Review

Roles of nuclear trafficking in infection by cytoplasmic negative-strand RNA viruses: paramyxoviruses and beyond

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Genome replication and virion production by most negative-sense RNA viruses (NSVs) occurs exclusively in the cytoplasm, but many NSV-expressed proteins undergo active nucleocytoplasmic trafficking via signals that exploit cellular nuclear transport pathways. Nuclear trafficking has been reported both for NSV accessory proteins (including isoforms of the rabies virus phosphoprotein, and V, W and C proteins of paramyxoviruses) and for structural proteins. Trafficking of the former is thought to enable accessory functions in viral modulation of antiviral responses including the type I IFN system, but the intranuclear roles of structural proteins such as nucleocapsid and matrix proteins, which have critical roles in extranuclear replication and viral assembly, are less clear. Nevertheless, nuclear trafficking of matrix protein has been reported to be critical for efficient production of Nipah virus and Respiratory syncytial virus, and nuclear localization of nucleocapsid protein of several morbilliviruses has been linked to mechanisms of immune evasion. Together, these data point to the nucleus as a significant host interface for viral proteins during infection by NSVs with otherwise cytoplasmic life cycles. Importantly, several lines of evidence now suggest that nuclear trafficking of these proteins may be critical to pathogenesis and thus could provide new targets for vaccine development and antiviral therapies.

Introduction

Negative-sense RNA viruses (NSVs) including the paramyxoviruses measles virus (MeV), mumps virus (MuV) and respiratory syncytial virus (RSV); the rhabdovirus Rabies virus (RABV) and the orthomyxovirus influenza virus constitute a major health burden worldwide. NSVs also include a number of highly pathogenic emerging and re-emerging viruses such as the filovirus Ebola virus (EBOV) and the paramyxoviruses Nipah virus (NiV) and Hendra virus (HeV). There are no currently licensed vaccines for use in humans to prevent infection by emerging NSVs. Furthermore, while effective vaccines are available for MeV and RABV, these viruses continue to cause significant loss of human life [annual fatality rates of approximately 150 000 (World Health Organisation, 2014) and 60 000 (Fooks et al., 2014), respectively] largely due to economic and political barriers to effective distribution and coverage and limitations in medical infrastructure in some geographical regions that prevent effective regimen delivery. Thus, the development of novel approaches to treat or prevent NSV infections is a high priority.

With a few exceptions, such as the segmented NSV influenza virus (Amorim & Digard, 2006), non-segmented NSV bornavirus (Briese et al., 1992) and several plant rhabdoviruses (Redinbaugh & Hogenhout, 2005), NSVs carry out their basic replication entirely within the cytoplasm. Nevertheless, roles for the nucleus in infection by NSVs with cytoplasmic replication cycles have been known for some time. Studies of MeV in the 1970s indicated that, while viral RNA synthesis does not occur in the nucleus (Schluederberg & Chavanich, 1974), viral titres are reduced in enucleated cells (Follett et al., 1976). The detection of nucleocapsid (N) protein-rich inclusion bodies in nuclei of MeV-infected cells (Nakai & Imagawa, 1969) and of matrix (M) protein in the nuclei and nucleoli of Sendai virus (SeV)-infected cells (Yoshida et al., 1976) provided initial evidence that NSV proteins might specifically target the nucleus for roles distinct from genome replication. Following these early indications, a substantial body of data suggesting that nuclear trafficking of proteins might be a common feature of NSVs with otherwise cytoplasmic life cycles has...
emerged. These include members of the Paramyxovirinae sub-family of the Paramyxoviridae family (which have come to represent a paradigm in this area) and rhabdoviruses of the genera Lyssavirus and Vesiculovirus.

While the precise role of the nucleus in infection by cytoplasmic NSVs has remained somewhat enigmatic, several lines of evidence now indicate critical roles for viral protein nuclear targeting, including in regulating viral replication/assembly and in subverting host immunity, such that nuclear trafficking could be a key component in pathogenicity. Here, we will review prototypical examples of the interactions formed by cytoplasmic NSVs with the nucleus and the nuclear transport machinery. We also consider current hypotheses regarding the roles of these interactions in infection and disease and their potential as targets for new antiviral approaches.

The infectious cycle and protein coding strategies of cytoplasmic NSVs

NSVs have relatively small genomes (generally <20 kb), encoding a limited number of key proteins required for replication and assembly. These typically include one or two proteins that mediate attachment/fusion with the host cell (e.g. attachment, G; fusion, F), proteins important to virion assembly/structure (e.g. M) and components of the RNA-dependent RNA polymerase (RdRp) complex (polymerase, L; N; and phosphoprotein, P) that mediates transcription and replication of the negative-sense RNA genome. The basic phases of infection common to cytoplasmic NSVs are summarized in Fig. 1 using the Paramyxovirinae replication cycle; other than for the 'atypical' NSVs noted above, none of these processes occurs within the nucleus.

Most NSVs express a number of additional ‘accessory’ proteins, which are not essential for infection in vitro, but have been implicated in processes required for efficient replication and pathogenicity in vivo (Audsley & Moseley, 2013). Accessory proteins are encoded by genes additional to the usual genome complement, such as non-structural 1 and 2 proteins and short hydrophobic protein variously found in members of the Paramyxoviridae and a diverse array of accessory genes found in certain rhabdoviruses (Goodbourn & Randall, 2009; Walker et al., 2011), or are encoded as alternative products within conserved structural protein genes. In RABV and viruses of the subfamily Paramyxovirinae, accessory proteins are encoded in the P gene in addition to the principal encoded protein P, the viral polymerase co-factor. 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C-termini (Hausmann et al., 1999). Many paramyxovirus P genes also contain alternative open reading frames encoding one or more C proteins. A number of functions have been attributed to P-gene-encoded accessory proteins, but most commonly involve antagonism of the type I IFN-mediated innate immune response to viral infection [reviewed in Audsley & Moseley (2013) and in Ito et al. (2016)].

Molecular mechanisms of nucleocytoplasmic trafficking

In spite of apparently non-essential roles of the nucleus in genome replication, both structural and accessory proteins of NSVs have been reported to enter the nucleus (Fig. 1 and below). Nuclear trafficking of cellular proteins is critical to biological processes including growth, development, gene expression, immunity and apoptosis and thus is tightly regulated. In eukaryotic cells, the double-membrane nuclear envelope restricts molecular movement between the cytoplasmic and nuclear compartments such that transport occurs exclusively through nuclear envelope-embedded nuclear pore complexes (NPCs) (Lim et al., 2008). NPCs are composed of 40–50 nucleoporin proteins (Nups) and create aqueous channels that permit passive diffusion of proteins less than approx 40–50 kDa (Swanson & McNeil, 1987). Movement of larger proteins or complexes requires active transport through interaction with specific nuclear import and export proteins [importins (IMPs) and exportins (EXPs), respectively]. These interactions provide the principal mechanism controlling nucleocytoplasmic protein trafficking/localization and must be successfully exploited by viral proteins for specific targeting of the nucleus.

