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The role of nuclear pores and importins for herpes simplex virus infection

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Microtubule transport and nuclear import are functionally connected, and the nuclear pore complex (NPC) can interact with microtubule motors. For several alphaherpesvirus proteins, nuclear localization signals (NLSs) and their interactions with specific importin-α proteins have been characterized. Here, we review recent insights on the roles of microtubule motors, capsid-associated NLSs, and importin-α proteins for capsid transport, capsid docking to NPCs, and genome release into the nucleoplasm, as well as the role of importins for nuclear viral transcription, replication, capsid assembly, genome packaging, and nuclear capsid egress. Moreover, importin-α proteins exert antiviral effects by promoting the nuclear import of transcription factors inducing the expression of interferons (IFN), cytokines, and IFN-stimulated genes, and the IFN-inducible MxB restricts capsid docking to NPCs.

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Introduction

Alphaherpesviruses such as the human herpes simplex viruses (HSV-1, HSV-2) and varicella-zoster virus (VZV) or the veterinary pseudorabies virus (PrV) and bovine herpesvirus (BoHV-1) replicate their genomes and assemble their capsids in the nuclei of keratinocytes, mucosal epithelial cells, fibroblasts, neurons, and some immune cells. After the fusion of the viral envelopes with host membranes, the inner tegument proteins remain associated with the incoming capsids. In particular, during the infection of neurons, microtubules are critical for the transport of incoming capsids from the cell periphery to the nuclei. Ultimately, the capsids dock at the nuclear pore complexes (NPCs) to release their genomes into the nucleoplasm (Figure 1a).

In addition to inducing genome release from the incoming capsids into the nucleoplasm, the NPCs need to import all host and viral proteins necessary for early viral transcription (Figure 1b), viral replication (Figure 1c), late viral transcription (Figure 1d), nuclear capsid assembly and capsid egress (Figure 1e) into the nucleoplasm. After nuclear egress, capsid-associated inner tegument proteins mediate capsid targeting to the organelles of the secondary envelopment. There, manifold interactions among capsid, tegument, and envelope proteins orchestrate the final assembly of the mature virions. Fusion of the virion-containing vesicles with the plasma membrane releases the virions for infecting neighboring naive cells.

Despite their multiple interactions, we know little about the host nucleocytoplasmic transport factors (NTFs) and the nucleoporins (Nups) on which herpesviruses rely for infection. Here, we review the contributions of the NPCs, the importins, a subgroup of NTFs, and the nuclear localization signals (NLSs) of HSV-1 proteins to infection.

Viruses and nuclear pore complexes

Viruses replicating in the nuclei of postmitotic cells dock their capsids at the NPCs and release their genomes into the nucleoplasm for viral transcription and replication [1–3]. Moreover, herpesvirus infections depend on the nuclear import of transcription factors, enzymes of viral DNA replication, and proteins mediating capsid assembly, genome packaging into progeny capsids, or nuclear capsid egress. The NPCs are the sole gateways for bidirectional trafficking in and out of the nucleus [4–7]. Any given NPC contains about 1000 proteins of more than 30 unique Nups that form cytoplasmic filaments, an outer cytoplasmic ring, an inner ring, a nuclear ring, and the nuclear basket.

Abbreviations

BoHV	bovine herpesvirus
CATCs	capsid-associated tegument complexes
HSV	herpes simplex virus
MTs	microtubules
NES	nuclear export signal
NLS	nuclear localization signal
NPC	nuclear pore complex
NTF	nuclear transport factor
Nup	nucleoporin
PrV	pseudorabies virus
vDNA	viral DNA
VZV	varicella-zoster virus

The cytoplasmic filaments regulate the transport direction across the NPCs, and provide docking sites for NTFs, the small GTPase Ran, and the Ran GTPase-activating protein 1 (RanGAP1) for nuclear import, and remodel mRNA ribonucleoprotein particles after nuclear export [4,6]. The cytoplasmic filaments are composed of Nup358, Nup214, Nup98, Nup88, Nup62, Nup42 (also called hCG1), Gle1, DDX19, and Rae1 [4,6]. Of those, in particular, Nup358 and Nup214 are known to interact with viral capsids (1-2). At each NPC, eight bundles of five Nup358 molecules project as far as 60 nm into the cytosol from the conserved heterohexameric Nup complexes, which link to the outer rings. **Nup358** comprises an N-terminal NPC-binding domain, an oligomerization element, 4 Ran-binding domains (RBD, hence its other name RanBP2), an E3 ligase domain between RBD3 and RBD4, and a C-terminal prolyl-isomerase domain [4–6]. **Nup214** has an N-terminal β-propeller domain, a central coiled-coil domain, and a large C-terminal FG-repeat domain, and forms a complex with Nup88 and Nup62 [4,6].

Nucleocytoplasmic transport is essential to properly distribute proteins and RNAs between the cytosol and the nucleoplasm. Molecules with a diameter of less than about 5 nm diffuse freely through NPCs [7], but NTFs are needed for the transport of larger complexes [7–10]. NPCs can modulate their resting diameter in response to physiological cues, and even allow passage of cone-shaped HIV capsids with their broad end of 60 nm [11,12].

Herpesvirions — envelope, tegument, capsid, and DNA genomes

The virions of alphaherpesviruses have host-derived envelopes containing about 15 viral membrane proteins, whose cytosolic domains link to about 25 tegument proteins, which in turn connect on the vertices to the icosahedral capsids enclosing the double-stranded DNA genomes [13,14]. Cryoelectron tomography and single-particle reconstruction studies of virions reveal their inherent asymmetric organization. One of the twelve vertices is occupied by the portal and the portal cap, and HSV-1, and possibly other herpesviruses, have a thick proximal and a thin distal tegument pole [15–20].

