is recruited to the mRNP by the transcription export (TREX) complex and serine and arginine-rich proteins. TREX is typically recruited to the mRNA during transcription and RNA processing, whereas serine and arginine-rich proteins bind to particular motifs in the mRNA (61, 65, 66). As is the case with NTF2, NXF1/NXT1 directly binds to FG-Nups and thus facilitates movement across the pore. Following the completion of translocation, the DEAD-box protein Dbp5 and mRNA export factor RAE1/Gle1, which are associated with the cytoplasmic filaments of the nuclear pore, are thought to remove the transport receptors from the mRNP in an ATP-dependent manner and prevent the mRNA from returning to the nucleus (67–69) (Fig. 1*B*). This exchange of mRNA-associated proteins during export is commonly referred to as mRNP remodeling, and this likely plays key roles in regulating mRNA export and mRNA translation (70, 71).

Other critical complexes are TREX2 (72–76), whose components bind to the nuclear basket components TPR and Nup153 (77, 78), and are required for efficient nuclear mRNA export (79–81); however, the exact details of how these function remain unclear although they likely play roles in mRNP remodeling.

The interaction of the innate immune response with nuclear pore proteins and nuclear transport receptors

The innate immune system is the first line of host defense and evolutionarily conserved across vertebrates. It utilizes a limited number of pattern-recognition receptors (PRRs) to detect and defend against microbial pathogens. PRRs recognize various conserved molecular structures termed pathogen-associated molecular patterns (PAMPs) (82). As DNA and RNA either carry genetic information or act as replication intermediates for all microbial pathogens, they serve as PAMPs and are the major targets identified by the innate immune system. In general, PRRs that sense nucleic acids are divided into membrane-bound Toll-like receptors (TLRs), cytosolic DNA sensors, and RNA sensors.

Membrane-bound TLRs recognize dsRNA, ssRNA, or unmethylated CpG DNA in the endosomal lumen, initiating signaling axes that culminate in the activation and nuclear translocation of transcription factors including interferon (IFN)-regulatory factor 3 (IRF3), interferon regulatory factor 7, and/or NF-κB (consisting of p65 and p50), to stimulate the transcription of specific genes such as type I interferon (IFN-I), and proinflammatory cytokine genes (83) (Fig. 2).

Nucleic acid–sensing TLRs are expressed and function mostly in human immune cells. In addition, there are more general cytosolic DNA and RNA sensors that are ubiquitously expressed that can activate innate immune responses in response to viral infection.

Cytoplasmic RNAs derived from pathogens are mainly detected by retinoic acid–inducible gene I (RIG-I) or by

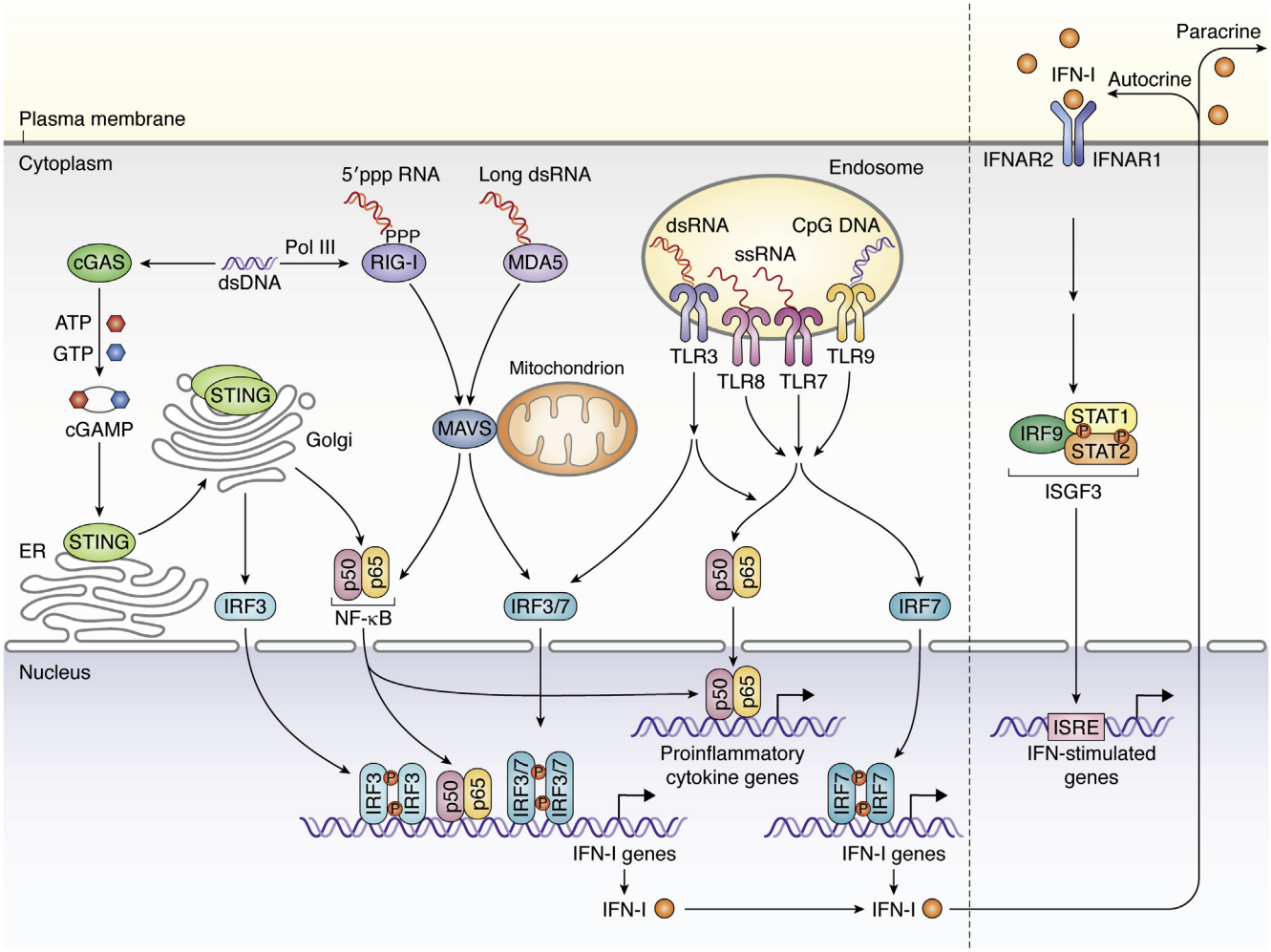

Figure 2. Nucleic acid recognition by distinct pattern-recognition receptors (PRRs) and activation of interferon (IFN)-stimulated genes (ISGs). Upon recognition of pathogen-derived nucleic acids, all the PRRs initiate distinct signaling cascades that culminates in the activation and nuclear translocation of transcription factors, including IFN-regulatory factor 3 (IRF3), IRF7, and/or NF-κB (consisting of p65 and p50), to stimulate the transcription of specific genes, such as type I interferon (IFN-I), and proinflammatory cytokine genes. IFN-I then initiates antiviral signaling in the infected cell and neighboring cells by directly binding to the interferon I receptor (IFNAR) at the cell surfaces, which culminates in the expression of numerous interferon-stimulated genes to repress the replication and assembly of pathogens. cGAMP, cyclic dinucleotide GMP–AMP; cGAS, cyclic GMP-AMP synthase; ER, endoplasmic reticulum; IFN-I, type I interferon; IFNAR, IFN-I receptor; ISGF3, IFN-stimulated gene factor 3; ISRE, interferon-stimulated response element; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation–associated gene 5; RIG-I, retinoic acid–inducible gene I; STAT, signal transducer and activator of transcription; STING, stimulator of IFN genes; TLR, Toll-like receptor.