Protein nuclear import

IMPs comprise >20 IMPβ and 7 IMPα subtypes in humans, which can provide selective regulation of nuclear transport due to differing cargo specificity and cell-type/developmental stage-dependent expression (Kelley et al., 2010; Mosamma-parast & Pemberton, 2004). In classical nuclear import, cargo proteins containing a nuclear localization signal (NLS) interact with IMPβ directly (Fig. 2a) or via IMPα in the context of an IMPαβ heterodimer (Fig. 2b) (Palmeri & Malim, 1999; Pouton et al., 2007). IMPβ mediates docking and translational and cargo–IMP complexes through the NPC via transient interactions with FxFG repeats in Nups that line the pore (Baylis et al., 2000). A differential nuclear to cytoplasmic concentration (high to low, respectively) of the GTP-bound form of the guanine nucleotide-binding protein Ran drives directional transport, whereby IMP–cargo complexes are formed in the cytoplasm in the absence of RanGTP but are dissociated in the nucleus following binding of RanGTP to IMPβ (Moroi-anu et al., 1996; Rexach & Blobel, 1995). To initiate a new round of transport, IMP–RanGTP complexes are recycled to the cytoplasm where GTP hydrolysis by Ran is promoted by cytoplasmic Ran GTpase-activating protein (RanGAP) and Ran-specific binding protein 1 (RanBP1) proteins, dissociating the complex (Bischoff et al., 1995).
Fig. 1. Cytoplasmic NSV infection cycle. Key events in cellular infection by NSVs are shown schematically, based on a typical virus of the Paramyxovirinae subfamily. (1) Host cell attachment occurs via interaction of F (fusion protein) and G/H/HN (attachment protein) with membrane receptors, which differ in a virus-specific manner and contribute to cellular tropism. Cellular entry of most paramyxoviruses occurs at a neutral pH, independent of endocytosis; Nipah virus (NiV) and Newcastle disease virus (NDV) may also use endocytic pathways (not shown). (2) F undergoes a conformational change that effects fusion of virus and cell membranes, and uncoating occurs, releasing the ribonucleoprotein (RNP; N-bound RNA) into the cytoplasm. (3) Transcription of viral genes is mediated by the viral RNA-dependent RNA polymerase (RdRp; L protein) and co-factor P protein through interaction with RNP. Viral mRNA is then translated by the cellular machinery. (4) Genome replication is mediated by the RdRp complex; the switch from transcription to replication mode is not fully understood but may depend on availability of unassembled N protein. For replication, the RdRp reads through termination/polyadenylation signals to produce a positive-sense antigenomic RNA template (dashed line) for new full-length negative-sense genome. (5) For virion assembly, G and F are delivered to the plasma membrane via the endoplasmic reticulum/Golgi secretory pathway and an active form of F (F1/2) is produced by proteolysis of the precursor (F0) by cellular proteases (not shown). (6 and 7) N and M of a number of paramyxoviruses are known to traffic between the nucleus and cytoplasm, with M export late in infection thought to be important to virion assembly at the plasma membrane (6) for budding (7). For full review, see Lamb & Parks (2007). NiV, Nipah virus; HeV, Hendra virus; MeV, measles virus; MuV, mumps virus; SeV, Sendai virus; CDV, canine distemper virus; RPV, rinderpest virus.
IMPα-recognized NLSs are commonly enriched for basic residues (K and R) organized in a monopartite or bipartite format and are generally modular in that they are sufficient to target heterologous protein to the nucleus. Monopartite NLSs encompass single short sequences, such as the prototypic IMPα-recognized NLS of the simian virus 40 large tumour antigen (T-ag) protein (PKKKRKV) (Kalderon et al., 1984), with K-R/K-X-R/K the generally accepted consensus sequence (Chelsky et al., 1989). Bipartite NLSs contain two basic residue-rich sequences separated by a spacer (generally 10–12 residues) (Nath & Nayak, 1990; Robbins et al., 1991), such as the IMPα-recognized NLS of nucleoplasmin (KRpaatkkagqaKKKK; spacer in lower case), in which the basic residue sequences are the key determinants of IMP binding/NLS function (Robbins et al., 1991). NLSs recognized directly by IMPβ, such as the human T-cell lymphotropic virus 1 Rex NLS (MPKTRRRPRRSQRKRPPPT) (Palmeri & Malim, 1999), are generally more variable and longer than those recognized by IMPα.

Some NLSs diverge significantly from the classical sequences, either containing basic residues not conforming to consensus motifs or entirely lacking basic residues [e.g. the NLSs of the nucleoproteins of influenza virus (TKGTKRSYEQM) and canine distemper virus (CDV) (TGILISIL), respectively] (Sato et al., 2006; Wang et al., 1997). Conformational NLSs that function only in the context of the native protein/domain have also been described, such as the predicted NLS of the globular C-terminal domain (CTD) of RABV P protein, comprising basic residues distantly localized in the primary sequence but closely aligned within the domain fold (Moseley et al., 2007a; Pasdeloup et al., 2005; Rowe et al., 2016). Several IMP-independent modes of nuclear import have also been
reported, including Ca^{2+}-dependent transport of sex-determining region Y protein, that is mediated by calmodulin and heat shock cognate protein 70 (Kaur & Jans, 2011; Kaur et al., 2013), as well as carrier-independent transport of β-catenin and human T-cell lymphotropic virus 1 Tax, which interact directly with Nups (Fagotto et al., 1998; Yokoya et al., 1999).

**Protein nuclear export**

Active nuclear export occurs via cargo interaction with EXPs (members of the IMPβ superfamily) (Fig. 2c), which in contrast to cargo–IMP interactions for nuclear import is RanGTP dependent and thus occurs within the nucleus to form a ternary transport complex of RanGTP-bound EXP with cargo. Following cytoplasmic delivery, GTP hydrolysis effects cargo release and free EXP is recycled to the nucleus (Poon & Jans, 2005). The best-characterized nuclear export pathway is mediated by EXP-1 (also known as chromosome region maintenance 1, CRM1), the only EXP with a well-established inhibitor, leptomycin B (LMB) (Kudo et al., 1998). CRM1-recognized nuclear export signals (NESs) are generally 9–15 residue leucine-rich sequences conforming to the consensus sequence L–×(2,3)–×(LIVFM)–×(2,3)–L–×–(LI) (la Cour et al., 2004). Other characterized EXPs include EXP-6 that exports actin (Stuven et al., 2003) and EXP-5 that exports the eukaryotic translation elongation factor 1A (Calado et al., 2002) and pre-micrornas (Yi et al., 2003). EXP-independent NES-driven nuclear export by the calcium-binding protein calreticulin has also been reported for the glucocorticoid receptor (Holaska et al., 2001).