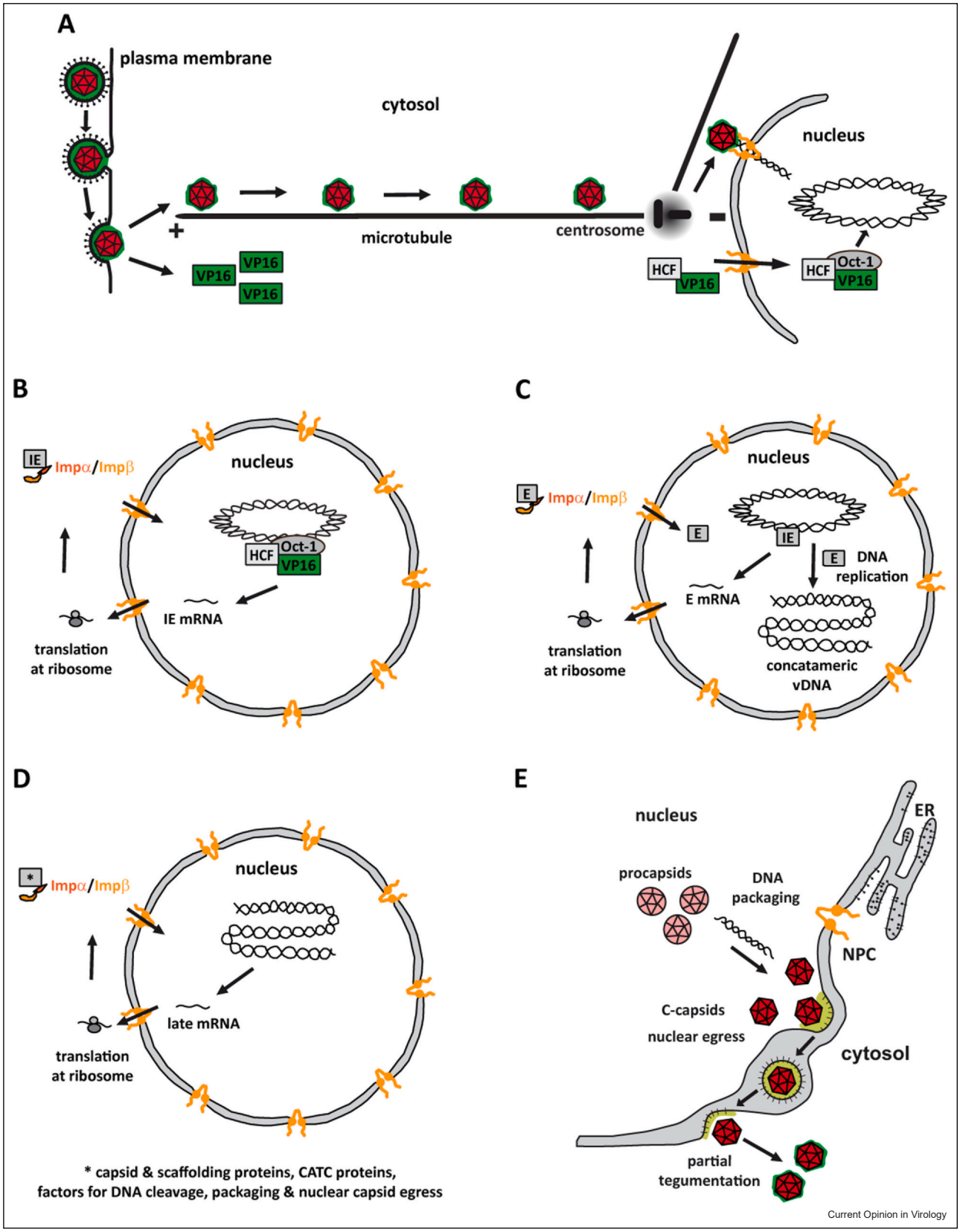
The capsid shells contain 150 hexamers of VP5 called hexons, 11 pentamers of the capsid protein VP5 called pentons, a dodecamer of pUL6 forming the portal, as well as triplexes of VP19C and VP23, which cross-link the hexons, pentons, and portal with each other [15,16,18,21]. The capsid-associated tegument complexes (CATCs) form star-shaped brackets connecting the portal and the pentons to the adjacent hexons and triplexes. The CATCs consist of one copy of pUL17, two copies of pUL25, and two copies of the C-terminal tail of the inner tegument protein pUL36 [15,19,20,22–25]. Recent studies indicate that two pentamers of pUL25 or its homologs also cap and seal the portal [17,26,27]. This portal cap likely prevents the premature release of the viral genome from the capsid [21,28–30].

Nuclear transport factors and importins

Specific NTFs bind to short linear peptide motifs named NLSs or nuclear export signals (NESs) and accompany cargoes through the NPCs. Many import cargoes bind directly to a member of the large karyopherin-β family of NTFs, for example, the sterol regulatory element-binding protein SREBP2 binds directly to importin β1, while others require an importin α as an adaptor to interact with importin β1 [7,10,31]. While for many NTFs only a few cargoes are known, up to hundreds have been reported for the importin αs [10,31]. Classical NLSs are enriched in basic residues, and either monopartite or bipartite with a linker of variable length and sequence (Table 2 [32,33]). Importin β1 in turn mediates binding of the ternary complex of importin β1 • importin α • cargo to the phenylalanine–glycine FG-rich Nups exposed in the NPC channel [34].

Flies encode for three, mice for six, and humans for seven importin αs (Table 1). Regardless of the functional differences, mice lacking a given importin α are viable, indicating some functional redundancy among mammalian importin αs [35–41]. They share an N-terminal importin β1-binding domain, which upon importin β-binding promotes importin-α binding to an nuclear localization signal (NLS), 10 armadillo repeats (ARM) characterized by 42–43 rather hydrophobic residues, and a short acidic C-terminal domain that binds their export

Figure 1


A
plasma membrane
cytosol
nucleus
microtubule
centrosome
VP16
VP16
VP16
HCF
VP16
HCF
Oct-1
VP16
B
IE
Impα/Impβ
nucleus
HCF
Oct-1
VP16
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factors for DNA cleavage, packaging & nuclear capsid egress
E
nucleus
ER
procapsids
DNA
packaging
NPC
C-capsids
nuclear egress
cytosol
partial
tegumentation
Current Opinion in Virology

HSV replication and the NPCs. **(a)** After viral fusion at a host membrane, HSV capsids (red) covered with the inner tegument (green) travel along microtubules to the centrosome and further to the NPCs (orange). The tegument protein VP16 dissociates from capsids and depends on the host protein host cell factor for nuclear import. In the nucleus, VP16 and host cell factor form a complex with Oct-1, which induces the transcription of HSV immediate–early genes. **(b)** HSV immediate–early transcripts are exported into the cytoplasm and translated at ribosomes. HSV-1 immediate–early proteins harbor NLSs and use importin α/βs for translocation into the nucleoplasm, where they induce the transcription of early genes. **(c)** HSV early proteins are involved in nucleotide metabolism and viral replication and require importins for nuclear import. **(d)** After DNA replication, late HSV genes encoding structural proteins are efficiently expressed. Capsid and scaffolding proteins, CATC proteins and factors for genome cleavage and packaging into capsids, and components of the nuclear egress complex are imported into the nucleoplasm. **(e)** After capsid assembly and DNA packaging, capsids move to the inner nuclear membrane and associate with the primary tegument (light green) for primary capsid envelopment. After fusion of the resulting primary virions with the outer nuclear membrane, capsids are released into the cytosol, where they acquire most of their tegument.

factors Cas and RanGTP [42,43]. ARM2, ARM3, and ARM4 form the major, and ARM6, ARM7, and ARM8 are the minor NLS-binding sites. A third binding site for noncanonical NLSs is located on ARM9 and ARM10 as well as on the C-terminal domain. The importin αs display striking differences in cargo recognition *in vivo*, despite their high sequence homology, and binding to similar, if not identical NLSs containing proteins *in vitro* [42–45]. For example, the nuclear Ran exchange factor RCC1 has an almost ninefold higher binding affinity for importin α3 than for α1; although both have a highly similar NLS-binding groove, importin α3 interacts stronger than α1 with the RCC1 surface flanking the NLS [45].

The small GTPase Ran is ultimately responsible for the transport direction across the NPCs by generating a gradient of low Ran^{GTP} and high Ran^{GDP} in the cytosol,

Table 1

Different importin αs and their interactions with nuclear proteins of HSV-1 and PrV.

