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A Review of the Role of Shuttling Proteins in Herpesvirus Replication and Pathogenesis

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Keywords: Apoptosis, Herpesvirus, Innate immune escape, Nuclear export signal, Shuttling proteins.

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Abstract

Herpesviruses are complex DNA viruses that rely on host-cell machinery for replication and pathogenesis. One critical aspect of their lifecycle involves the transport of viral and host proteins between the nucleus and cytoplasm. This review aims to explore the role of shuttling proteins in herpesvirus infection, focusing on their transport mechanisms, interaction with nuclear transport receptors, and contribution to viral replication and immune evasion. the viral lifecycle is significantly impacted by the transportation of different proteins between the cytoplasm and nucleus throughout viral infection. Shuttling proteins usually consist of nuclear localization signals as well as nuclear export signals for the purpose of mediating proper positioning for themselves and other proteins. They are crucial components in nucleocytoplasmic information transmission inside the cells. The nuclear pore complex on nuclear envelope facilitates the nucleocytoplasmic transport mechanism, which is mediated by certain protein carriers. Ongoing research has progressively clarified which herpesvirus proteins function via nucleocytoplasmic shuttling. An outline of how shuttling proteins use nuclear transport receptors as well as nucleocytoplasmic shuttling signals for nucleocytoplasmic transport is given in the presented work. This research offers a resource for

comprehending herpesvirus infection pathogenesis and formulating novel anti-viral approaches. It also explains how herpesvirus shuttling proteins contribute to efficient infection of viruses through altering their life-cycle and engaging in innate immunity.

Keywords: Apoptosis, Herpesvirus, Innate immune escape, Nuclear export signal, Shuttling proteins.

مراجعة لدور البروتينات الناقلة في تضاعف فيروس الهربس و مسبباته المرضية

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المستخلص

تُعدّ عملية النقل النووي–السيتوبلازمي للبروتينات من الآليات الحيوية التي تؤثر بشكل مباشر على دورة حياة الفيروس. وتعتبر فيروسات الهربس من الفيروسات التي تمتلك تلك الاليات. حيث ان تعتمد العديد من البروتينات الفيروسية على إشارات الدخول والخروج النووي لضمان تموضعها الصحيح داخل خلية المضيف. هذه الاشارات تتيح للفايروسات أداء وظائفها في التعديل المناعي والتكاثر. علاوة على ذلك, تلعب بروتينات النقل دورًا محوريًا في تنظيم هذه الحركة عبر المسام النووية وذلك باستخدام مستقبلات نقل متخصصة. في هذا السياق، يهدف العمل الحالي إلى تسليط الضوء على دور بروتينات النقل في آلية العدوى بفيروسات الهربس. بالإضافة الى ذلك التركيز على كيفية استخدامها لإشارات النقل النووي والمستقبلات المرتبطة بها, وذلك لفهم تأثيرها في تعديل دورة حياة الفيروس والتفاعل مع المناعة الفطري.

الكلمات المفتاحية: موت الخلايا المبرمج، فيروس الهربس، الهروب من المناعة الفطرية، إشارة التصدير النووي، البروتينات الناقلة.

1. Introduction

Herpesviruses are enveloped viruses that have got large double-stranded DNA genomes found in nucleus. Herpesviruses are big, spherical, and have icosahedral symmetry, measuring between 150 and 200 nanometers. With an average range diameter of 100 nm, the icosahedral protein capsid is made up of 162 hollow hexagonal and pentagonal capsomeres with an electron-dense core that houses the double-stranded DNA genome. The nucleocapsid is made up of 125–240 kbp nucleotides. **[1]**, as shown in **Figure 1**. They belong to the Herpesviridae family. These viruses have a biphasic lifecycle with lytic and latent phases, this enables them to cause chronic infections in their hosts. They could infect humans as well as other vertebrates. There are three subfamilies of herpesviruses based on characteristics including Alpha, Beta,

and Gamma. These characteristics play a crucial role in disease processes, cell tropism and latency sites. Alpha-herpesviruses are responsible for infecting animal. For example, Marek's disease virus, pseudorabies virus, and bovine herpesvirus-1. Moreover, they cause human infections, such as varicella-zoster virus and herpes simplex virus type 1 and 2. In sensory neurons, such viruses typically remain latent [2].