**Regulation of nucleocytoplasmic trafficking**

Mechanisms regulating nucleocytoplasmic trafficking include differential expression of IMP/EXP subtypes at the mRNA and protein level through development and/or in specific cell types (Yashahara et al., 2009), interactions of IMPs with negative regulators of nuclear import such as etoposide-induced protein (Jul-Larsen et al., 2009), and differential expression of NESs between isoforms of cargo protein, such as the IFN-regulated promyelocytic leukaemia (PML) protein (Jul-Larsen et al., 2010) and RABV P protein (see below). Direct rapid modulation of NLS/NES function can also occur through the formation of intramolecular and intramolecular interactions, often regulated by post-translational modifications, which expose or mask trafficking signals or create NLSs/NESs de novo. For example, nuclear import of nuclear factor κ light-chain enhancer of activated B cells (NFkB; a major mediator of antiviral cytokine production) is regulated by interaction with inhibitor of NFkB (IκB), which masks the NFkB NLS until phosphorylation of IκB induces its proteasomal degradation (Beg et al., 1992). Similarly, IFN-dependent phosphorylation and heterodimerization of signal transducers and activators of transcription STAT1 and STAT2 (that mediate antiviral IFN-dependent signalling) both mask a NES and assembles a NLS (Reich & Liu, 2006). Post-translational modification can also directly regulate the affinity of NLSs for IMPs/EXPs as shown for simian virus 40 T-ag (Hubner et al., 1997; Xiao et al., 1997), human cytomegalovirus ppUL44 protein (Alvisi et al., 2005) and Epstein-Barr virus nuclear antigen 1 (Kitamura et al., 2006), where phosphorylation proximal to the NLS enhances interaction with IMPs. Alternatively, phosphorylation can inhibit nuclear import by conferring interaction with cytoplasmic retention factors, as observed for the T-ag and ppUL44 NLSs, with BRCA-1 binding protein 2 (Fulcher et al., 2010).

Regulation by ubiquitination and SUMOylation has been reported for tumour-suppressor proteins p53, BRCA-1 associated protein 1 and phosphatase and tensin homologue. The ubiquitin-conjugating enzyme E20 binds and ubiquitinates a subset of proteins (including BRCA-1 associated protein 1), which have bipartite NLSs with a conserved patch of aliphatic hydrophobic amino acids in their linker region; the ubiquitination results in increased cytoplasmic localization (Mashatul et al., 2014). Monoubiquitination of p53 also increases cytoplasmic localization by inhibiting IMP interaction (Marchenko et al., 2010) and increasing nuclear export by the p53 CTD NES (Rodriguez, 2014). For phosphatase and tensin homologue, monoubiquitination increases nuclear import (Rodriguez, 2014) while SUMOylation of different lysine residues can enhance or inhibit nuclear localization (Rodriguez, 2014).

Interactions of cargo proteins with the cellular cytoskeleton have been reported to enhance or inhibit nuclear localization of diverse cellular and viral proteins (Brice & Moseley, 2013). Increased import most likely occurs through a mechanism involving facilitated delivery to the perinuclear region by the microtubule-associated dynein motor (Moseley et al., 2007b; Roth et al., 2007), while inhibition involves sequestration to cytoplasmic microtubule/microfilament networks (Roth et al., 2009). Such mechanisms appear to require the expression of specific dynein or microtubule association sequences and, thus, have been observed only in specific subsets of nuclear trafficking cellular (e.g. SMADS, p53 and parathyroid hormone-related protein) and viral (e.g. RABV P3) proteins (Brice & Moseley, 2013; Moseley et al., 2007b, 2009; Roth et al., 2007). Analogous regulable interactions of proteins with intranuclear structures such as cellular genomic DNA and nuclear bodies can provide another means to control protein localization within the nuclear compartment (Bernardi et al., 2004; Roth et al., 2009).

**Nuclear trafficking of proteins of cytoplasmic NSVs**

Among cytoplasmic NSVs, the Paramyxovirinae are particularly well defined with respect to protein nuclear trafficking, having the greatest number of documented nuclear localizing proteins (see Table 1) and characterized mechanisms to exploit the host trafficking machinery, including defined NLSs/NESs (Table 2). The signals/mechanisms employed vary significantly across the subfamily Paramyxovirinae and between genera (Table 2), with greatest conservation observed between closely related viruses (i.e. within the...
same genus), possibly indicative of genus-specific nuclear functions. Importantly, recent reports have indicated that protein nuclear targeting may be common among other cyttoplasmic NSVs, with multiple intricately regulated sequences identified in proteins expressed by the rhabdoviruses rabies virus and vesicular stomatitis virus (VSV), suggestive of broad significance of the nuclear interface to this class of viruses.

The precise roles of nucleocytoplasmic trafficking in infection remain only partially understood but include sequestration of viral proteins into the nucleus until required in the cytoplasm for processes including replication and assembly. This is likely to be particularly important for proteins known to be inhibitory to viral transcription such as MeV C, SeV Y1 (a C protein isoform), NiV W and MeV M (Bankamp et al., 2005; Iwasaki et al., 2009; Reutter et al., 2001; Sleeman et al., 2008; Suryanarayana et al., 1994). Of note, mutagenic inhibition of nuclear export of NiV M (see below) inhibits budding of NiV M-derived virus-like particles and NiV virions (Wang et al., 2010), while inhibition of RSV M nuclear export by mutation or LMB treatment reduces viral titres (Ghildyal et al., 2009), presumably due to the requirement for cytoplasmic delivery of M to assemble new virions at the plasma membrane. Interestingly, mutagenic inhibition of NiV M nuclear import also reduced virus-like particle budding in protein expression experiments (Wang et al., 2010), indicating that regulated nucleocytoplasmic shuttling is important to efficient formation of virions. Nuclear sequestration can also conceal proteins from cytoplasmic host sensors of viral infection. MeV N, for example, can stimulate cytoplasmic IFN regulatory factor IRF-3 (tenOever et al., 2002), an activator of host antiviral responses, such that nuclear localization of N might minimize N–IRF-3 interaction.

An increasing body of evidence also suggests that nuclear localization enables NSV proteins to interact directly with host nuclear factors and/or to affect host protein nucleocytoplasmic trafficking, in order to modulate the biology of the infected cell (Table 1). Notably, early studies indicated that nucleation of cells does not substantially affect titres of RSV or VSV (Follett et al., 1974, 1976), both of which express nuclear trafficking proteins (Table 2) consistent with the idea that nuclear trafficking might not be critical to viral replication/assembly events but rather to modulating intranuclear processes. Based on these considerations, it is possible that an evolutionarily ‘early’ exploitation of the nucleus as a passive depot to regulate cytoplasmic levels of viral proteins might have provided opportunities for viruses to develop mechanisms to interfere with specific intranuclear events. Of particular interest in this respect are interactions of viral proteins with nucleocytoplasmic transcription factors involved in antiviral signalling, where NSV protein trafficking appears to have major roles in antagonising type I IFN responses.

### Nucleocytoplasmic trafficking of structural proteins

**M protein.** M proteins of several members of the *Paramyxoviridae* family, including NiV, HeV, SeV, Newcastle disease virus (NDV) (subfamily *Paramyxovirinae*) and RSV (subfamily *Pneumovirinae*), as well as of the rhabdovirus VSV, have been detected in the nuclei of infected cells (Ghildyal et al., 2002; Lyles et al., 1988; Peeples et al., 1992; Pentecost et al., 2015; Wang et al., 2010). For the *Paramyxovirinae*, nuclear localization of M appears to be time dependent, occurring early in the infection cycle, with export observed at later time points (Fig. 1), as M becomes necessary in the cytoplasm for virion assembly (Fig. 1) (Peeples et al., 1992; Wang et al., 2010). In addition, nuclear localization of SeV M *in vitro* occurs following temperature shifts from 37°C, indicating that M trafficking may be altered in response to cellular stress. Moreover, SeV M nuclear localization appears to be associated with the capacity to establish and maintain persistent infection in cell culture systems suggesting a biologically significant role of M within the nucleus (Ito et al., 2004; Nishio et al., 2003).