Nuclear transport factor		Importin-αP RCH family clade α2		Importin-αQ QIP family clade α3			Importin-αS SRP family clade α1		Imp β1	Transportin
Drosophila	Gene	Pen		Kap-alpha3			Kap-alpha1		Fs(2)Ket	Tnpo
	Protein	Imp α2		Imp α3			Imp α1		β1	Trn
Mus musculus	Gene	Kpna2	Kpna7	Kpna4	Kpna3	Kpna1	-	Kpna2	Kpnb1	Tnpo1
	Protein	α2	α8	α4	α3	Imp α1	-	Imp α6	β1	Trn1
Homo sapiens	Gene	KPNA2	KPNA7	KPNA4	KPNA3	KPNA1	KPNA5	KPNA6	KPNB1	TNPO1
	Protein	**α1**	**α8**	**α3**	**α4**	**α5**	**α6**	**α7**	**β1**	**Trn1**
Interaction of proteins of alphaherpesviruses with different importin-α isoforms with										
PrV–ICP0 (pRL2)[130]		+	nd	+	nd	-	nd	+	+	+
HSV-1–pUL2 [166]		+	nd	-	nd	+	nd	+	+	+
PrV–pUL2 [167]		+	nd	+	nd	+	nd	-	+	+
HSV-1–pUL6 [139]		+	nd	-	nd	-	nd	+	-	+
HSV-1–pUL15 [144]		nd	nd	nd	nd	+	nd	nd	nd	nd
VZV–ICP8 (pORF29) [168]		+	nd	nd	nd	nd	nd	nd	-	nd
HSV-1–pUL30 [134]		nd	nd	nd	nd	+	nd	nd	nd	nd
HSV-1–pUL31 [152]		+	nd	+	nd	nd	+	nd	nd	nd
HSV-1–pUL31 [154]		+	nd	-	nd	-	nd	-	-	+
PrV–pUL31 [155]		+	nd	+	nd	+	nd	+	+	+
HSV-1–pUL34 [153]		+	nd	+	nd	nd	+	nd	+	nd
HSV-1–VP19C (pUL38) [142]		nd	nd	nd	nd	-	nd	nd	+	nd
HSV-1–pUL42 [169]		+	nd	+	nd	nd	nd	+	nd	nd
PrV–VP11/12 (pUL46) [170]		-	-	-	-	+	-	+	nd	nd
BoHV-1–VP8 (pUL47) [171]		-	nd	-	nd	-	nd	-	+	nd
VZV–VP16 (pORF10) [172]		nd	nd	nd	nd	-	nd	nd	+	nd
HSV-1–ICP27 (pUL54) [131]		nd	nd	nd	nd	nd	nd	nd	+	nd
PrV–ICP27 (pUL54) [132]		nd	nd	nd	nd	+	nd	nd	+	-
VZV–ICP27 (pORF4) [173]		nd	nd	nd	nd	+	nd	nd	+	nd
PrV–ICP22 (pUS1)[174]		+	nd	-	nd	-	nd	+	-	-

While the evolutionary relationship among the nuclear transport factor (NTF) of the importin-α family within as well as among species has been clarified with increasing sequencing information, the numbering of their gene and protein names has remained diverse in the literature. We use in this review the numbering of the human importin αs also on their murine orthologs but compiled in this table also to murine numbering. For further information, we would like to refer our readers to these reviews [42,43], Supp. Table 1 in Ref. [10]. Biochemical interaction data of proteins of the alphaherpesviruses HSV-1, VZV, PrV, or BoHV-1 with different isoforms of importin α. (+) Interactions were experimentally validated (+) by co-immunoprecipitation, glutathione S-transferase (GST) pulldown, yeast two-hybrid, gel mobility assays, or cocrystallization or not (-) in parallel assay. Often, the experiments included only a limited number, and not all importin-α isoforms (nd, not determined).

Table 2

Predicted and experimentally validated NLSs in HSV-1 proteins [175–192].