melanoma differentiation–associated gene 5 (84). This then leads to the stimulation of mitochondrial antiviral signaling protein on the mitochondrial membrane to activate IRF3 or NF-κB (p50/p65) and promote their nuclear translocation to induce the production of IFN-I and other antiviral molecules such as inflammatory cytokines (85) (Fig. 2). In addition, cyclic GMP–AMP synthase (cGAS) recognizes cytosolic dsDNA, either derived from DNA viruses or generated through the reverse transcription of retrovirus RNA genomes, and activates the production of cyclic dinucleotide GMP-AMP (cGAMP) from ATP and GTP (86–89). Being a small molecule, cGAMP not only activates downstream signals in the infected cell but also is packaged into new virions, where it can activate signals in subsequently infected cells (90–93). cGAMP binds to stimulator of IFN genes (STING), causing its relocalization from the ER to the ER–Golgi intermediate compartment and the Golgi complex. There, STING recruits kinases to activate IRF3 and NF-κB (p50/p65), which go on to activate transcription of *IFN-I* and proinflammatory cytokine genes (94–97) (Fig. 2).

Once induced, newly synthesized IFN-I then functions *via* autocrine and paracrine signaling by directly binding to the interferon I receptor at the cell surface to initiate signaling, which in turn leads to the phosphorylation of signal transducer and activator of transcriptions (STATs) and the formation of STAT1/STAT2 heterodimer. The heterodimer further recruits interferon-regulatory factor 9 (IRF9) to form the interferon-stimulated gene factor 3 (ISGF3) complex. The ISGF3 complex then rapidly translocates into the nucleus in an importin-dependent manner and binds to IFN-stimulated response elements of IFN-stimulated genes to activate their transcription (98) (Fig. 2). Consequently, this stimulates the production of proteins that establish a robust immune response to repress the replication and assembly of pathogens (99, 100).

The innate immune system has been extensively reviewed elsewhere (101–103), so in the next section, we will discuss how these systems interface with the nuclear pore complex. As described previously, during the innate antiviral immune response, the production of IFNs, proinflammatory cytokines, and IFN-stimulated genes depends on the nuclear translocation of key innate immunity signal transducers. Unsurprisingly, signal transducers, such as IRF3, NF-κB (p50/p65), and STATs, interact with distinct Nups and/or nuclear transport receptors in order to traffic from the cytoplasm to the nucleus through nuclear pore complexes in response to the activation of PRRs (Table 1).

IRF3

IRF3 is a key transcription factor employed by various innate immunity pathways including RIG-I-like receptors, cGAS/STING signaling, and TLR3 signaling. It turns on the transcription of *IFN-I* genes in response to the activation of PRRs. To date, three nuclear transport receptors, importin-β1, importin-α3, and importin-α4, are known to promote IRF3 nuclear transport (104, 105) (Table 1).

NF-κB

NF-κB (p50/p65) is another crucial downstream signal transducer that transcriptionally activates IFN-I and proinflammatory cytokines upon PPR activation. It is imported into the nucleus by importin-β1, importin-α3, and importin-α4 (104–106) and requires the Nups Nup88, Nup214, Nup98, Nup153, RanBP2/Nup358, and POM121. The close link between nuclear porins and NF-κB appears to be ancient as it is conserved in *Drosophila* (107–111). Interestingly, several chromosome translocation mutations result in the formation of fusion proteins involving Nups and a diverse set of proteins, which often impact the nucleocytoplasmic transport of NF-κB (p50/p65) and the activation of innate immune responses (Table 1). NF-κB activity can be inhibited by CRM1, which mediates p65 nuclear export (112). Recently, it has been suggested that overexpression of Nup62 stabilizes overexpressed Nup88 and its interaction with NF-κB (p65) to induce inflammatory signaling (113). Another recent study revealed that POM121 inhibits the nuclear translocation of phosphorylated p65 (phos-p65) and consequently impairs the macrophage inflammatory response (114). Another report suggested that RanBP2/Nup358, which is one of the main components of the cytoplasmic filaments on the nuclear pore complex, and Nup153, which is part of the nuclear pore basket, formed a complex (RanBP2/Nup358–RanGDP-Nup153–IκBα-SUMO) in response to tumor necrosis factor α (TNF-α) stimulation. This complex facilitates the nuclear import of IκBα, allowing its binding to NF-κB (p50/p65). IκBα binding, in turn, masks the nuclear localization signal and the DNA-binding domain of NF-κB (p50/p65), thus downregulating innate immune responses (115).

STATs

The nucleocytoplasmic trafficking of STATs (such as STAT1 and STAT2) plays a central role in activating IFN-stimulated gene expression to repress viral replication and

Table 1
The interaction of Nups or nuclear transport receptors with signal transducers of innate immunity

Signal transducers	Nups/nuclear transport receptors	Roles of nuclear pore–associated proteins in innate immune responses	References
IRF3	Importin-β1	The main nuclear import receptor for IRF3	(104)
	Importin-α3 and importin-α4	Promoting nuclear import of IRF3	(105)
NF-κB	Importin-β1	The main nuclear import receptor for NF-κB (p65)	(104)
	Importin-α3 and importin-α4	Promoting nuclear import of NF-κB	(105, 106)
	Nup214–Nup88 complex	Promoting the translocation of NF-κB (p65) from the cytoplasm to the nucleus	(107, 109)
	Nup62	Stabilizing Nup88 and its interaction with NF-κB (p65) to induce inflammatory responses	(113)
	SET-Nup214 and DEK-Nup214	Inhibiting NF-κB activation by tethering the complex, including p65 and its inhibitor IκB, in the nucleus	(108)
	Nup98-HOXA9 and Nup98-DDX10	Causing nuclear accumulation of NF-κB (p65), thus promoting NF-κB–mediated transcription	(110)
	Nup98-IQCG	Inhibiting the CRM1-mediated nuclear export of p65, thus enhancing the transcriptional activity of NF-κB	(111)
	POM121	Inhibiting phosphorylated p65 (phos-p65) nuclear translocation, thus the macrophage inflammatory response	(114)
	Nup153 and RanBP2/Nup358	Promoting IκBα nuclear import and subsequently terminating NF-κB activation	(115)
STATs (including STAT1 and STAT2)	Importin-α3	Promoting the nuclear import of unphosphorylated STAT2/IRF9 complex	(130)
	Importin-α4	Promoting the nuclear import of unphosphorylated STAT2/IRF9 complex	(130)
	Importin-α5	Promoting the nuclear import of activated STAT1 and STAT2	(124, 125)
	Importin-α6	Promoting the nuclear import of activated STAT1 and STAT2	(125)
	Importin-α7	Promoting the nuclear import of activated STAT1 and unphosphorylated STAT2/IRF9 complex	(125, 130)
	Nup153 and Nup214	Promoting the nucleocytoplasmic translocation of latent STAT1	(116, 117)
	CRM1	Promoting the nuclear export of the unphosphorylated STAT1 and STAT2	(130)

assembly. In general, this process is accomplished *via* two distinct pathways. In the first, tyrosine-phosphorylated STAT dimers utilize importins to enter the nucleus upon cytokine stimulation. In the second pathway, latent unphosphorylated STATs employ karyopherin-independent and energy-free translocation mechanisms by directly interacting with FG-Nups without cytokine stimulation (116–118). To date, several transport receptors and Nups have been shown to contribute to the nucleocytoplasmic transport of phosphorylated or unphosphorylated STATs (Table 1).