Bipartite sequences typical of classical IMPα/β-recognized NLSs have been defined in NiV M and NDV M (Table 2). Mutation of either of the basic residue clusters of the NDV M NLS reduced import, with combined mutation having an additive effect (Coleman & Peeples, 1993). However, the predicted minimal NDV M NLS was not sufficient to target heterologous protein to the nucleus (Coleman & Peeples, 1993), indicating that additional sequences or the tertiary protein structure are important. Mutagenic analysis of NiV M indicated that nuclear targeting is dependent on the positive charge at K\textsubscript{258} of the NLS (Pentecost et al., 2015; Wang et al., 2010). Since the equivalent regions of HeV, SeV and MuV M also contain conserved basic residues, with nuclear localization dependent on the lysine corresponding to NiV M K\textsubscript{258}, it appears that the bipartite NLS is conserved between these proteins (Pentecost et al., 2015).

NiV, HeV, SeV and NDV M proteins interact with a broad range of IMPs, with NiV M shown to associate with IMPα1, IMPα3, IMPα4, IMPα5, IMPα6, IMPβ4, IMPβ5, IMPβ7 and IMPβ9 (Pentecost et al., 2015), although it is not known whether these interactions are direct, and determination of the roles of specific IMPs in M nuclear import awaits further research. Importantly, not all protein IMP-binding sequences are functional NLSs and can instead act as negative regulators of nuclear import. Indeed, EBOV VP24 protein binds to IMPα5, IMPα6 and IMPα7, inhibiting IMPα-dependent STAT1 nuclear import to antagonize antiviral IFN signalling (Mateo et al., 2010; Xu et al., 2014), while the accessory protein α1 of the rhabdovirus *Bovine ephemeral fever virus* binds to IMPs, potentially to effect sequestration to the Golgi (Joubert et al., 2014).

In spite of similarities in their NLSs, the nuclear export mechanisms are not conserved between NiV and NDV M proteins. For nuclear export, NiV M contains two leucine-rich sequences typical of CRM1-recognized NESs.
### Table 1. Paramyxovirinae proteins that localize in the nucleus and their cellular interacting partners

<table>
<thead>
<tr>
<th>Protein</th>
<th>Virus</th>
<th>Localization</th>
<th>Infected cells</th>
<th>Viral</th>
<th>Interacting partners</th>
</tr>
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<tbody>
<tr>
<td>M</td>
<td>NiV</td>
<td>Cyt &amp; Nuc (Ciancanelli &amp; Basler, 2006; Pentcost et al., 2015; Wang et al., 2010)</td>
<td>Cyt/PM or Nuc/Nucleolar† (Pentcost et al., 2015; Wang et al., 2010)</td>
<td>NR</td>
<td>For full protein interactome, see Pentcost et al. (2015). Also of note: AP3B1 (Golgi, Cyt, Nuc) (Sun et al., 2014)</td>
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<td>HeV</td>
<td>Cyt &amp; Nuc (Pentcost et al., 2015)</td>
<td>Cyt/PM or Nuc/Nucleolar†, ‡ (Monaghan et al., 2014)</td>
<td>NR</td>
<td>For full protein interactome, see Pentcost et al. (2015). Also of note: AP3B1 (Golgi, Cyt, Nuc) (Sun et al., 2014); ANP32B (Nuc) (Bauer et al., 2014)</td>
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<tr>
<td>N/NDV</td>
<td>Cyt &amp; Nuc (Coleman &amp; Peeples, 1993)</td>
<td>Cyt/PM or Nuc/Nucleolar† (Peeples et al., 1992)</td>
<td>NR</td>
<td>For full protein interactome, see Pentcost et al. (2015). Also of note: AP3B1 (Golgi, Cyt, Nuc) (Sun et al., 2014); ANP32B (Nuc) (Bauer et al., 2014)</td>
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<td>SeV</td>
<td>Cyt &amp; Nuc (Pentcost et al., 2015)</td>
<td>Cyt/PM or Nuc/Nucleolar†, ‡ (Nishio et al., 2003; Yoshida et al., 1976); Cyt &amp; Nuc/ Nucleolar (Pentcost et al., 2015).</td>
<td>NR</td>
<td>For full protein interactome, see Pentcost et al. (2015). Also of note: AP3B1 (Golgi, Cyt, Nuc) (Sun et al., 2014); ANP32B (Nuc) (Bauer et al., 2014)</td>
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<td>MeV</td>
<td>Cyt &amp; Nuc (Sato et al., 2006; Sugai et al., 2014; Takayama et al., 2012)</td>
<td>Cyt or Nuc§,</td>
<td></td>
<td>(Chui et al., 1986)</td>
<td>P, L, RNA</td>
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<td>CDV</td>
<td>Cyt &amp; Nuc§ (Sato et al., 2006)</td>
<td>Cyt or Nuc (Oglesbee &amp; Krakowka, 1993)</td>
<td>P, L, RNA</td>
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<td>W</td>
<td>NIV</td>
<td>Nuc (Shaw et al., 2004)</td>
<td>Nuc‡ (Lo et al., 2010)</td>
<td>NR</td>
<td>STAT1 and STAT2 (Cyt or Nuc§) (Shaw et al., 2004); PLK1 (Cyt &amp; Nuc) (Ludlow et al., 2008)</td>
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<td>V</td>
<td>hPIV2</td>
<td>Cyt &amp; Nuc (Watanabe et al., 1996)</td>
<td>Cyt &amp; Nuc (Nishio et al., 1999; Watanabe et al., 1996)</td>
<td>L and N (Watanabe et al., 1996)</td>
<td>MDAA5 (Cyt) (Andrejeva et al., 2004); LGP2 (Cyt) (Childs et al., 2012); STAT1 and STAT2 (Cyt or Nuc§) (Ulane &amp; Horvath, 2002); DDB1 (Cyt or Nuc§) (Ulane &amp; Horvath, 2002); Cul4A (Cyt) (Ulane &amp; Horvath, 2002); IKKs: and TBK1 (Cyt) (Lu et al., 2008)</td>
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<td>MeV</td>
<td>Cyt &amp; Nuc (Palosaari et al., 2003; Ramachandran et al., 2008)</td>
<td>Cyt &amp; Nuc (Wardrop &amp; Briefis, 1991)</td>
<td>N &amp; P# (Liston et al., 1995; Tober et al., 1998)</td>
<td>MDA5 (Cyt) (Childs et al., 2007); LGP2 (Cyt) (Childs et al., 2012); STAT1, STAT2 and STAT3 (Cyt or Nuc§) (Palosaari et al., 2003); JAK1 (PM) (Caignard et al., 2007); IRF-9</td>
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<td>Protein</td>
<td>Virus</td>
<td>Localization</td>
<td>Infected cells</td>
<td>Interacting partners</td>
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<td></td>
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<td><strong>Protein expression studies</strong></td>
<td><strong>Infected cells</strong></td>
<td><strong>Viral</strong></td>
<td><strong>Cellular</strong></td>
</tr>
<tr>
<td>TioV</td>
<td>Cyt &amp; Nuc (Caignard et al., 2013)</td>
<td>NR</td>
<td>NR</td>
<td>(Cyt or Nuc) (Palosaari et al., 2003); IRF-3 (Cyt or Nuc) (Irie et al., 2012); p65 (Cyt or Nuc) (Schuhmann et al., 2011); IFNAR (PM) (Yokota et al., 2003)</td>
<td></td>
</tr>
<tr>
<td>MprPV</td>
<td>Cyt&gt;Nuc (Hagmaier et al., 2007)</td>
<td>NR</td>
<td>NR</td>
<td>MDA5 (Cyt) (Childs et al., 2007); LGP2 (Cyt) (Childs et al., 2012); MDA5 (Cyt) (Childs et al., 2007); LGP2 (Cyt) (Childs et al., 2012); STAT1 and STAT2 (Cyt/Nuc) (Hagmaier et al., 2007)</td>
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<tr>
<td>RPV</td>
<td>Cyt &amp; Nuc (Nanda &amp; Baron, 2006)</td>
<td>Cyt &amp; Nuc (Sweetman et al., 2001)</td>
<td>N and L (Sweetman et al., 2001)</td>
<td>MDA5 (Cyt) (Chinnakannan et al., 2014); STAT1 (Nanda &amp; Baron, 2006) and STAT2 (Chinnakannan et al., 2014) (Cyt or Nuc)</td>
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<tr>
<td>MuV</td>
<td>Cyt &amp; Nuc (Caignard et al., 2013; Puri et al., 2009)</td>
<td>NR</td>
<td>NR</td>
<td>MDA5 (Cyt) (Andrejeva et al., 2004); LGP2 (Cyt) (Childs et al., 2012); DDB1 (Cyt or Nuc) (Ulane et al., 2003); Cul4A (Cyt) (Ulane et al., 2003); STAT1, STAT2 and STAT3 (Cyt or Nuc) (Kubota et al., 2001; Ulane et al., 2003); IKKα and TBK1 (Cyt) (Lu et al., 2008); RACK1 (Cyt or Nuc) (Kubota et al., 2002)</td>
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<td>PIV5</td>
<td>Cyt &amp; Nuc (Precious et al., 1995)</td>
<td>Cyt &amp; Nuc (Paterson et al., 1995)</td>
<td>N (Randall &amp; Bermingham, 1996)</td>
<td>MDA5 (Cyt) (Audsley et al., 2016)</td>
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<td>JPV</td>
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<td>NR</td>
<td>MDA5 (Cyt) (Audsley et al., 2016)</td>
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<tr>
<td>BeiPV</td>
<td>Cyt &amp; Nuc (Audsley et al., 2016)</td>
<td>NR</td>
<td>NR</td>
<td>MDA5 (Cyt) (Audsley et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>MeV C</td>
<td>Cyt or Nuc (Nishie et al., 2007; Sparrer et al., 2012)</td>
<td>Nuc (Bellini et al., 1985)</td>
<td>NR</td>
<td>SHCBP1 (Nuc&gt;Cyt) (Ito et al., 2013); IFNAR1 (PM) (Yokota et al., 2003)</td>
<td></td>
</tr>
<tr>
<td>C isoforms</td>
<td>Nuc (Rothlisberger et al., 2010)</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDV C</td>
<td>PM, Cyt &amp; Nuc (Irie et al., 2008, 2013)</td>
<td>Cyt (Portner et al., 1986; Yamada et al., 1990)</td>
<td>L (Horikami et al., 1997)</td>
<td>STAT1 (Cyt or Nuc) (Garcin et al., 2002); Alix/AIP1 (Cyt) (Irie et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>SeV C and C’</td>
<td>Nuc&gt;Cyt (Irie et al., 2008, 2013)</td>
<td>N (Yamada et al., 1990), L (Grogan &amp; Moyer, 2001; Horikami et al., 1997)</td>
<td>STAT1 (Cyt or Nuc) (Garcin et al., 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SeV Y1</td>
<td>Nuc&gt;Cyt (Irie et al., 2008, 2013)</td>
<td>NR</td>
<td>N (Yamada et al., 2002, 2013)</td>
<td>STAT1 (Cyt or Nuc)</td>
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Table 1. cont.