Protein [Gene] NLS score of PSORT II	**1. PSORT II** **2. cNLS Mapper** **3. NLStradamus**	**Experimental validation** Reference
ICP34.5 [RL1] NLS score 1.39	^{3}**RRRR**^{6} (m 5), ^{10}**PRRPRPP**^{16} (m 5) ^{200}**RR**GSWA**RER**ADRA**R**F**RRR**V^{218} (bi) ^{3}**RRRR**HRGP**RRPR**PPGPTGAVPTAQSQVTSTPNSEP^{37} (bi 3.6) ^{208}**R**AD**R**A**R**F**RRR**VAEAEAVIGPCLGPEA**R**A**R**ALARG^{241} (b; 4.5) ^{3}**RRRR**HRGP**RRPR**PPGP^{18}; ^{101}**PRPR**PPPPG^{109} ^{200}**RR**GSWA**RER**AD**R**A**R**F**RRR**V^{218}	^{1}MARRRRHRGPRRPRPP^{16}; nucleolar localization ^{200}RRGSWARERADRARF**RRR**V^{218} functional NLS [175]
ICP0 [RL2] NLS Score 0.77	^{502}**PRKRR**GS^{508} (m 5) ^{501}**RPRKRR**GSGQ^{510} (m 14) ^{251}RTP**R**APP**RR**^{259} ; ^{503}**RKRR**^{506}	^{501}RP**RKRR**GS^{508}; functional NLS [176]
gL [UL1] NLS Score 0.09	^{215}**RRRR**^{218} (m 5) ^{188}QP**K**PLTTPPPIIATSDPTP**RR**DAAT**K**S**RR**^{216} (bi 3.1) ^{206}P**RR**DAAT**K**S**RRRR**PHS**RR**L^{224}	-
pUL2 [UL2] NLS Score 1.49 uracil-DNA glycosylase	^{9}PSP**RRR**P^{15} (m 5); ^{69}**PRR**P**R**GC^{75} (m 5) ^{2}**KR**ACS**R**SPSP**RRR**PSSP**RR**TPP**R**DGTPP^{29} (bi 3.4) ^{11}**PRR**PSSP**RR**TP^{22}	^{1}M**KR**ACSRSPSP**RRR**PSS^{17}; functional NLS [177]
pUL3 [UL3] NLS Score: 0.03	^{179}**RKPRK**^{183} (m 4) - -	^{179}**RKPRK**^{183}; functional NLS [178]
pUL5 [UL5] NLS Score -0.03 helicase-primase	^{47}PIL**KR**I**R**^{53} (m 3); ^{744}**RRR**H^{747} (m 3) ^{45}VQPIL**KR**I**R**EL^{55} (m 4) -	nuclear upon co-expression with pUL8 & pUL52 [179]
pUL6 [UL6] NLS score -0.13 capsid portal	^{171}PIL**RKR**Q^{177} (m 4) ^{5}**RSR**APTT**R**A**R**GDTEALCSPEDGWV**K**VHPTP^{34} (bi 3.6) ^{640}ASP**R**G**RSRSR**SPG**R**TA**R**GAPDQGGGIGH**R**DG**RR**DG**RR**^{676}	^{171}PIL**RKR**Q^{177}; not confirmed [139]
pUL7 [UL7]	- ^{14}TIL**K**QAIAGD**RS**LVEAAEAISQQTLL**R**LACE^{44} (bi 3.29) -	-
pUL9 [UL9] Ori-binding protein	- - -	^{793}KREFAGARFKLR^{804}; functional NLS [180]
gM [UL10]	- ^{367}HSAL**KR**V**R**SS^{376} (m 3) ^{364}H**R**AHSAL**KR**V**R**SSM**R**GS**R**DG**R**H**R**P^{387}	-
pUL12 [UL12] NLS Score: 0.5 desoxyribonuclease	^{34}P**KR**P**R**PN^{40} (m 5) ^{13}**R**TVT**KR**PWALAEDTP**R**GPDSPP**KRR**P**R**PN^{40} (bi 10.9) -	^{35}**KR**P**R**P^{39}; functional NLS [181]
pUL13 [UL13] NLS score -0.04 protein kinase	^{466}P**K**I**RR**AF^{472} (m 5) ^{483}H**K**AILSSVALPPEL**K**PLLVLVS**R**LCHTNP^{511} (bi 3.7) ^{65}H**R**SGL**RER**L**R**AGLS**R**WR**R**MS**R**SSH**RR**A^{90}	-
pUL15 [UL15] NLS Score 0.64 terminase	^{182}PP**KKR**A**K**V^{189} (m 5) ^{181}GPP**KKR**A**K**VD^{190} (m 13) -	^{183}P**KKR**A**K**V^{189}; functional NLS [182] co-crystal with imp α5 [144]
pUL23 [UL23] NLS Score -0.22 thymidine kinase	^{30}**R**P**RR**^{33} (m 4) ^{18}**R**S**R**GHNN**RR**TAL**R**P**RR**QQ**K**ATEV**R**LEQ**K**MPTLL**R**VY^{53} (bi 3.0) ^{214}ID**R**LA**KR**Q**R**PG^{224} (m 5.5) -	^{25}**RR**TAL**R**P**RR**^{33}; functional NLS [183] ^{236}RR^{237}, ^{317}K**R**^{318}; functional NLS [184]
pUL24 [UL24] NLS score: 1.08	^{199}**RRRR**GGAA**R**GSA**S**P**KR**S^{216} (bi) ^{194}**R**TQ**R**A**RRRR**GGAA**R**GSA**S**P**KR**SH^{217} (bi 3.0) ^{197}**R**A**RRRR**GGAA**R**GSA**S**P**KR**SHSGA**R**^{221}	not confirmed [185]
VP21, VP24 [UL26] NLS score -0.16 capsid	^{426}**KRR**^{429} (m 5) ^{421}V**R**GSG**KRRR**YE^{431} (m 10.5) -	^{426}**KRR**^{429}; functional NLS [186]
VP22a [UL26.5] NLS score -0.16 capsid	^{120}**KRR**^{123} (m 5) ^{115}V**R**GSG**KRRR**YE^{125} (m 10.5) -	^{120}**KRR**^{123}; functional NLS [186]
gB [UL27] NLS Score: 1.55	^{6}P**AR**G**RR**W^{12} (m 4) ^{67}P**K**P**KK**N**KK**P**K**^{76} (m 5); ^{882}**RKRR**^{885} (m 5) ^{857}E**R**TEH**KAKKK**GTSALLSA**K**VTDMVM**RKRR**NT^{887} (bi 4.9) ^{880}VM**RKRR**NTNY^{889} (m 7) ^{67}P**K**P**KK**N**KK**P**K**PP**R**P^{83}	-
ICP8 [UL29] NLS Score -0.13 ssDNA binding protein	^{709}PVL**RRR**V^{805} (m 4) ^{1167}FAG**RKR**AFHGDDPFGEGPPD**KK**GDLT^{1192} (bi 4.6) -	^{1169}G**RKR**AFHGDDPFGEGPPD**KK**GDLTLDML^{1196} functional NLS [187]
pUL30 [UL30] NLS Score 0.80 DNA polymerase	^{124}**PRR**S**R**LW^{130} (m 5) ^{1114}PA**KR**P**R**E^{1120} (m 4); ^{1133}**KPRK**^{1136} (m 4) ^{1111}LPSPA**KR**P**R**ET^{1121} (m 7) -	^{124}PRRSRLW^{130}; not confirmed [134] ^{1114}PA**KR**P**R**ETPSPADPPGGAS**KPRK**^{1136} functional NLS [134]
pUL31 [UL31] NLS score 0.02 nuclear egress complex	^{8}**RR**G**S**RPGPYHG**K**E**RRR**S^{24} (bi) ^{12}**R**PGPYHG**KERRR**S**R**SSAAGGTLGVV**RR**AS**RK**SLPP^{46} (bi 3.5) ^{8}**RR**G**S**RPGPYHG**KERRR**S**R**SSAAGGTLGVV**RR**AS**RK**SLPP^{46}	^{21}RRRSRSSAAGGTLGVVRRASRK^{42} functional NLS [152]
pUL33 [UL33] terminase	- ^{83}GPI**K**APDDAAAPQTPDTACVHGELLA**RKR**E^{112} (bi 3.5) ^{107}LA**RKR**E**R**FAAV^{117} (m 3.5) -	^{107}LARKRERFAAV^{117} predicted NLS [144]
	^{178}**RR**ILC**R**AAEQAIT**RRRR**^{194} (bi)	^{178}**RR**ILCRAAEQAIT**RRRR**^{194}

Table 2 (*continued*)