Upon IFN-I stimulation, both STAT1 and STAT2 are phosphorylated by Janus kinases and subsequently form a STAT1/STAT2 heterodimer. During heterodimer formation, STAT1 undergoes a conformational change that exposes a dimer-specific nuclear localization signal within its DNA-binding domain (119, 120). Unlike conventional NLSs, which binds to importin-α (121–123), this dimer-specific nuclear localization signal interacts with importin-α5 (124), importin-α6, and importin-α7 (125), which then facilitates the nuclear translocation of the STAT1/STAT2/IRF9 complex (also known as the ISGF3 complex). In the nucleus, the ISGF3 complex binds to the IFN-stimulated response element promoter site to activate the transcription of various IFN-stimulated genes. In addition, the binding of STAT1 to target DNA releases importin-α5 back to the cytoplasm for recycling (120, 126).

One important concept that has emerged from the literature is that upon cytokine stimulation, the amount of nuclear accumulated STATs is often influenced by their nuclear retention rather than by the rate of their nuclear import (117). It was observed that phosphorylated STAT1 can reside in the nucleus for around 30 min, and that the duration of its nuclear accumulation is affected by several phosphatases, such as 45-kDa T cell protein tyrosine phosphatase splice variant and SH2 domain–containing protein tyrosine phosphatase 2 (127–129). Once dephosphorylated, STAT1 interacts with CRM1 *via* an exposed leucine-rich NES in its DNA-binding domain and in turn is exported back to the cytoplasm for subsequent activation–inactivation cycles (130).

It was assumed that latent unphosphorylated STATs are trapped in the cytoplasm and do not shuttle in and out of the nucleus in resting cells. However, this idea has been challenged by several studies (116, 117, 131). It should be noted that only a third of all the STAT1 protein is tyrosine phosphorylated at any given time during cytokine stimulation (127). Although the conventional STAT1 nuclear localization signal requires phosphorylation to be active, unphosphorylated STAT1 still shuttles between cytosol and nucleus by directly binding to FG-Nups like Nup153 and Nup214 in a cytokine-independent manner (116, 117).

STAT2 is another critical transcription factor in the IFN-I signaling pathway. Unlike other STATs, STAT2 is constitutively bound by the transcriptional activator IRF9. Upon IFN-I stimulation, phosphorylated STAT2 joins the ISGF3 complex and is transported into the nucleus as described previously. Thus, STAT1 is essential for the nuclear translocation of activated STAT2 (130). Like STAT1, the recycling of STAT2 to the cytoplasm is catalyzed by its dephosphorylation by 45-kDa T cell protein tyrosine phosphatase splice variant and SH2 domain–containing protein tyrosine phosphatase 2. This causes the dissociation of STAT2/IRF9 from both STAT1 and the DNA. STAT2, which has its own nuclear export sequence, is then ferried out of the nucleus by CRM1. Like STAT1, STAT2 is believed to translocate into the nucleus even when it is unphosphorylated. However, unlike other STAT family members, this is not mediated by STAT2 directly, but rather its binding partner IRF9 (132), suggesting that the nuclear translocation of the unphosphorylated STAT2 differs from that of unphosphorylated STAT1. Indeed, the import of STAT2 is dependent on IRF9 interactors, which include importin-α3, importin-α4, and importin-α7 (130).

The interaction of viruses with nuclear pore proteins and nuclear transport receptors

As discussed previously, the nucleocytoplasmic shuttling of signal transducers is regulated by distinct nuclear pore proteins and nuclear transport receptors. This regulation is critical for the host innate immune response upon viral infection. Therefore, it is not surprising that different viruses subvert the nucleocytoplasmic transport machinery to evade antiviral innate immunity. In the next sections, we discuss different viral proteins that interact and interfere with nuclear pore complexes and nuclear transport receptors, organized by viral families (summarized in Table 2).

Herpesviruses

Members of herpesviruses belong to Herpesviridae, which is a large family of dsDNA viruses that cause diseases in a wide range of hosts, including humans. There are nine herpesvirus types known to infect humans, including herpes simplex viruses (HSVs) type 1 and 2 (HSV-1 and HSV-2 or human herpesvirus 1 [HHV-1] and HHV-2), varicella-zoster virus (or HHV-3), Epstein–Barr virus (EBV or HHV-34), human cytomegalovirus (or HHV-5), HHV-6A and HHV-6B, HHV-7, and Kaposi's sarcoma–associated herpesvirus (KSHV or HHV-8). Most humans are infected with HSV-1, a member of alphaherpesvirus subfamily, which causes clinical symptoms, such as cold sores, in roughly one-third of all humans.

It has been observed that certain proteins encoded by HSV-1 interact with distinct nuclear pore proteins and nuclear transport receptors. The capsid (CA)-tethered tegument protein pUL36, and the minor CA protein pUL25, binds to RanBP2/Nup358 and Nup214, respectively, to dock the viral capsids at the cytoplasmic face of the nuclear pore, which subsequently facilitates the uncoating and release of the viral genome into the nucleus (133–136). pUL25 may have other functions, as viruses bearing a mutant form of this protein did not efficiently trigger cGAS signaling in infected cells. This mutation did not affect the attachment of CA to the nuclear pores but instead significantly delayed the expression of viral proteins (137). It has also been reported that HSV-1 infection inhibits Nup153 expression (138) and alters its subcellular localization, sending this nuclear basket protein to the

Table 2
Viral subversion of nuclear pore proteins/nuclear transport receptors to interfere with host antiviral innate immune responses

Virus group (Baltimore classification)	Virus family	Virus	Nups and nuclear transport receptors employed by the virus (References)	Proposed interaction between virus and nuclear pore–associated proteins that interferes with host antiviral innate immune responses
I (dsDNA viruses)	*Herpesviridae*	HSV-1	RanBP2/Nup358 and Nup214 (133–136)); Nup62 (141); Nup153 (138–140); TAP/NXF1 (142–144)	Unknown
		EBV	Nup62 and Nup153 (150, 151)	Unknown
	Adenoviridae	Adenoviruses	Nup214, RanBP2/Nup358, and Nup62 (155); CRM1 (156); TAP/NXF1 (157–159)	Unknown
	Poxviridae	VACV	Importin-α1 (164)	Blocking the nuclear translocation of NF-κB
	Papillomaviridae	HPV	Importin-α1, importin-β2, and importin-β3 (167, 168)	Unknown
IV ((+) ssRNA viruses)	*Coronaviridae*	SARS-CoV	Importin-α1 and importin-β1 (176, 177)	Blocking the nuclear translocation of STAT1
		MERS	Importin-α3 (178)	Blocking the nuclear translocation of NF-κB-p65 subunit
		SARS-CoV-2	Nup37, Nup54, Nup58, Nup62, Nup88, Nup93, Nup160, Nup188, Nup210, Nup214, Nup98-RAE1, NUTF2, IPO5, IPO8, RanBP6, importin-β1, CRM1, XPOT, THOC3, RanBP2/Nup358 (112, 179–184)	Blocking the nuclear export of host antiviral mRNAs and nuclear translocation of STAT1
	Picornaviridae	HRV	Nup62, Nup98, Nup153, Nup214, and RanBP2/Nup358 (194–200)	Unknown
		PV	Nup62, Nup98, and Nup153 (194–198)	Unknown
		EMCV	Ran-GTPase, Nup62, Nup153, and Nup214 (203, 204)	Unknown
		TMEV	Ran-GTPase, Nup98 (205)	Unknown
	Flaviviridae	Dengue virus	Importin-α and importin-β (206); Nup153, Nup98, and Nup62 (207)	Unknown
		ZIKA	Nup98, Nup153, and TPR (207); importin-α7 (208)	Unknown
		HCV	Importin-β1 (104, 209)	Blocking the nuclear translocation of STAT1, IRF3, and NF-κB-p65
		JEV	Importin-α3 and importin-α4 (105)	Blocking the nuclear translocation of NF-κB and IRF3
V ((−) ssRNA viruses)	*Filoviridae*	EBOV	Importin-α5, importin-α6, and importin-α7 (125, 221–223)	Blocking the nuclear import of PY-STAT1
	Orthomyxoviridae	IAV	Importin-α/β (225), CRM1 (226, 227), NXF1/NXT1, RAE1, and Nup98 (235–237)	Blocking the nuclear export of host antiviral mRNAs
	Rhabdoviridae	VSV	RAE1 and Nup98 (250, 252, 253)	Unknown
	Bunyaviridae	HTNV	Importin-α1, importin-α2, and importin-α3 (256)	Blocking the nuclear translocation of NF-κB
VI (ssRNA-RT viruses)	*Retroviridae*	HIV-1	Importin-α1, importin-α3, importin-α5 (262–265, 267, 282), importin-7 (266), transportin-3 (268), CRM1 (269), Nup62, Nup153, Nup98, Nup214, RanBP2/Nup358, and hCG1 (223, 270–278, 281)	Blocking the nuclear translocation of NF-κB and IRF3
VII (dsDNA-RT viruses)	*Hepadnaviridae*	HBV	Importin-α/β (284, 285), importin-α5 (289), and Nup153 (286)	Blocking the nuclear translocation of STAT1/2