<table>
<thead>
<tr>
<th>Protein</th>
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<th>Interacting partners</th>
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<td>Viral</td>
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<td></td>
<td></td>
<td></td>
<td>1990, L (Grogan &amp; Moyer, 2001; Horikami et al., 1997)</td>
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<td>Protein expression studies</td>
<td>Infected cells</td>
<td></td>
<td></td>
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<tr>
<td>2008, 2013</td>
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</tr>
</tbody>
</table>

*Excludes components of the nuclear transport machinery (see Table 2 for interactions with IMPs and EXPs); localization of host protein in parentheses. ‘&’ indicates that protein has been detected simultaneously in both locations; ‘or’ indicates protein detected in multiple locations but not concurrently (i.e. localization changes dependent on experimental conditions).

†Time dependent.
‡Cell-type dependent.
§Temperature/stress dependent.
||Strain dependent.
¶Dependent on activation state.
#Conflicting reports for interaction.

(106LLEECLSLKV<sub>115</sub> and 268LGSIGGLSL<sub>276</sub>) and mutation of these sequences could prevent export of NiV M (Wang et al., 2010). However, only 106LLEECLSLKV<sub>115</sub> was sufficient to drive export of heterologous protein, suggesting that this is the principal NES of NiV M (Wang et al., 2010). Alanine mutagenesis of key leucine residues in corresponding sequences in the M proteins of HeV, SeV and MuV also increased their nuclear accumulation (Pentecost et al., 2015), indicative of conservation of the NES in multiple paramyxoviruses. As HeV M export is inhibited by LMB, it appears that this NES is CRM1 dependent (Bauer et al., 2014). Equivalent mutations in NDV M and MeV M, however, did not cause nuclear accumulation (Pentecost et al., 2015), suggestive of divergence in the NESs used by these proteins. Indeed, NDV M export appears to involve three independent NESs that are insensitive to LMB treatment (Table 2). Each of these NESs was sufficient for nuclear export of NDV M, although their capacity to export heterologous protein differed (Duan et al., 2013), potentially indicating differences in EXP binding affinities.

Regulation of the nuclear localization of M protein by post-translational modification has been reported for NiV, HeV, SeV and MuV, for which nuclear export was inhibited by depletion of free ubiquitin using the proteasome inhibitor MG132 or enhanced by overexpression of ubiquitin (Pentecost et al., 2015; Wang et al., 2010). Similar regulation was not observed for NDV and MeV M proteins, indicative of specific roles for ubiquitination in trafficking of M proteins from different viral species (Pentecost et al., 2015). Enhanced nuclear localization of NiV M was also observed following mutation of K<sub>258</sub> within the NLS to arginine, which is unlikely to significantly impact NLS activity but resulted in reduced ubiquitination of M (Pentecost et al., 2015; Wang et al., 2010). While the mechanism has not been defined, Pentecost & colleagues (2015) hypothesized that ubiquitination within the NLS may obstruct IMP binding, thereby inhibiting nuclear import. This could provide the means to prevent nuclear re-entry at later stages of infection following relocalization of M from the nucleus to the cytoplasm (Fig. 1). Alternatively, ubiquitination of M within the nucleus may directly enhance nuclear export activity, although the molecular mechanism for such an effect is currently unclear (Pentecost et al., 2015).