pUL34 [UL34] NLS Score 0.50 nuclear egress complex	- -	functional bipartite NLS [153]
pUL36; [UL36] NLS Score 2.87 VP1-3, CATC, large tegument protein	^{294}PPT**R**A**RR**D^{301} (m 4) ^{402}**PKRRR**PT^{408} (m 5); ^{430}PA**KT**K**KK**^{436} (m 4) ^{1370}**PR**DF**RKR**^{1376} (m 3); ^{1924}PQML**RRR**^{1930} (m 3) ^{2658}**RR**H**RR**^{2662} (m 3) ^{400}GLP**KRRR**PTW^{409} (m 8) ^{423}**KT**K**R**SAPPA**KTKKK**STPK**G**K^{442} ^{2654}GS**R**A**RR**H**RR**A**R**^{2664}	^{402}**PKRRR**PTWTPPSSVEDLTSGEK TKRSAP^{430}PA**KTKKK**STPKGK^{442} functional bipartite NLS [72,188]
VP19C [UL38] capsid triplex	- - -	^{50}PRGSGPRRAAS^{60} functional NLS [142]
pUL39 [UL39] NLS Score -0.13 ribonucleotide reductase	^{1045}P**L**R**R**F**K**T^{1051} (m 4) - -	-
pUL41 [UL41] NLS Score -0.29 virus-host-shutoff	^{385}**RRR**H^{388} (m 3) ^{15}LV**KRR**GLGAPAGYFTPIAVDLWNVMYTLV^{43} (bi 3.2) -	-
pUL42 [UL42] NLS Score: 0.04 DNA polymerase	^{391}PTT**KR**G**R**^{397} (m 3); ^{410}**KK**P**K**^{413} (m 4) - -	^{391}PTT**KR**G**R**SGGEDARADAL**KK**P**K**^{413} functional bipartite NLS [169]
gC [UL44] NLS Score -0.29	^{508}**R**H**RR**^{511} (m 3) - -	-
VP11/12 [UL46] NLS Score -0.16	^{487}**RRRR**^{490} (m 5) ^{31}PE**RR**IFGGCLLPTPEGLLSAAVGAL**R**Q**R**SD^{60} (bi 3.3) ^{463}**RR**DNEPPPLP**R**P**R**LHSTPAST**RR**F**RRRR**A^{491}; ^{713}EG**RR**S^{717}	-
VP13/14 [UL47] NLS Score 2.87	^{6}PAG**RRRR**^{12} (m 5); ^{60}PPV**RRRR**^{66} (m 5); ^{69}P**R**A**RRRR**^{75} (m 5) ^{73}**RRR**ASEAPPTSH**RR**AS**R**^{89} (bi); ^{524}H**RRR**^{527} (m 3) ^{9}**RRRR**AST**R**P**R**ASPVADEPAGDGVGFMGYL**R**AVF^{42} (bi 3.2) ^{9}**RRRR**A^{13}; ^{63}**RRRR**EGP**R**A**RRRR**ASEAPPTSH**RR**AS**R**Q**R**PGP^{94}	^{63}**RRRR**EGPRA**RRRR**^{75} functional NLS [189]
VP22 [UL49] NLS Score 0.2	- ^{82}P**R**T**RR**PV^{88} (m 5); ^{295}**R**P**RR**^{298} (m 4) ^{280}S**R**PTE**R**P**R**APA**R**SAS**R**P**RR**P^{299}	-
gN [UL49A]	- ^{3}PP**RR**VC**R**AGLLFVLLVALAAGDAGP**R**GEPP^{32} (bi 3.2) -	-
pUL50 [UL50] NLS Score 0.39 dUTPase	^{111}P**KR**T**R**EF^{117} (m 5); ^{180}PA**RRR**G**R**^{186} (m 5) - -	-
ICP27 [UL54] NLS Score 0.21	^{162}P**RRR**APR^{168} (m 5) - ^{121}GG**K**VA**R**LQPPPT**K**AQPA**R**GG**RR**G**RRR**GR**G**RGGPGAA DGLSDP**RRR**APR**T**N**R**NPGGP**R**PG^{179}	^{110}ARRPSCSPERHGGKVARLQPPPTKAQPA^{137} functional bipartite NLS [190] ^{141}RRGRRRGRGRGGPGAADGLSDP**RRR**A^{166} contributing NLS [191]
pUL56 [UL56]	- - ^{75}**R**G**R**S**R**QAAQ**R**AA**RR**A**RRR**AE**RR**AQ**RR**^{100}	-
ICP4 [RS1] NLS Score 2.49	^{164}PP**RRRR**H^{170} (m 5); ^{727}**RKRK**^{730} (m 5); ^{746}P**KTKK**SG^{752} (m 5) ^{724}RE**GRKRK**SPGP^{734} (m 7) ^{157}SP**R**PPAQPP**RRRR**HG**R**W**R**^{174} ^{231}APG**R**TPPPPGPPPLSEAAP**K**P**R**AAAR**T**PAASAG**R**IE**RRR**A**R**AA^{273} ^{723}P**R**EG**RKRK**SPGPA**R**PPGGGGP**R**PP**KTKK**SGADAPG^{757}	^{726}G**RKRK**SP^{732}; functional NLS [176]
ICP22 [US1] NLS Score 3.20	^{16}**RR**PAL**R**SPPLGT**RKRK**^{32} (bi); ^{121}P**RPKRA**R^{127} (m 5) ^{11}PCV**KARR**PAL**R**SPPLGT**RKRKR**PS^{34} (bi 11.2) ^{119}IP**PRPKRA**RVN^{129} (m 10) ^{14}**KARR**PAL**R**SPPLGT**RKRKR**PS**R**P^{36}	^{16}**RR**PALRSPPLGT**RKRKR**^{31}; ^{118}DIPP**RPKRA**RVNL**R**^{131} 2 functional bipartite NLSs [192]
pUS3 [US3] NLS Score 0.13 protein kinase	^{141}PGI**RRR**S^{147} (m 4) ^{3}C**RK**FC**R**VYGGQG**RRK**EEAVPPET**K**PS**R**V^{30} (bi 3.2) -	-
gD [US6] NLS Score -0.04	^{372}P**KR**I**R**LP^{378} (m 5) ^{370}**K**AP**KR**I**R**LP^{378} (m 3.5) -	-
gI [US7] NLS Score 0.30	^{343}P**KS**R**RR**SS**R**TPMPSLTAIAEESEPAGAAGLP^{373} (bi 3.4) -	-
gE [US8]	- ^{24}**K**TSW**RR**VSVGEDVSLLPAPGPTG**R**GPTQ**K**LLW^{55} (bi 3.2) -	-
pUS9 [US9] NLS Score 0.47	^{56}**RRRRRR**^{61} (m 5) - -	-
pUS10 [US10]	- ^{1}MI**KRR**GNVEI**R**VYYESV**R**TL**R**S**R**SHL**K**PSD^{30} (bi 3.3) -	-
pUS11 [US11]	- - ^{85}P**R**TP**R**VP**R**EP**R**VP**R**PPP**R**EP**R**EP**R**VP**R**AP**R**DP**R**VP**R**DP**R**DP**R** Q P**R**SP**R**EP**R**SP**R**EP**R**SP**R**EP**R**TP**R**TP**R**EP**R**TA**R**^{158}	-

To predict NLSs (nuclear localization sequences) in capsid (red), tegument (green), or membrane (gray) proteins as well as transcription factors and enzymes (left column), we used the sequence of the HSV-1 strain 17^+ (RefSeq: NC_001806), and the 3 algorithms (middle column) PSORT II (top lines, Protein Subcellular Localization Prediction Tool; https://www.genscript.com/psort.html?src=leftbar [193]), cNLSMapper (middle lines, https://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi [194]), or NLStradamus (bottom lines, https://bioconda.github.io/recipes/nlstradamus/README.html [195]). The NLS score of PSORT is indicated in the left column. A cNLSMapper score (indicated as m x or bi x in the middle lines of the middle column) of > 7 indicates that this protein is mostly in the nucleus. With these algorithms, we did not detect any NLSs in pUL4, pUL8, pUL11, pUL14, pUL16, pUL17, VP23, VP5, pUL20, pUL21, gH, pUL25, pUL28, pUL32, VP26, pUL37, pUL40, pUL43, pUL45, VP16, pUL51, pUL52, gK, pUL55, pUS2, gG, gJ, pUS8A, or ICP47. We also compiled the references for experimental data confirming functional NLSs (right column). Prediction of multiple NLSs in pUL36 may be of interest concerning the role of pUL36 fragments of different lengths. (-) indicates that we could not predict any NLS with these algorithms (middle column), or not find any references on experimental validation (right column).

and high Ran^{GTP} and low Ran^{GDP} in the nucleoplasm [7,10,46]. In the nucleus, its guanine-nucleotide exchange factor RCC1 is tethered to chromatin and ensures a high amount of nuclear RanGTP. The binding to Ran^{GTP} changes the conformation of importin β1 and leads to cargo release in the nucleoplasm. In the cytosol, the GTPase-activating protein RanGAP1, in conjunction with Ran-binding protein 1 (RanBP1) or Nup358 (RanBP2), stimulates guanosine-5'-triphosphate (GTP) hydrolysis and thus conversion of Ran^{GTP} to Ran^{GDP}. Ultimately, this polarized distribution of Ran-interacting proteins generates a steep gradient of Ran^{GTP}/Ran^{GDP} between the nucleoplasm and the cytosol.