cytoplasm (139). This observation suggests that HSV-1 interferes with nuclear import by repressing Nup153 (140). ICP27 is another HSV-1 protein that interacts directly with Nup62 and blocks host protein import *via* importin-α, importin-β1, and importin-β2 nuclear import pathways (141). In addition, ICP27 interacts with both the RNA export receptor TAP/NXF1 and the TREX complex adaptor protein Aly/REF, to preferentially export HSV-1 RNA over endogenous mRNAs (142–144). Moreover, ICP27-like proteins encoded by other related herpesviruses, including human cytomegalovirus, KSHV, EBV, varicella-zoster virus, also facilitate nuclear export of viral mRNAs (145–149).

The gamma-herpesvirus EBV also modulates nucleocytoplasmic transport. One of its proteins, BGLF4, is a serine–threonine protein kinase that directly binds and phosphorylates Nup62 and Nup153 to modulate nuclear pore complex organization (150, 151). Consequently, BGLF4 blocks general nuclear import by impairing importin-β1 nuclear targeting, while simultaneously facilitating the nuclear import of certain EBV lytic proteins (151).

Even though herpesviruses contain proteins that interact with nuclear pore complexes and nuclear transport receptors (as described previously), whether these interactions engage in the regulation of host innate immunity is unknown.

Adenoviruses

The family Adenoviridae is a large group of nonenveloped dsDNA viruses that infect a broad range of vertebrate hosts, including humans, and cause diseases, such as respiratory, gastrointestinal, urogenital, and ocular diseases. The entry of adenoviruses (AdVs) into the cell starts with the association of viral fiber proteins with host cell receptors (known as coxsackievirus AdV receptors) (152), followed by receptor-mediated endocytosis and virion escape into the cytoplasm (153). Subsequently, these virions travel along microtubules through their interaction with the molecular motor dynein (154). In this way, virions make their way to the nuclear envelope where they dock onto the cytoplasmic filaments of the nuclear pore by interacting with Nup214. Then the viral CA is disassembled in a kinesin-I–dependent manner (155),

releasing the viral genome into the nucleus. In addition, studies have indicated that AdVs displace cytoplasmic nuclear pore filament proteins (RanBP2/Nup358, Nup214, and Nup62) into the cytoplasm to increase nuclear envelope permeability and thereby facilitate the nuclear import of viral DNA (155). In the nucleus, viral DNA is transcribed into adenoviral early mRNA, which is exported to the cytosol in a CRM1-dependent manner (156), and adenoviral late transcripts, which are exported by the export receptor TAP/NXF1 (157). Two adenoviral proteins, E1B-55K and E4orf6, not only inhibit NXF1-mediated host mRNAs export but also promote adenoviral late mRNA export by binding to the host protein E1B-AP5 (also known as hnRNPUL1), which interacts with NXF1 (158, 159).

The stepwise process of docking AdV CAs at the nuclear pore and uncoating of the virion to release viral genome into the nucleus may allow AdV to evade host antiviral innate immune responses by restricting viral DNA exposure in the cytoplasm. In addition, the disruption of host nucleocytoplasmic trafficking system may help interfere with the nuclear import of crucial factors involved in innate immunity (160–162). Further studies are needed to determine whether the interaction between AdVs and host nucleocytoplasmic transport system contributes to viral evasion of the host innate immune response.

Poxviruses

The family Poxviridae is a large group of dsDNA viruses that replicate in the cytoplasm and infect humans, vertebrates, and arthropods. Poxviruses are currently divided into 22 genera and 83 species. Among them, variola virus and vaccinia virus (VACV) are commonly known. Variola virus causes an acute contagious disease called smallpox and was responsible for a large number of deaths throughout human history. VACV, a lab-grown strain of poxvirus, was used as a live vaccine that helped to eradicate smallpox. VACV can stimulate a strong immune response and encodes a number of proteins that inhibit NF-κB activation to evade the host immune response (163). Recently, it was shown that VACV protein A55 competes with NF-κB for importin-α1 binding, thereby preventing the nuclear import of NF-κB and inhibiting downstream gene transcription (164) (Fig. 4).

Papillomaviruses

Papillomaviruses (Papillomaviridae family) are a large family of small, nonenveloped, and icosahedral DNA viruses with a single molecule of 8 kb double-stranded circular DNA (165). Human papillomavirus (HPV) includes low-risk HPVs, such as HPV-6 and HPV-11, causing benign exophytic condylomas, and high-risk HPVs, such as HPV-16, HPV-18, HPV-31, and HPV-45, which are associated with anogenital cancers, oropharyngeal cancers, and skin cancers (166).

To date, the L1 major and L2 minor CA proteins of HPV-11 and HPV-16 have been revealed to interact with nuclear transport receptors. The HPV-11 L1 protein binds to importin-α1 and enters into the nucleus through the classical importin-α1/β1-mediated import system (167). In addition, L1 from both HPV-11 and HPV-16 binds to importin-β2 and importin-β3, inhibiting their nuclear import activities (167). The L2 minor CA protein of HPV-16 is transported into the nucleus by interacting with either importin-β2, importin-β3, or the importin-α1/importin-β1 heterodimer (168). Although HPV-11 and HPV-16 associate with host nuclear import pathways, whether these interactions subvert host antiviral immune systems remain unknown.

Coronaviruses

Coronaviruses (CoVs) are enveloped, single-stranded, positive-sense RNA viruses under the family of Coronaviridae. CoV genomes are approximately 30 kb with the first two-thirds of the genome encoding two large polyproteins important for replicase function and the last third of the genome encoding multiple structural and accessory proteins. CoVs are divided into four genera named *alpha-*, *beta-*, *gamma-*, and *delta-coronavirus* (169) and can infect a wide range of hosts, such as bats, birds, mice, dogs, as well as humans (170, 171). Among these, three novel beta-CoVs, including severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, and SARS-CoV2, are highly pathogenic in humans, causing worldwide outbreaks and pneumonia in the past 2 decades (172–174). According to the coronavirus disease 2019 (COVID-19) map from Johns Hopkins University, SARS-CoV2 has infected over 160 million individuals globally and has caused 3.4 million deaths by mid-May 2021. In addition, four CoVs are responsible for up to 20% of all severe cases of the common cold (175).