In addition to the Paramyxovirinae, nuclear trafficking of M protein has been reported for VSV and RSV. VSV M contains two independent NLSs that can target heterologous protein to the nucleus (Table 2), but no NES has been experimentally confirmed. RSV M contains both an IMPβ1-recognized NLS and a CRM1-dependent NES (Table 2).

Although the specific mechanisms/regulation of nucleocytoplasmic transport of M protein diverge somewhat across cytoplasmic NSVs, the conservation of nuclear trafficking per se is suggestive of (an) important role(s) in infection. Other than potential roles in regulating cytoplasmic levels of M, several intranuclear roles have been suggested. VSV M is thought to target a component of the NPC to prevent general nucleocytoplasmic transport, with consequent effects on mRNA export and/or import of transcription factors (Her et al., 1997; Petersen et al., 2000) potentially contributing to known functions of VSV M in preventing transcription from multiple cellular promoters (Ferran & Lucas-Lenard, 1997; Petersen et al., 2000). RSV M has been suggested to use similar mechanisms to inhibit transcription (Ghildyal et al., 2003), in part through interaction with nuclear proteins (e.g. zinc finger protein 2 and SMAD3). While intranuclear functions of other paramyxovirus M proteins have not been described, recent characterization of the interactome of the NiV, HeV, SeV and NDV M proteins (Pentecost et al., 2015) identified a number of nuclear proteins, suggesting that M proteins may have several roles in the nucleus, which the new proteomic data should help to elucidate.
Table 2. NLSs and NESs identified in proteins from cytoplasmic NSVs

<table>
<thead>
<tr>
<th>Genus</th>
<th>Virus</th>
<th>Protein</th>
<th>NLS</th>
<th>Import pathway</th>
<th>Ref.</th>
<th>NES</th>
<th>Export pathway</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henipavirus</td>
<td>NiV</td>
<td>W</td>
<td>69KKAR482 +</td>
<td>IMPx3, IMPx4</td>
<td>Shaw et al. (2005)</td>
<td>NR</td>
<td>N</td>
<td>Rodrigues et al. (2004)</td>
</tr>
<tr>
<td>Henipavirus</td>
<td>NiV</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>NiV</td>
<td>M</td>
<td>24RRRAVKYYSDFICRRK208</td>
<td>ND</td>
<td>Wang et al. (2010)</td>
<td>100LEELSLK115</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rubulavirus</td>
<td>hPIV2</td>
<td>V</td>
<td>Residues 1-46</td>
<td>ND</td>
<td>Watanabe et al. (1996)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NDV</td>
<td>M</td>
<td>26KKGKKVYDNIKKRIRR283</td>
<td>ND</td>
<td>Coleman &amp; Peeples (1993)</td>
<td>100LEYKVF178 (NES1)</td>
<td>261LFLHIGLM268 (NES2)</td>
<td>– – –</td>
</tr>
<tr>
<td>Avulavirus</td>
<td>APMV</td>
<td>M</td>
<td>28KKTRKGDARSVLQ41KVKRV102</td>
<td>ND</td>
<td>Samuel et al. (2010)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SeV</td>
<td>Y1</td>
<td>14KMKTEWLR37</td>
<td>Ran GTPase-</td>
<td>Irie et al. (2012)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>MeV</td>
<td>C</td>
<td>41PPARKKRQQ86</td>
<td>ND</td>
<td>Nishie et al. (2007)</td>
<td>30LEKAMTLK105</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>CDV</td>
<td>N</td>
<td>30TGIISIL37</td>
<td>ND</td>
<td>Sato et al. (2006)</td>
<td>26ASLHSTLAFairoK159</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>RPV</td>
<td>N</td>
<td>30TGALISIL37</td>
<td>ND</td>
<td>Sato et al. (2006)</td>
<td>26ASLRSLAKR135</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>MeV</td>
<td>N</td>
<td>30TGALISIL7</td>
<td>ND</td>
<td>Sato et al. (2006)</td>
<td>429SENELPRGKEDBRV480</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Morbillivirus</td>
<td>FmOPV</td>
<td>N</td>
<td>TGAISSL (proposed)</td>
<td>ND</td>
<td>Woo et al. (2012)</td>
<td>LLRSLAFF (proposed)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pneumovirus</td>
<td>RSV</td>
<td>M</td>
<td>Residues 110-183</td>
<td>IMPβ1</td>
<td>Ghildyal et al. (2003)</td>
<td>19IPYSGGLAVTV296</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lyssavirus</td>
<td>RABV</td>
<td>P1-P2</td>
<td>21KKYK214/216R206 (C-NLS)</td>
<td>ND</td>
<td>Pasdeloup et al. (2005)</td>
<td>48LPEDMKRL126 (N-NES)</td>
<td>Residues 208-264 (C-NES)</td>
<td>CRM1</td>
</tr>
<tr>
<td>Lyssavirus</td>
<td>RABV</td>
<td>P4-P5</td>
<td>21KKYK214/216R206 (C-NLS)</td>
<td>ND</td>
<td>Pasdeloup et al. (2005)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lyssavirus</td>
<td>RABV</td>
<td>P3</td>
<td>Residues 53-174 (N-NLS)</td>
<td>IMPx2/1</td>
<td>Oksayan et al. (2012b); Pasdeloup et al. (2005); Bowe et al. (2016)</td>
<td>Residues 208-264 (C-NES)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vesyulovirus</td>
<td>VSV</td>
<td>M</td>
<td>Residues 23-57</td>
<td>ND</td>
<td>Glodowski et al. (2002)</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>M</td>
<td>Residues 47-229</td>
<td>ND</td>
<td>–</td>
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</tr>
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</table>
Notably, the M proteins of NDV, NiV, HeV, SeV and MuV have also been detected within nucleoli (Pentecost et al., 2015), with nuclear localization of NiV, HeV, SeV and MuV enhanced by ubiquitin depletion, suggesting that ubiquitination is required for nucleolar egress (Pentecost et al., 2015). Since nuclear and nucleolar localization involve distinct mechanisms [for review, see Rawlinson & Moseley (2015)], this suggests the presence of specific targeting activity within the proteins to enable as yet undefined roles within the nucleolus. Notably, nucleolar interaction by other viruses has been linked to regulation of apoptosis, stress responses and transcription (Rawlinson & Moseley, 2015), such that a major aspect of nuclear localization of certain NSV proteins may relate to modulation of nucleolar functions. A recent siRNA screen of HeV infection identified a significant number of nuclear and nucleolar proteins as important to infection, with the most significant effect observed for knockdown of the nucleolar marker fibrillarin, which was also shown to bind to HeV M protein (Deffrasnes et al., 2016). Fibrillarin knockdown impaired HeV RNA and protein synthesis and inhibited infection by a number of paramyxoviruses with cytoplasmic replication cycles, with this effect on HeV and NiV dependent on fibrillarin’s methyltransferase activity. In contrast, fibrillarin knockdown did not affect infection by the nuclear-replicating NSV influenza, suggestive of specific functions of an M protein-targeted nucleolar factor in cytoplasmic NSV replication (Deffrasnes et al., 2016).