Nuclear localization signals in herpesviral proteins

Alphaherpesviruses encode many proteins containing canonical NLSs. For HSV-1, for example, the NLSs of 21 proteins have been validated experimentally, and for another 22, NLSs have been predicted (Table 2). The NLSs of herpesviral proteins could recruit the importin α • importin-β1 complex to target incoming capsids to NPCs, connect capsids to phenylalanine-glycine (FG) repeats on Nups, and/or mediate nuclear import of factors for viral transcription, genome replication, capsid assembly, and nuclear capsid egress. Although the precise protein composition of incoming capsids remains unclear, VP26, pUL17, pUL25, pUL21, pUS3, pUL36, and pUL37 most likely remain exposed on the capsids until arrival at the NPCs and are therefore candidates to bind to microtubule (MT) motors, NTFs, and Nups [47–52].

Importins for capsid transport along microtubules

Alphaherpesviruses fuse their envelopes with endosomal or plasma membranes in keratinocytes, mucosal cells, or fibroblasts, while they seem to enter neurons at axon terminals or presynaptic membranes exclusively by fusion with the plasma membrane [49,53–55]. The incoming capsids use transport along MTs to reach the nuclei (Figure 1a) [56–59]. To study the axonal transport of incoming capsids, neurons cultured in compartmentalized chambers are selectively infected from the axonal side [60–63]. Fluorescent tags on capsid and tegument proteins, as well as electron microscopy, are used to identify unequivocally cytosolic capsids and to localize them on MTs or NPCs [64–68].

MT motors such as dyneins and kinesins transport host and viral cargoes toward the centrosomes in the cell center or the periphery, respectively [69–71]. HSV-1, VZV, and PrV capsid transport occurs processively toward the neuronal soma, but is interrupted by short, transient backward bursts toward the axon terminal, and capsids transiently accumulate around centrosomes before docking to NPCs, suggesting that they use both types of MT motors [51,64,66,68,72–76]. Furthermore, isolated HSV-1 capsids covered by inner tegument, but not untegumented or fully tegumented capsids, can translocate along MTs in the presence of ATP and recruit dynein, its cofactor dynactin, kinesin-1, and kinesin-2 in cell-free assays [50,75,77–79]. If dynein function is perturbed, the incoming HSV-1 capsids accumulate in the cell periphery, while inhibition of kinesin-1 leads to capsid clustering around the centrosomes [50,73,75,78,80]. Intriguingly, HSV-1 and PrV package kinesin-1 into the tegument during secondary envelopment, and depend on it for capsid docking to the NPCs during cell entry [75]. It may seem counterintuitive to run forward and backward along MTs instead of moving steadily from the cell periphery to the NPCs. However, many host cargoes also depend on bidirectional transport to avoid traffic jams and to change subcellular localization in response to specific stimuli.

The nonessential HSV-1–**VP26** and PrV–**pUL21** capsid proteins interact with the dynein light chains Tctex-1 and Tectex-3, or roadblock-1, respectively [52,81,82]. Incoming capsids of PrV–ΔpUL21, but not of HSV-1–ΔVP26 or PRV–ΔVP26, are impaired in retrograde axonal transport [52,83,84]. Further studies need to clarify whether VP26 and pUL21 recruit dynein to

capsids, as VP26 and pUL21 contribute also to proper virion assembly, and as dynein light chains function in other complexes too [71,84–87].

The essential inner tegument proteins **pUL36** and **pUL37** of HSV-1 and their homologs in other alphaherpesviruses contribute to MT transport and are essential for secondary envelopment but not for nuclear egress [65,67,88–96]. Notably, a proline-rich region in the C-terminal third of PrV–pUL36 can directly associate with dynein and dynactin; however, mutating this sequence impairs nuclear capsid targeting only mildly [96]. Furthermore, HSV-1– and PrV–pUL36 contain acidic tryptophan motives with similarities to binding motives for the light chains of kinesin-1 on host cargoes and the vaccinia virus protein A36 [75,97,98]. Moreover, the nonessential HSV-1–**pUS11**, which has no homolog in VZV, PrV, or BoHV-1, can interact with the heavy chain of kinesin-1, although the relevance of this interaction remains to be elucidated [99].

MT transport and nuclear import have long been considered to function independently, but there is increasing evidence that several Nups interact with MTs and MT motors [5]. Nup358, for example, has a binding site for the heavy chain of kinesin-1 between RBD3 and RBD4, and it can interact with dynein and kinesin-1 via the adaptor bicaudal-2 [100,101], suggesting that both dynein and kinesin-1 may also contribute to capsid docking to NPCs. Moreover, recent studies show that axonal importin α • importin-β1 complexes can bind to the NLSs of transcription factors, for example, of NFκB or STAT3, and in turn, recruit dynein and dynactin for axonal transport of such signaling complexes [102,103].

Similarly, axonal capsids of alphaherpesviruses might also recruit importin α • importin β1 via exposed NLSs on the capsid surface, and thus indirectly dynein for axonal transport of incoming capsids. When the capsid-associated HSV-1–pUL36 lacks the NLS or is unable to recruit kinesin-1, the amount of incoming HSV-1, VZV, and PrV capsids that accumulate transiently around the centrosomes is increased, and viral gene expression is decreased (Figure 1a) [51,72–75]. Thus, at least the NLS of pUL36 does not seem to be required for dynein-mediated transport to centrosomes of epithelial cells. However, the pUL36–NLS or other capsid-associated NLSs may be crucial for dynein-mediated axonal transport. Moreover, the interaction of pUL37 with dystonin might promote transport from the centrosomes to the NPCs [104,105].

Taken together, alphaherpesvirus capsids can simultaneously recruit dynein for transport to the MT minus ends at the centrosomes, and kinesin-1 for transport to the MT plus ends that are mostly located in the cell periphery, but may also point toward the NPCs [5,101]. The largely unidirectional capsid transport runs in cells and *in vitro* imply that the activity of capsid-associated MT motors of opposing directionality is tightly regulated to prevent a tug-of-war among them.

Importins for genome release at the nuclear pore complexes

Electron microscopy of infected cells, fluorescence microscopy of labeled incoming genomes, as well as cell-free assays with isolated capsids and nuclei are used to dissect the functions of capsid and tegument proteins for capsid assembly and genome packaging from those for NPC docking and genome release. Incoming HSV-1 and PRV capsids associate with the cytosolic NPC filaments and occasionally reveal electron-dense threads reaching from one capsid vertex toward the lumen of connected NPCs, which we interpret as viral genomes being injected into the nucleus (Figure 1a) [51,106–108]. In Vero cells, 50–60% of incoming HSV-1 genomes are released from capsids, and 60–70% upon incubating capsids directly with isolated nuclei and cytosol [107]. Adding antibodies against Nups or importin β1 blocks HSV-1 capsid binding to isolated nuclei, and inhibiting ATPase and GTPase activities in this system reduces genome uncoating [107].