It is well known that human CoVs have evolved mechanisms to evade the host antiviral innate immunity. Certain proteins encoded by the three highly pathogenic human CoVs can antagonize host innate immune response by interacting with distinct nuclear pore proteins or nuclear transport receptors, which disrupt the nuclear transport of critical factors in innate immune signaling pathways. More specifically, the viral ORF6 protein of SARS-CoV inhibits IFN signaling by interacting with importin-α1 and importin-β1, tethering them to the rough endoplasmic reticulum/Golgi membrane, thus blocking the nuclear translocation of STAT1 without affecting its phosphorylation (176, 177) (Fig. 3). Likewise, during MERS-CoV infection, the accessory protein 4b of MERS-CoV disrupts NF-κB nuclear translocation by outcompeting it for importin-α4 binding (178) (Fig. 4).

An interaction map of SARS-CoV2 proteins revealed that they associate with human proteins involved in several biological processes, including the innate immune response (179). In particular, the nonstructural proteins (NSPs) 13 and 15, and ORF9b, bind to components of the IFN pathway and the NF-κB pathway. Other factors bind to the nucleocytoplasmic transport machinery, including NSP4 (which interacts with GP210), NSP9 (which associates with Nup54, Nup58, Nup62, Nup88, and Nup214), NSP15 (which binds to NTF2), and ORF6 (which interacts with the Nup98–RAE1 complex) (179). Indeed, this last interaction has been confirmed by several

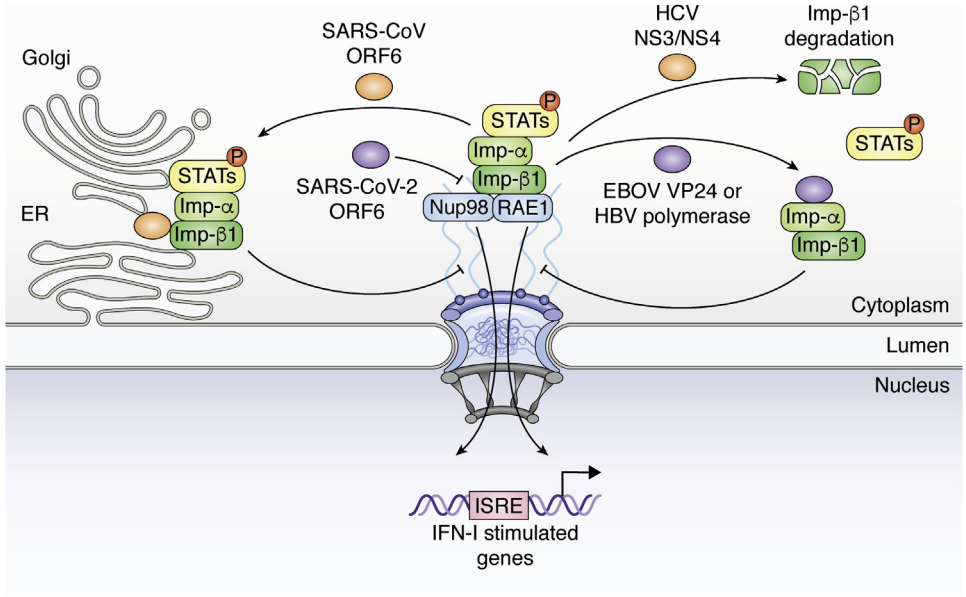

Figure 3. Viruses interfere with host IFN-I/JAK/STAT signaling by subverting nuclear transport of activated STAT1. The SARS-CoV protein ORF6 inhibits IFN signaling by interacting with importin-α1 and importin-β1, tethering them to the endoplasmic reticulum–Golgi membrane, thus blocking the nuclear translocation of STAT1 without affecting its phosphorylation. SARS-CoV2 ORF6 also blocks STAT1 nuclear translocation by interacting with the Nup98–RAE1 complex and disrupts the interaction between Nup98 and importin-β1/importin-α1/PY-STAT1 complex, thus preventing the docking of this complex at the nuclear pore. During HCV infection, viral NS3/4A complex binds to and cleaves importin-β1, which in turn blocks the nuclear translocation of STAT1 to restrict host antiviral immune response. VP24 of EBOV interacts with importin-α5, importin-α6, and importin-α7 at the regions where PY-STAT1 binds, and this prevents the formation of importin-α/PY-STAT1 transport complex, thereby blocking the nuclear import of activated STAT1 to counteract IFN signaling. In addition, HBV polymerase prevents the nuclear localization of STAT1/2 and the expression of interferon-stimulated genes by inhibiting importin-α5. EBOV, Ebola virus; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; HBV, hepatitis B virus; HCV, hepatitis C virus; PY-STAT1, tyrosine-phosphorylated STAT1; SARS CoV, severe acute respiratory syndrome coronavirus; STAT, signal transducer and activator of transcription; VP24, virus protein 24.

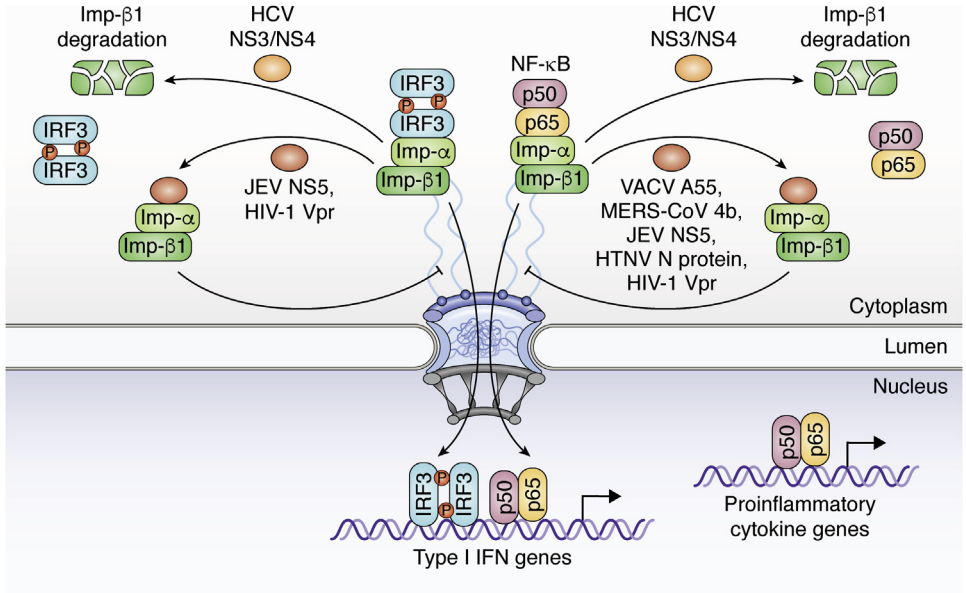

Figure 4. Viruses interfere with host IRF3 or NF-κB signaling by subverting their nuclear transport. During VACV infection, protein A55 prevents NF-κB nuclear translocation and inhibits NF-κB–dependent gene transcription by interacting with importin-α1 and disturbing the interaction between NF-κB and importin-α1. The accessory protein 4b of MERS-CoV disrupts NF-κB nuclear translocation by outcompeting it for importin-α4 binding and consequently interferes with NF-κB–dependent innate immune response. A complex of NS3/4A from HCV binds to and cleaves importin-β1, which in turn blocks the nuclear translocation of IRF3 and NF-κB-p65 to restrict host antiviral immune response. JEV NS5 protein competes with NF-κB-p65 and IRF3 for importin-α3 and importin-α4 binding and thus suppressing host innate immunity. HTNV nucleocapsid (N) protein interacts with importin-α1, importin-α2, and importin-α3, to block NF-κB nuclear translocation and inhibit NF-κB activity. In addition, HIV-1 viral protein R interacts with importin-α5 to prevent the nuclear translocation of IRF3 and NF-κB after immune activation. HCV, hepatitis C virus; HTNV, Hantaan virus; IRF3, IFN regulatory factor 3; JEV, Japanese encephalitis virus; MERS-CoV, Middle East respiratory syndrome coronavirus; VACV, vaccinia virus; Vpr, viral protein R.