N protein. Nuclear localization has been reported for N proteins of Paramyxovirinae of the genus Morbillivirus, including CDV, Rinderpest virus (RPV) and MeV (Sato et al., 2006). These proteins contain a broadly conserved NLS that can mediate nuclear localization of heterologous protein but lacks K or R residues and thus does not conform to classical IMPα/β-recognized sequences; the IMP-binding properties of this NLS have not been investigated (Table 2). CDV and RPV also contain a NES, which is not conserved in MeV (Table 2). Notably, morbillivirus P protein binds to two separate sites in N protein, including within the region containing residues 4–188 (Bankamp et al., 1996), which overlaps with the NLS. Thus, P binding could mask the NLS to retain N in the cytoplasm (Sato et al., 2006) so that N only enters the nucleus when it is not part of the RdRp complex (Fig. 1), representing a mechanism to coordinate its cytoplasmic and nuclear roles.

In protein expression studies, MeV, CDV and RPV N proteins have been shown to prevent STAT1 and STAT2 nuclear accumulation to inhibit type I IFN signalling (Takayama et al., 2012). In MeV-infected cells, STAT1 co-localizes with N in cytoplasmic aggregates (Palosari et al., 2003) indicating a comparable function in infected and transfected cells. Mutagenesis of sequences within the MeV N NLS (\(\gamma_6\)TGAL\(\gamma_7\) and \(\gamma_4\)GIL\(\gamma_7\)) prevented its nuclear import and significantly reduced its capacity to inhibit IFN signalling (Takayama et al., 2012). This is consistent with roles for N protein trafficking in immune evasion, although direct confirmation of this awaits further research, for example using a heterologous NLS to rescue nuclear import of mutant MeV N.

Nucleocytoplasmic trafficking of accessory proteins: multipronged targeting of the host IFN responses?

Although structural proteins such as MeV N can inhibit IFN signalling, this aspect of infection by paramyxoviruses, lyssaviruses and other NSVs is mediated principally by accessory proteins (Audsley & Moseley, 2013; Ito et al., 2016). Type I IFN (IFN-α/β) induction (Fig. 3) and signalling (Fig. 4) is driven by cytoplasmic, endosomal and plasma membrane receptors, which initiate signalling pathways that ultimately stimulate latent cytoplasmic transcription factors to translocate to the nucleus and drive antiviral gene expression (summarized in legends to Figs 3 and 4). Importantly, many accessory proteins of NSVs target key processes in the cytoplasm and nucleus, with several lines of evidence indicating that their nucleocytoplasmic trafficking is required for efficient shut down of antiviral responses, by enabling virus to inhibit IFN responses at multiple stages.

The RABV P protein isoforms are perhaps the best-characterized accessory proteins with respect to mechanisms of nuclear trafficking, with multiple sequences identified that are likely to mediate dynamic shuttling to produce the predominantly cytoplasmic CRM1-dependent localization of full-length P (also called P1) and P2, and the more nuclear localization of P3–P5 [reviewed in Oksayan et al. (2012a)]. Notably, several key trafficking sequences show broad conservation in the lyssavirus genus, indicative of important roles in viral biology (Jacob et al., 2000; Pasdeloup et al., 2005; Wiltzer et al., 2012). RABV P protein contains two nuclear trafficking modules/domains, located in the N-terminal region (NTR) (Oksayan et al., 2012b) and globular CTD (Pasdeloup et al., 2005; Rowe et al., 2016), both of which incorporate a CRM1-dependent NES and an IMP-binding NLS in overlapping or closely associated sequences, enabling efficient co-regulation of import and export. Truncation of the NTR to generate isoforms impacts localization both by inactivating/deleting the N-terminal NES and, in P3, by activating the N-terminal NLS de novo resulting in strong nuclear import (Oksayan et al., 2012b). Trafficking by the NTR also appears to involve an additional CRM1-dependent NES, but this has not been fully characterized (Oksayan et al., 2012b).

Trafficing function of the CTD, which is present in all RABV P isoforms, is proposed to be regulated by a phosphorylation-dependent mechanism that switches between conformations favouring export or import by the NES and non-conventional conformational NLS (Moseley et al., 2007a, b; Oksayan et al., 2012b; Pasdeloup et al., 2005; Rowe et al., 2016). Notably, the CTD can interact directly with IMPα/β heterodimer and IMPβ alone, suggesting that the conformational NLS has non-classical function in
coupling to multiple transport pathways (Rowe et al., 2016), which parallels the broad IMP-binding specificity of some paramyxovirus M proteins (Pentecost et al., 2015). Regulation of P protein trafficking is also likely to derive from its role as an interaction hub at the virus–host interface (Ito et al., 2016), such that NLSs/NESs may be regulated by competing intermolecular interactions. In particular, the binding of IMPs and RABV N protein to overlapping sequences in the P protein CTD (Moseley et al., 2007a, b; Pasdeloup et al., 2005; Rowe et al., 2016) might coordinate trafficking by a mechanism similar to that suggested for morbillivirus N–P protein interaction (Sato et al., 2006).

P protein additionally contains two sequences for association with the cytoskeleton, which modulate nuclear localization by either inhibiting nuclear import through sequestration to microtubules (as reported for P3) or facilitating nuclear import through interaction with a component of the microtubule-associated dynein motor. Switching between these modes of interaction may be dynamically regulated by P protein dimerization (Brice & Moseley, 2013; Brzozka et al., 2005; Moseley et al., 2007a, b; 2009). P3 can also localize into the nucleoli through a signal within the CTD (Oksayan et al., 2015), which is likely to relate to interaction with the nucleolar protein nucleolin. Although the specific function of the interaction is not known, knockdown studies indicate that nucleolin is required for efficient virus production (Oksayan et al., 2015).

The main data relating to P protein trafficking in IFN antagonism indicate key roles in inhibiting STAT1/2 signalling. The STATs can be bound by all isoforms by a site in the conserved P protein CTD (Wiltzer et al., 2012), enabling cytoplasmic P1, as well as presumably P2, and microtubule-associated P3 to effect their exclusion from the nucleus (Brice & Moseley, 2013; Brzozka et al., 2005; Moseley et al., 2009; Vidy et al., 2005; Wiltzer et al., 2012). P1 also binds and inhibits STAT3 nuclear translocation in response to the IL6 family cytokine oncostatin M (Lieu et al., 2013; Oksayan et al., 2012a). Nuclear localization of P3–P5 may additionally enable intranuclear blockade of IFN responses by preventing interaction of STATs with target DNA (Vidy et al., 2005) and enabling association of P protein with the IFN-stimulated gene product PML protein in PML nuclear bodies, potentially impacting IFN effector functions (Blondel et al., 2002). P1 proteins of several lyssaviruses additionally inhibit IFN induction by preventing phosphorylation of IRF-3, which occurs in the cytoplasm (Brzozka et al., 2005; Masatani et al., 2016; Rieder et al., 2011). Importantly, P protein nuclear trafficking has been linked to pathogenesis through the observation that mutations impairing NES-mediated nuclear export of P1 and associated STATs can
reduce IFN antagonist function and virulence in mice (Ito et al., 2010), having potential significance to vaccine development.