More specifically, treating cells with antibodies or siRNA directed against **Nup358** and **Nup214** reduces capsid docking to NPCs [88,109]. Docking to NPCs might be mediated via capsid-associated pUL36 • dynein or pUL36 • kinesin-1 light chain • kinesin-1 heavy-chain binding to Nup358. Capsids of adenovirus and HIV-1 interact with NPCs via kinesin-1 [110,111]. Moreover, docking to NPCs might be mediated via a capsid-associated NLS • importin α • importin-β1 binding to Nup358 and Nup214. HSV-1 capsids with the inner tegument on their surface, as well as tegument-free capsids, can bind to isolated nuclei, and this binding requires intact NPCs and importin β1, but a role for importin α could neither be shown nor excluded [107,112]. Tegumented capsids recruit importin α5 and importin β1 from the cytosol of resting or interferon (IFN)-induced macrophages [113]. In contrast, targeting of incoming HSV-1 capsids to the nucleus is not impaired in murine fibroblasts lacking importin α1, α3, or α4, or in neurons lacking importin α1 [61]. Thus, incoming capsids might recruit importin α5 and possibly others, bind indirectly or directly to importin β1, and target Nup358 via capsid-associated MT motors to achieve stable docking at NPCs.

HSV-1 pUL36 is crucial for genome release independent of its functions in MT transport as a single-point mutation in **pUL36** at residue 1453 blocks the release of the viral genome into the nucleoplasm but does not prevent binding to the NPCs [114]. Moreover, proteolytic

cleavage of pUL36 seems to trigger genome release into the nucleoplasm [115]. In contrast, deleting **pUL37** or microinjected antibodies against pUL37 does not block capsid docking to NPCs [88,91]. HSV-1–**pUL25** can interact with Nup214 and Nup42 [109], and proper interaction of capsids with NPCs is disrupted, when pUL25 is overexpressed before infection, or when its very C-terminus is missing [108,116]. Moreover, genome-containing C capsids bind better to NPCs than the DNA lacking A or B capsids that have less pUL25 [117]. These data suggest that properly matured CATCs and/or the portal caps might promote the docking of incoming capsids to NPCs, or promote capsid destabilization upon their interaction with the NPCs.

The capsids most likely must align their portals toward the NPCs to allow the release of viral genomes into the nucleoplasm [17,21,26,56,118]. For the human cytomegalovirus, a betaherpesvirus, the portals seem to be primed for genome release already during envelope fusion with a host membrane [119]. Specific NPC interactions might dislocate the portal caps and destabilize the capsids such that their internal pressure drives the injection of the tightly packed viral genomes into the nucleoplasm until the capsid lumen reaches ambient pressure. Binding of nuclear host and viral proteins and possibly anomalous diffusion complete the translocation of the incoming herpesviral genomes into the nucleoplasm [115,118,120–122]. HSV-1 genome uncoating at the NPCs and transcription start as early as 30 min after inoculation, and many nuclear viral and host proteins, for example, transcription factors, RNA polymerase II, or nuclear DNA sensors, interact quickly with the incoming viral genomes [51,108,123–126].

Importins for nuclear viral transcription and genome replication

While the host RNA polymerase II transcribes all herpesviral genes in the nucleus, several herpesviral proteins contribute to the synthesis of all immediate–early, early, and late viral gene products (Figure 1b). Mostly for HSV-1, but less for other alphaherpesviruses, several NLSs have been mapped and characterized (Table 2). The tegument protein HSV–**VP16** (pUL48), which dissociates from incoming capsids, complexes with the host cell factor HCF-1 and Oct-1 to activate immediate–early promoters for transcription (Figure 1a). VP16 seems to lack an NLS on its own but piggy-backs onto HCF-1 for import into the nucleus, where VP16 • HCF-1 bind to Oct-1 already associated with the promoters of the incoming genomes [127,128]. While the NTFs of HCF-1 and Oct-1 are unknown, Oct-4 interacts with importin α1 and Oct-6 with α6 [129], but the nuclear import of VP16 is not impaired in murine fibroblasts lacking importin α1, α3, or α4 [61].

For all immediate–early HSV-1 proteins important for early and late transcription (Figure 1b), namely the E3 ubiquitin ligase **ICP0** (pRL2), the major transactivator **ICP4** (pRS1) and its regulators **ICP22** (pUS1) and **ICP27** (pUL54), some interacting NTFs (Table 1) and NLSs have been identified (Table 2). PrV–ICP0 can interact with transportin, importin β1, **α1**, α3, and α7 but not with α5 [130], HSV-1–ICP27 with importin β1 [131], and PrV–ICP27 with importin β1 and α5, but not with transportin [132]. Using a targeted siRNA screen against 17 host factors in human cells, we identified importin α1, α6, β1, and transportin-1 as factors contributing to efficient HSV-1 infection [61]. In contrast, importin 11, importin 8, transportin 3, and importin 9 appeared to repress HSV-1 reporter expression [61]. Murine fibroblasts lacking importin α1 produced less infectious virions. While the levels of ICP0 and ICP4 remain the same, their nuclear import is less efficient in the absence of importin **α1**, and for ICP0 also in the absence of **α3** in fibroblasts [61]. In addition to the nuclear import of viral or host proteins necessary for transcription, transportin might also mediate the nuclear import of viral genomes directly [133].

The formation of nuclear DNA replication compartments results in host chromatin marginalization toward the nuclear envelope, and requires 7 HSV-1 proteins (Figure 1b). The origin-binding protein **pUL9**, the single-strand-binding protein **ICP8** (pUL29), and the viral DNA polymerase subunits **pUL30** and **pUL42** contain classical NLSs (Table 2). HSV-1–pUL30 binds to importin **α5** and β1, but other αs have not been tested, and HSV-1–pUL42 interacts with importins α1, α3, and α7 [134]. Efficient nuclear import of HSV-1–ICP8 and the HSV-1–pUL30/42 requires importins α1 and α3 but is increased in the absence of α4 [61]. The **pUL5, pUL8, and pUL52** subunits of the helicase/primase complex of HSV-1, Epstein–Barr virus, and Kaposi sarcoma herpesvirus remain cytosolic when expressed in isolation, but their assembly exposes or generates a composite NLS for nuclear import [135,136]. So far, no interactions with importins have been reported for them, although HSV-1–pUL5 has a predicted NLS (Table 2). Overall, these data indicate that, in particular, importins α1 and β1 are important for the nuclear import of HSV-1 proteins required for early transcription and DNA replication.