other groups (180–183) and was shown to disrupt bidirectional nucleocytoplasmic transport, likely inhibiting host response to viral infection (183). This interaction also blocks nuclear translocation of STAT1 to antagonize IFN signaling (181). Since RAE1 plays a role in mRNA export, ORF6 also alters the mRNA export and expression of innate immunity proteins. Aside from Nup98–RAE1 complex, ORF6 also interacts with many other key members of the nuclear pore machinery, including Nups, such as RanBP2/Nup358, Nup160, Nup188, Nup210, Nup37, and Nup93, importins, such as importin-5 (also known as importin-β3), importin-8, RanBP6, and importin-β1, exportins, such as CRM1 and exportin-T (also known as XPO3), as well as spliceosome components, such as THO complex subunit 3, a member of TREX complex known for splicing-coupled mRNA export (182).

In addition, it was observed that SARS-CoV2 infection resulted in a significant reduction in RanBP2/Nup358 protein level, which was assumed to repress NF-κB activation as discussed previously (115, 184). It is also known that SARS-CoV2 infection leads to the development of "cytokine storms" in most patients with severe COVID-19, in which immune cells and nonimmune cells secrete excessive amounts of cytokines, such as interleukin-6 (IL-6), TNF-α, and cause serious damage to hosts (185–188). In general, the hyperactivation of the NF-κB pathway is one of the major mechanisms leading to the phenotype of cytokine storms. SARS-CoV2 infection was shown to stimulate the IL-6 amplifier by activating both NF-κB and STAT3, which subsequently lead to the hyperactivation of NF-κB by STAT3, and the induction of multiple inflammatory and autoimmune diseases (189, 190). Interestingly, mutations in RanBP2/Nup358 are also known to cause cytokine storms in response to influenza infection, and this is likely because of alterations in its ability to sumolyate proteins (see Orthomyxovirus section later). Indeed, sumoylation of NF-κB or STAT1 has been shown to inhibit their activation (191–193). Although it remains unclear whether RanBP2/Nup358 engages in the sumoylation of NF-κB or STATs to repress antiviral innate immune responses, the aforementioned information suggests that RanBP2/Nup358 may play a critical role in the induction of cytokine storms by SARS-CoV2 infection. Further studies focusing on the crosstalk between RanBP2/Nup358, innate immunity, and SARS-CoV2 may help us develop novel drug targets and antiviral approaches against SARS-CoV2.

Picornaviruses

The Picornaviradae family is a group of nonenveloped, single-stranded, positive-sense RNA viruses that infect vertebrate hosts, including mammals and birds, and cause diseases, including poliomyelitis, paralysis, meningitis, and hepatitis. Picornaviruses are currently divided into 47 genera including the notable genera *Enterovirus* and *Cardiovirus*. It has been found that infection of human rhinovirus (HRV) and poliovirus (PV) in the *Enterovirus* genus and encephalomyocarditis virus (EMCV) and Theiler's murine encephalomyelitis virus (TMEV) in the *Cardiovirus* genus results in alterations in the nuclear pore complex composition and nucleocytoplasmic transport pathways (7, 160). The proteases $3C^{pro}$ and $2A^{pro}$ encoded by HRV and PV, and leader (L) protein encoded by EMCV and TMEV, which processes a single viral polyprotein into several proteins, are found to disrupt nucleocytoplasmic trafficking. More specifically, $2A^{pro}$ from PV mediates the proteolytic degradation of Nup62, Nup98, and Nup153, whereas HRV $2A^{pro}$ cleaves Nup98 and Nup62 (194–198). In addition, $3C^{pro}$ and its precursor 3CD from HRV were shown to target Nup62, Nup153, Nup214, and Nup358 for proteolysis (199, 200). The degradation of Nups mediated by protease $3C^{pro}$ and $2A^{pro}$ results in an increase in the bidirectional permeability of nuclear envelope (201, 202) and the disruption of nuclear import pathways mediated by importin-α/importin-β1 and importin-β2/transportin-1 (195, 197). However, the mechanisms employed by cardioviruses like EMCV and TMEV, which lack a 2A-like protease, are quite different. The L protein from EMCV binds tightly to, and interferes with, the activity of Ran-GTPase and promotes the hyperphosphorylation of Nup62, Nup153, and Nup214. This results in the cytoplasmic accumulation of nuclear proteins by increasing nuclear envelope permeability and altering protein export (203–205). In addition, TMEV L protein prevents the nuclear export of most mRNAs, likely by promoting Nup98 phosphorylation (203–205). Overall, the modulation of nucleocytoplasmic trafficking by picornaviruses may contribute to its subversion of host antiviral innate immune responses.

Flaviviruses

Members of the Flaviviridae family are divided into four genera, including genus *Flavivirus*, *Hepacivirus*, *Pegivirus*, and *Pestivirus*. They are mainly transmitted through arthropod vectors, such as ticks and mosquitoes. Several important human pathogens in this virus family cause health challenges worldwide, and examples include the yellow fever virus, West Nile virus, dengue virus (DENV), Zika virus (ZIKV), Japanese encephalitis virus (JEV), hepatitis C virus (HCV), and tick-borne encephalitis virus. The genome of each flavivirus member consists of a monopartite, linear, single-stranded, and positive polarity RNA molecule, which encodes a single polyprotein cleaved by both host and viral proteases into three structural (C, E, and prM) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins.

Several NSPs, such as NS2B, NS3, NS3/4A, NS5, have been found to associate with the nuclear pore complex and/or nuclear transport receptors to modulate nucleocytoplasmic transport pathways. During DENV infection, NS5 is transported into the nucleus by interacting with importin-α/β or by directly binding to importin-β. However, NS3 was shown to compete with NS5 for binding to importin-α/β, and this may reduce nuclear import of the latter, which may play a role in viral RNA processing in the cytoplasm (206). In addition, the DENV NS3 has protease activity and has been shown to degrade Nup153, Nup98, and Nup62 (207). Likewise, ZIKV NS3 mediates the degradation of Nup98, Nup153, and TPR

(207). Moreover, ZIKV infection inhibits the ubiquitin-proteasomal degradation of importin-α7, thereby increasing its level, which in turn promotes viral replication (208). In addition, it has been shown that a complex composed of NS3 and its cofactor NS4A from HCV, termed NS3/4A, binds to and cleaves importin-β1, which in turn blocks the nuclear translocation of STAT1, IRF3, and NF-κB-p65 and restricts host antiviral immune response (104, 209) (Figs. 3 and 4). In JEV, the NS5 protein was shown to compete with NF-κB-p65 and IRF3 for importin-α3 and importin-α4 binding and thus suppressing host innate immunity (105) (Fig. 4).

Another way in which flaviviruses, especially HCV, evade the immune system is to replicate in membrane-delimited vesicles in the cytoplasm, termed viral factories (210). These structures prevent the detection of viral genomes by various innate immune pathways. Viral factories resemble mininuclei in that their delimiting envelope is derived from the ER, and any transport between the factory and cytosol must pass through nuclear pore–like structures that likely contain several Nups. In those formed by HCV, these pore-like structures contain Nup153, RanBP2/Nup358, Nup155, Nup98, and Nup53 (211, 212). Similar viral factories are thought to be important for the replication of VACV (213) where the putative pores contain Nup62 and RanBP2/Nup358 (214, 215). Other viruses that use viral factories that resemble mininuclei with nuclear pore–like structures include various flaviviruses, such as ZIKV, DENV, and West Nile virus (216–218).