P gene-encoded accessory proteins (V, W and C) of paramyxoviruses of the genera Henipavirus, Rubulavirus, Respirovirus and Morbillivirus and of the proposed genus Jeilongvirus have been shown to localize into the nucleus or to undergo nucleocytoplasmic trafficking (Table 1). Dynamic localization has been reported for MeV C, which is nuclear early during infection and cytoplasmic later (Nishie et al., 2007), similar to localization of M protein of NiV, NDV and SeV. NLSs have been mapped within hPIV2 V, MeV C, SeV Y1 and NiV W, and NESs have been described for NiV V and MeV C (Table 2). Of note, several basic residues predicted to form part of a NLS in hPIV2 V are required for binding to cytoplasmic MDA5 to antagonize IFN induction (Ramachandran & Horvath, 2010), which may suggest a mechanism to regulate cytoplasmic and nuclear roles analogous to N–P protein interactions of MeV and RABV.

Nuclear localization of NiV W, SeV Y1 and MeV C has been linked to antagonism of IFN induction and IFN signalling pathways (summarized in Figs 3 and 4) (Irie et al., 2013; Shaw et al., 2005; Sparrer et al., 2012). NiV W binds to non-phosphorylated STAT (Shaw et al., 2004) and can accumulate in the nucleus due to a NLS in its C-terminal region (Table 2). However, differential nuclear localization has been detected in NiV-infected endothelial and neuronal cells, where greater nuclear accumulation in the latter correlated with the capacity to antagonize IFN signalling. Coupled with the observation that non-phosphorylated STAT1 is sequestered to the nucleus of infected Vero cells (Ciancanelli et al., 2009), these data suggest key roles for nuclear W protein. Shaw and colleagues (Shaw et al., 2005) also identified a role for NiV W nuclear import in antagonism of TLR3-dependent IFN induction through mutagenesis of the endogenous NLS of NiV W. Moreover, attachment of a heterologous NLS to drive nuclear accumulation of NiV V (which is otherwise cytoplasmic due to a NES and lacks TLR3 antagonist function) imparted the ability to inhibit TLR3 responses (Shaw et al., 2005). Thus, both NiV V and W proteins could antagonize TLR3-dependent induction of IFN dependent on their localization into the nucleus, indicating that the shared NTR of V and W contains TLR3-antagonist function that requires nuclear localization.

Roles for nuclear localization of SeV Y1 and MeV C protein have been suggested by the finding that mutation of their NLSs impairs the capacity to antagonize STAT1/2 signalling.
and IFN induction, respectively. Notably, the mutations affecting the MeV C NLS were identified in an attenuated vaccine strain (Schwartz MeV), where they reduced nuclear localization of C protein compared with wild-type MeV (Table 2) (Nishie et al., 2007; Sparrer et al., 2012). Although vaccine attenuation is likely to be multigenic in origin, these data support a link between C protein nuclear localization, IFN antagonism and pathogenicity, with potential application in vaccine attenuation strategies.

**Perspectives: the potential of NSV protein nuclear trafficking as a therapeutic target**

Our appreciation of the roles of viral protein nuclear trafficking in the biology of cytoplasmic NSVs has seen significant advances in recent years, with the available data pointing to key roles in infection by viruses of significance to human health including RSV, RABV and NiV. Studies using introduced or naturally occurring mutations of viral protein trafficking signals or pharmacological modification of trafficking have identified significant effects on the functions of structural proteins in viral replication/assembly and of accessory proteins in subverting immunity. Although in vivo studies are limited, there emerge data correlating these effects with pathogenicity, suggesting that NSV protein nuclear transport has the promise to yield new targets for vaccine attenuation strategies or for antivirals.

Methods to target protein nuclear trafficking include direct inhibition of NSV protein interactions with cellular trafficking factors or more general inhibition of cellular nuclear transport pathways. While direct data for the former approach are currently lacking, studies assessing the latter to treat infection by RNA viruses have proven promising, including the finding that titres of the positive-sense RNA virus dengue virus that replicates in the cytoplasm can be reduced by an inhibitor of IMPα/β-dependent nuclear import (Tay et al., 2013). Similarly, Verdinexor, a member of a new class of orally available, low-toxicity CRM1 inhibitors known as selective inhibitors of nuclear export, can reduce titres of influenza virus *in vitro* and *in vivo* and can reduce pathogenicity in a mouse model (Perwitasari et al., 2014). Given the evidence that nuclear export of P protein is important to IFN resistance of RABV (Ito et al., 2010) and that export of M protein is important to viral production/assembly by RSV and NiV (Ghildyal et al., 2009; Wang et al., 2010) and, potentially, HeV, MuV and SeV (Pentecost et al., 2015), selective inhibitors of nuclear export could prove effective against diverse NSVs. The feasibility of using compounds that modulate post-translational modifications important to regulation of nuclear trafficking has also been suggested by the finding that bortezomib, a Food and Drug Administration-approved proteasome inhibitor, can reduce titres of NiV *in vitro* (Wang et al., 2010), but awaits further studies of efficacy *in vivo* to determine the potential of such compounds as antivirals.

While there is now a substantial body of evidence indicating significant roles for nuclear trafficking in NSV biology, there is still much to be learned regarding this aspect of virology before its full potential for therapeutic or vaccine application can be realized. Therapeutic targeting of viral nucleocytoplasmic transport is still in the developmental stages and requires detailed knowledge of the cargo protein sequences/domains, molecular partners and regulatory mechanisms involved, in order to facilitate the development of attenuating mutations or drug screening and optimization. For example, the demonstration of binding of certain viral protein NLSs to IMPs (e.g. morbillivirus N proteins; Table 2), determination of the roles of specific IMP subtypes in trafficking [e.g. *Paramyxovirinae* M proteins (Pentecost et al., 2015)] and direct confirmation of the function of certain predicted NLSs in terms of conferring IMP-dependent nuclear localization into heterologous protein (e.g. NiV W) would provide important details on the molecular mechanisms involved. Furthermore, in spite of significant progress in delineating the sequences and cellular partners underlying trafficking of specific proteins such as NiV W, NiV M and RABV P, knowledge on other NSV proteins and viral species remains limited. A greater appreciation of the extent of conservation of trafficking across species, genera and families of cytoplasmic NSVs will provide important insights into the biology of this diverse viral order.

It is also important to note that much of the available data concerning NSV protein trafficking has been limited to overexpression studies of individual viral proteins. Although this has been valuable in elucidating the molecular mechanisms regulating trafficking, particularly for proteins of highly pathogenic viruses or viruses lacking a recombinant system, it is possible that the properties of transfected proteins may diverge from those of virus-expressed protein. Given the multifunctional nature of many viral proteins, it is also likely that mutagenesis affecting nuclear trafficking can have off-target effects, particularly on N and M proteins that have critical roles in replication and structure. With the development of new reverse genetics systems and increased understanding of the sequences involved in viral protein trafficking, it will be exciting to see future studies examining trafficking during infection both *in vitro* and *in vivo*, including defining the role of nuclear import of W proteins of NiV or HeV and the importance of the different trafficking sequences in RABV P. Such studies should greatly expand our understanding of molecular events at the virus–host interface and further validate NSV interactions with the nucleus as therapeutic targets. Furthermore, studies using new technologies for genomics and proteomics should help to delineate the precise molecular outcomes of NSV protein nuclear trafficking; this is of particular interest in the light of recent reports that paramyxovirus M proteins form complex interactomes including through associations with multifunctional nucleoli and intranuclear bodies, suggestive of novel roles beyond regulation of the cytoplasmic concentration of viral proteins, or dampening of immune responses.
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