Importins for capsid assembly, DNA packaging, and nuclear egress

As herpesvirus capsids are assembled in the nucleoplasm, all bonafide capsid proteins and proteins required for genome packaging and nuclear capsid egress are imported into the nucleus (Figure 1d and e) [21,137]. The portal most likely nucleates capsid assembly [138]. HSV-1–pUL6 interacts with transportin, importins α1

and α7, and when transportin and α7 are depleted together, HSV-1–pUL6 remains cytosolic [139]. The capsid proteins VP5, VP23, and VP26 are not imported if expressed alone. VP5 requires VP22a or VP23, and VP23 in turn VP19c for nuclear import. VP19c has an unusual NLS and an NES, interacts with importin β1, but not with **α5,** and expression of a dominant-negative importin β1, but not of α5, blocks nuclear import [140–143]. Furthermore, VP26 is only nuclear, if VP5 is present with either VP22a or VP23 [143].

In addition to UL6, the six HSV-1 genes UL15, UL17, UL25, UL28, UL32, and UL33 are required for genome packaging into the capsid [21]. The terminase consists of **pUL15**, **pUL28**, and **pUL33** and overcomes under ATP hydrolysis the intrinsic DNA repulsion during packaging. pUL15 recruits importin α5 for nuclear import of the terminase complex, and NLSs have been identified in pUL15 and pUL33 but not pUL28 [144]. Moreover, HSV-1–**pUL32** may play a role in localizing capsids to the sites of DNA packaging, but the determinants of its import are unknown [145]. DNA packaging generates an internal capsid pressure of tens of atmospheres that induces outward movements on the portals but also the other vertices [122,138]. The CATC proteins **pUL17**, **pUL25**, and possibly **pUL36**, are assembled onto nuclear C capsid to counterbalance this internal pressure [15,28,87,146]. At least the C-terminal domain but possibly even full-length HSV-1– and PrV–pUL36 seem to be incorporated into the CATCs of nuclear capsids. However, neither the N-terminal pUL36–NLS (Table 2) nor the entire pUL36 is required for nuclear capsid egress [25,72,91,93,147–149].

Unexpectedly, many HSV-1 membrane proteins also contain predicted NLSs, although except for the pUL34, none have been tested experimentally (gray in Table 2). The nuclear egress complex consists of the capsid-associated **pUL31** and type-II membrane protein **pUL34** and mediates nuclear capsid egress [150]. The biological role of potential NLSs in the other HSV-1 membrane proteins is unclear; some of them, for example, gB, gH/gL, might also be targeted to the inner nuclear membrane to mediate membrane fusion of primary enveloped virions with the outer nuclear envelope to complete nuclear egress (Figure 1e; [151]). Both pUL31 and pUL34 depend on specific NLSs to be targeted to the inner nuclear membrane [152,153]. HSV-1–pUL31 coprecipitates with transportin-1, importin α1, but not α3, α5, α7, or β1 [154], and interacts via its NLS with imp α1, α3, and α6 in yeast-2-hybrid assays [152]. Similarly, PrV–pUL31 coprecipitates with transportin-1, importin β1, α1, α3, α5, and α7 [155]. The nucleoplasmic tail of HSV-1–pUL34 interacts via its NLS with importins α1, α3, and α6 in yeast-2-hybrid assays, and coprecipitates with importins β1 and α6 [153].

In fibroblasts and primary neurons, the formation of nuclear HSV-1 capsid compartments is impaired in the absence of importin α1, and quantitative electron microscopy shows that the nuclei of these fibroblasts contain overall fewer but proportionally more mature capsids [61]. These observations suggest that importin α1 might be required for the nuclear import of capsid proteins and the nuclear egress complex. However, as upstream events such as efficient nuclear import of immediate–early and early HSV-1 proteins, as discussed above, are also impaired in cells lacking importin α1, a general delay in the progression of HSV-1 infection might also contribute to these later phenotypes.

Importins in innate immunity and restricting capsid docking to nuclear pore complexes

As the release of the viral genomes into the nucleoplasm is essential for infection, there are also intrinsic and inducible host restriction mechanisms that limit the capacity of incoming capsids to properly align with the NPCs, or that restrict the nuclear import of host proteins contributing to the induction of antiviral host responses.

In addition to supporting herpesvirus infection, several NTFs also indirectly modulate viral infection: they mediate the nuclear import and export of transcription factors contributing to the induction of IFN, pro-inflammatory cytokines, and IFN-stimulated genes such as the IFN regulatory factors IRF3 and IRF7 or NF-κB. On the other hand, viral proteins interfere with the nuclear import, and target, for example, importins to cytoplasmic organelles or induce their degradation [156]. VP24 of ebolavirus binds importins α5, α6, and α7 via a non-canonical NLS, and blocks the nuclear import of phosphorylated STAT without interfering with binding to conventional NLSs [157].

Moreover, the IFN-inducible GTPase **MxB** binds to HSV capsids and reduces capsid targeting to NPCs as well as the nuclear import of viral genomes [113,158,159]. MxB can disassemble HSV-1, HSV-2, and VZV capsids *in vitro* [113]. It remains to be tested whether capsids are also attacked in cells upon IFN induction and whether MxB could also disassemble other viral capsids [113]. Interestingly, endogenous MxB localizes to NPCs, and it can interact with transportin-1, Nup358, and Nup214, possibly via a basic N-terminal NLS-like ^{11}RRR^{13} motif [160–165]. However, upon IFN induction, all NPC-binding sites appear to be occupied, leading to MxB accumulation in the cytosol [160–165]. Therefore, we have speculated that endogenous, NPC-associated MxB might also destabilize capsids to some extent and promote portal cap dislocation at the NPCs [113]. On the other hand, the increased amount of cytosolic MxB upon IFN induction might lead to premature capsid destabilization and

inappropriate genome release into the cytosol instead of translocation to the nucleoplasm [113].

Conclusions

Nuclear import and NPCs are essential for many viral infections. Viral proteins and capsids interact in various means with the NPCs, the importins, and the nuclear envelope. Dissecting the relative importance of specific importins and Nups for infection of the neurotropic alphaherpesviruses remains a challenge. Perturbing these host proteins in transgenic animals or by RNAi or CRISPR/Cas could affect the function of other proviral or antiviral proteins and thus many stages of the infection cycle. However, coprecipitation assays allow the distinction of direct or indirect binding of tegument or capsid proteins to specific importins or Nups, for example, Nup358 or Nup214. Moreover, specific point mutations, for example, in the large tegument protein HSV-1–pUL36, have revealed functional domains and residues important for capsid targeting to NPCs or for capsid destabilization and genome release into the nucleoplasm.

In addition, cell-free-assays with isolated capsids of different tegument compositions can reconstitute functional capsid–host protein complexes, capsid transport along MTs, capsid binding to NPCs, or genome release into the cytoplasm. Such cell-free assays can be used to dissect potential functions of specific importin-α proteins in axonal capsid transport, capsid docking to NPCs, or nuclear import of tegument or capsid proteins. A further molecular understanding of the herpesvirus capsid interactions with importins and Nups might foster the development of drugs that specifically interfere with the nuclear translocation of essential viral factors without disturbing cellular homeostasis, and might promote the design of synthetic gene-harboring protein cages for novel therapies against brain cancers or neurodegenerative diseases.

Data Availability

All analyzed protein sequences and analysis software are already publicly available.

Declaration of Competing Interest

Nothing to declare.

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