In summary, flaviviruses employ several distinct mechanisms to disrupt the nuclear transport of important cellular factors involved in immunity and consequently dampen host antiviral response.

Filoviruses

The Filoviridae family is a group of single-stranded negative-sense RNA viruses, which is currently divided into four genera including genus *Cuevavirus*, *Dianlovirus*, *Ebolavirus*, and *Marburgvirus*. Among them, Ebola virus (EBOV) and Marburg virus are two commonly known filoviruses, which cause periodic outbreaks of severe hemorrhagic fever in humans, with high fatality rates of up to 90% (219, 220). During EBOV infection, the viral protein VP24 interacts with importin-α5, importin-α6, and importin-α7 at the regions where tyrosine-phosphorylated STAT1 (PY-STAT1) usually binds. This prevents the importin-α/PY-STAT1 transport complex formation and thus blocks the nuclear import of activated STAT1 to counteract IFN-α/β and IFN-γ signaling (125, 221–223) (Fig. 3). Thus, like other viruses discussed previously, EBOV can evade host antiviral innate immunity by interfering with nuclear transport pathways.

Orthomyxoviruses

The Orthomyxoviridae family includes influenza viruses that are divided into four types (A, B, C, and D), where influenza A viruses (IAVs) cause seasonal epidemics, commonly known as the flu (224). Upon infection, the viral particles are imported into the nucleus of host cells *via* the importin-α/β system for transcription and replication (225). The influenza viruses make use of CRM1 for the export of progeny viral particles (226). It is believed that influenza viruses outcompete host CRM1 substrates by localizing viral particle export complexes in close proximity to regulator of chromosome condensation 1. Thus, once RanGTP-bound CRM1 is generated, the viral particles gain preferential access to CRM1 before it diffuses to bind host cellular substrates, and as a result, the host CRM1-mediated export is impaired (227).

During influenza infection, the viral NSP NS1 protein is a major player for the viruses to evade innate immune responses. In the cytoplasm, NS1 inhibits the activation of RIG-I receptor signaling and prevents induction of IFN-β through binding to RIG-1 and the E3-ubiquitin ligase TRIM25 (228–231). In the nucleus, NS1 inhibits splicing and 3′-end processing of host mRNAs through its interaction with the U6 snRNP (232), the cleavage and polyadenylation specificity factor CPSF30 (233), and the poly(A)-binding protein PABP1 (234). NS1 further alters host gene expression by forming an inhibitory complex with the host mRNA export machinery NXF1/NXT1, Rae-1, and E1B-AP5, thus blocking the export of host mRNAs encoding for antiviral factors such as *RIG-1*, or mRNAs regulated by IFN such as *IFIT2* and *IFIT3* (235, 236). Besides sequestering host mRNA export machinery, influenza viruses also downregulate Nup98, which provides a docking site for the mRNA export factors at the nuclear pore, and this further inhibits the export of host antiviral mRNAs (235, 237).

Interestingly, dominant mutations in RanBP2/Nup358 are associated with acute necrotizing encephalopathy 1 (ANE1), where patients suffer from elevated levels of cytokine production after viral infection, oftentimes by influenza viruses (238, 239). These cytokine storms are typically restricted to the brain, unlike in COVID-19, where they are localized to the lung. How mutations in RanBP2/Nup358 contribute to disease remains unclear. Our recently published work suggests that RanBP2/Nup358 represses the translation of two ANE1-associated cytokine mRNAs, *IL-6* and *TNF-α*, by sumoylating argonaute proteins thereby enforcing microRNA-mediated silencing (240). Our data indicate that argonautes initially interact with the *IL-6* mRNA in the nucleus, and that after nuclear export, RanBP2-dependent sumoylation stabilizes argonaute binding to the mRNA as part of an mRNP remodeling event. It is thus possible that ANE1-associated mutations alter this remodeling event, which eventually leads to pathology, though further studies are needed. As a number of case studies have indicated that COVID-19 can sometimes result in ANE1-like cytokine storms in the brain (241–245), it would be important to determine whether these patients have mutations in RanBP2/Nup358.

Rhabdoviruses

The vesicular stomatitis virus (VSV), a member of the Rhabdoviridae family, causes acute vesicular disease in rodents, cattle, swine, horses, and sometimes humans (246). It is an enveloped, single-stranded, and negative-sense RNA virus that replicates in the cytoplasm (246). VSV suppresses host

antiviral responses by the viral matrix (M) protein, which has been found to block host gene expression at the levels of transcription (247–249), mRNA export (250–253), and translation (254, 255). Since M protein lacks enzymatic activity, it is believed that it inhibits host gene expression by interacting with host factors and interfering with their functions. It has been shown that during VSV infection, M protein interacts with host proteins RAE1 and Nup98. While some studies showed that the interaction among M, RAE1, and Nup98 prevents bulk poly(A) mRNA export (250, 253), another study found that this complex also binds to chromatin and partially mediates the ability of VSV to inhibit host transcription, without affecting host mRNA export or host translation in VSV-infected cells (252). Though it is still unclear how exactly M protein inhibits host gene expression, it appears to suppress this process at multiple levels, and this helps VSV evade the immune system.

Bunyaviruses

Bunyaviruses (Bunyaviridae family) are a large family of spherical and enveloped RNA viruses with a negative-sense and single-stranded RNA genome. Bunyaviruses are currently divided into five genera including genus *Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus*, and *Tospovirus*, which only infects plants. Many bunyaviruses can infect humans causing several mild to severe diseases. Unlike most bunyaviruses, which are arthropod borne, Hantaviruses are rodent borne. Hantaviruses were first discovered near the Hantaan River in Korea and now are found worldwide. Hantaviruses cause high fever, lung edema, as well as pulmonary failure in humans. Hantaan virus, a member of the *Hantavirus* genus, has been shown to inhibit NF-κB activity through the interaction between its nucleocapsid (N) protein and importin-α1, importin-α2, and importin-α3, and blocking NF-κB nuclear translocation (256) (Fig. 4). Thus, Hantaan virus is another example that virus can manipulate host antiviral innate immunity by disrupting nuclear transport pathways.

Retroviruses

HIV-1 belongs to the genus *Lentivirus* of the Retroviridae family and is an enveloped positive-strand RNA virus that causes AIDS. Upon infection, the HIV-1 virion delivers two single-stranded RNA genomes into the cytoplasm of host cells, which are then reverse transcribed into complementary DNA, associate with viral proteins and cellular machinery in the form of preintegration complex, and translocate into the nucleus for integration into the host genome (257).

Viral proteins such as the M protein, integrase, viral protein R (Vpr) contain NLSs (258–261), and many studies have demonstrated their interaction with nuclear transport receptors, such as various importin-α isoforms (*e.g.*, importin-α1, importin-α3, importin-α5) (262–267), importin-7 (266), and transportin-3 (268), which are important for the nuclear translocation of the preintegration complex. At later stages of HIV-1 infection, the Vpr helps recruit CRM1 *via* its NES to mediate the export of intron-containing viral transcripts (269). Many Nups have been indicated as important host cofactors for HIV-1 infection, including Nup62, Nup153, Nup98, Nup214, RanBP2/Nup358, and hCG1 (270–278). These Nups have been shown to interact with various viral proteins to assist with nuclear import of the preintegration complex, viral DNA integration, and nuclear export of viral factors. Besides co-opting the host Nups and nuclear transport machinery, HIV-1 also blocks the nuclear import of hnRNP A1, A2, and D. These proteins then act in the cytoplasm to increase internal ribosome entry site–mediated translation of viral proteins (279, 280).

Interestingly, it has been shown that the HIV-1 CA protein mutants N74D and P90A, which are unable to interact with CPSF6, RanBP2/Nup358, and cyclophilin A, end up triggering IFN production and ultimately prevent viral replication (281). Thus, the CA protein's interaction with CPSF6, RanBP2/Nup358, and cyclophilin A likely contributes to the evasion of immune sensors; however, the mechanism remains unclear. The authors proposed that the interaction between the CA protein and host factors may restrict viral complementary DNA production to the nuclear pore, allowing it to be rapidly imported into the nucleus and thus preventing the viral DNA from being detected by cytosolic sensors. Recently, it is found that the interaction of HIV-1 Vpr with importin-α5 prevents efficient recruitment and transport of transcription factors IRF3 and NF-κB into the nucleus after immune activation (282) (Fig. 4). By targeting activated transcription factors, Vpr can promote HIV-1 replication in innate immune-activated cells, even when immune stimulation is caused by other PAMPs unrelated to HIV-1.

Hepadnaviruses

Viral hepatitis refers to a liver inflammation caused by viral infection, and the most common causes are the five unrelated hepatitis viruses A, B, C, D, and E. Hepatitis B virus (HBV) belongs to the Hepadnaviridae family and is a partially dsDNA virus that replicates through an RNA intermediate and integrates into the host genome (283). Upon hepatitis B viral infection, its CA proteins become phosphorylated, which exposes the nuclear localization signal and allows for its interaction with importin-α/β and subsequent nuclear import (284, 285). Though both mature and immature viral capsids are imported, only the mature capsids disintegrate and release their DNA genome into the host nucleus, whereas the immature capsids are arrested at the nuclear basket (285). Nup153, which is localized to the nuclear basket, has been found to interact with the viral capsids, and knockdown of this protein increases the amount of genome release from immature capsids (286). Thus, it is believed that Nup153 prevents premature release of genome, and this may be an essential step in the viral replication cycle.

HBV employs multiple strategies to evade immune response, and the viral polymerase is thought to play a major role. It has been shown that the hepatitis B viral polymerase can interact with heat shock protein 90 (287) and the DEAD-box RNA helicase DDX3 (288), which prevents the activation

of pattern recognition receptor signaling and STING-mediated viral DNA sensing. Furthermore, the polymerase activity inhibits both importin-α5 and protein kinase C-δ and prevents the nuclear localization of STAT1/2 and the expression of IFN-stimulated genes (289) (Fig. 3).

Conclusion and perspectives

In sum, the nuclear transport of signal transducers, such as IRF3, NF-κB, and STATs, is mediated by different nuclear transport receptors and is indispensable for the activation of host antiviral innate immune responses. Although it remains unclear whether the disruption of nucleocytoplasmic transports by several viruses discussed previously interfere with the host innate immunity, the crosstalk between other viruses (such as SARS-CoV, MERS, HCV, JEV, EBOV, IAV, HIV-1, and HBV), nucleocytoplasmic trafficking pathways, and host antiviral innate immune responses suggest that such subversion of the nucleocytoplasmic transport systems may be a common immune evasion strategy by distinct viruses. Therefore, nucleocytoplasmic transport pathways are logical antiviral therapeutic targets.

To date, several drugs have been identified to target nucleocytoplasmic trafficking systems from high-throughput drug screening. A compound named quinoline carboxylic acid was discovered to restore mRNA export inhibition caused by influenza A viral protein NS1, through enhancing the expression of the nuclear transport receptor NXF1 (290). Ivermectin, a small-molecule inhibitor of nuclear import, was shown to interfere with DENV-2 and HIV-1 infection by blocking the nuclear import of DENV-2 NS5 protein and HIV-1 integrase (291). Beyond these two viruses, ivermectin was also able to inhibit infection caused by Venezuelan equine encephalitis virus (VEEV) (292), AdV (293), as well as ZIKV (294), and potentially several alphaviruses, such as Chikungunya virus, Sindbis virus, and Semliki forest virus (295). In addition, mifepristone, another inhibitor of importin α/β-mediated import, has been shown to reduce nuclear-associated CA and viral titer of VEEV in a relevant mammalian cell line (292) and inhibit AdV infection (293). Aside from nuclear import inhibitors, there are also nuclear export inhibitors, such as leptomycin B and verdinexor (KPT-335), that have been shown to interfere with viral infection. Indeed, leptomycin B was discovered to reduce viral titer of VEEV in a mammalian cell line (292). Later, it was shown to inhibit the nuclear export of HIV-1 Rev protein (296). Verdinexor was demonstrated to inhibit the infection of respiratory syncytial virus, VEEV, IAV, EBV, KSHV, AdV-5, as well as HPV-11 (297–300). More recently, studies revealed that selinexor, another nuclear export inhibitor that selectively binds CRM1, could be repurposed to directly target the interaction between the SARS-CoV-2 protein ORF6 and other host proteins such as Nup98-RAE1 (179, 182). Although none of the drugs has been approved in clinical trials for treating viral infections, these studies show that the comprehensive understanding of the crosstalk between nucleocytoplasmic transport and host antiviral immune responses to viral infection, together with high-throughput drug screening, will contribute to the development of novel antiviral therapies in the future.

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Abbreviations—The abbreviations used are: AdV, adenovirus; ANE1, acute necrotizing encephalopathy 1; CA, capsid; cGAMP, cyclic dinucleotide GMP–AMP; cGAS, cyclic GMP–AMP synthase; CoV, coronavirus; COVID-19, coronavirus disease 2019; CRM1, chromosomal maintenance 1; DENV, dengue virus; EBOV, Ebola virus; EBV, Epstein–Barr virus; EMCV, encephalomyocarditis virus; ER, endoplasmic reticulum; FG-Nups, Nups that contain phenylalanine–glycine repeats; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV, human herpesvirus; hnRNP, heterogenous nuclear ribonucleoprotein; HPV, human papillomavirus; HRV, human rhinovirus; HSV, herpes simplex virus; IAV, influenza A virus; IFN, interferon; IFN-1, type I interferon; IL-6, interleukin-6; IRF3, interferon-regulatory factor 3; IRF9, interferon-regulatory factor 9; ISGF3, interferon-stimulated gene factor 3; JEV, Japanese encephalitis virus; KSHV, Kaposi's sarcoma–associated herpesvirus; L, leader protein; M, matrix protein; MERS, Middle East respiratory syndrome; mRNP, messenger ribonucleoprotein; NES, nuclear export signal; NLS, nuclear localization signal; NSP, nonstructural protein; NTF2, nuclear transport factor 2; Nup, nucleoporin; NXF1, nuclear RNA export factor 1; NXT1, NTF2-like export factor 1; PAMP, pathogen-associated molecular pattern; PRR, pattern-recognition receptor; PV, poliovirus; RIG-I, retinoic acid–inducible gene I; SARS, severe acute respiratory syndrome; STAT, signal transducer and activator of transcription; STING, stimulator of IFN genes; TLR, Toll-like receptor; TMEV, Theiler's murine encephalomyelitis virus; TNF-α, tumor necrosis factor α; TPR, translocated promoter region; TREX, transcription export; VACV, vaccinia virus; VEEV, Venezuelan equine encephalitis virus; Vpr, viral protein R; VSV, vesicular stomatitis virus; ZIKV, Zika virus..

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