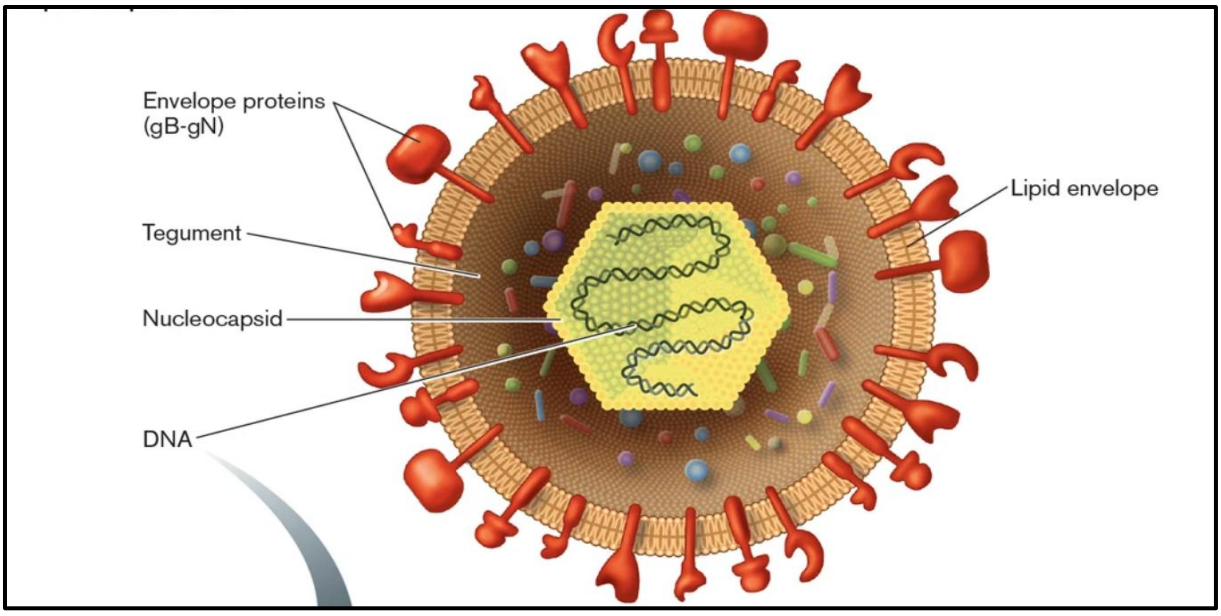
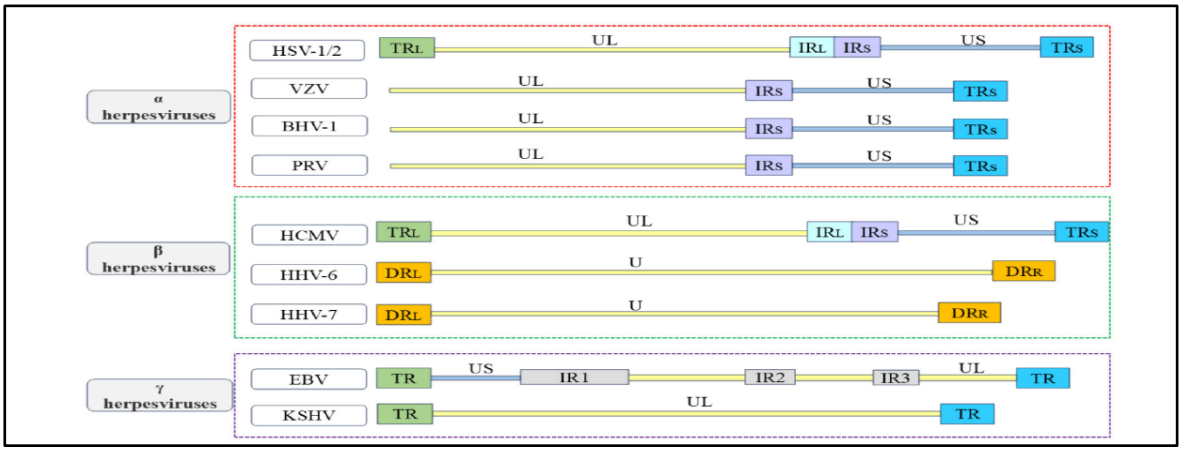


Figure 1: Structural Organization of Herpes Simplex Virus Type 1 (HSV-1) [1].

Human cytomegalovirus, human herpesvirus-6, and -7 are the primary members of the beta-herpesvirus group. They have a tendency to develop latency in mononuclear cells. Gamma-herpesviruses, on the other hand, usually remain dormant in lymphocytes. For example, Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus in humans, as well as alcelaphine herpesvirus 1 and herpesvirus saimiri in animals [3]. The capsid, a DNA core, a layer of tegument around it, and an outer lipid envelope make up herpesvirus particles structurally. The unique short and long regions of viral genome are surrounded by different repeat sequences. These consist of internal, direct, and terminal repetitions that differ amongst herpesvirus species and affect their genomic structure. The coordinated action of numerous viral proteins supports viral replication and propagation. These proteins must localize to specific cellular compartments by crossing barriers such as the plasma and nuclear membranes. Some strategies for viral life cycle and replication. In certain viral proteins there is the capacity of shuttling between cytoplasmic and nuclear compartments, such as important processes including regulation of host transcription, translation, immune evasion, mRNA transport, or virion assembly [4].

For instance, proteins such as UL47 of HSV-1 and BHV-1 may act in the nucleus or during the replication process and then migrate to the cytoplasm for virion assembly. These proteins, called the nucleocytoplasmic shuttling proteins, via their NLS (nuclear localization signals) and NES (nuclear export signals), alter their subcellular location. They are found throughout the cell and their spatial and temporal distribution directly affects the progression of the viral lifecycle; they are trafficked through interaction with host receptors and adaptor proteins [5]. Notably, many herpesvirus lytic genes are intronless. Since transcription takes place in the nucleus, the resulting mRNA must be efficiently exported to the cytoplasm. It is a process that is mediated by proteins like ICP27 which re-invigilate host-based RNA processing pathways in order to sustain viral transcripts to stay constant as well as be effectively translated. There are also other viral proteins, one of which is the HSV-1 VP19c, the HCMV UL94 and KSHV ORF45 that help in the virion assembly **[6].**

Herpesviruses maintain infection by the use of a defined set of specific proteins. The most notable of them (PRV UL46, HSV 1 g134.5, and HCMV UL94) align notably due to their ability



to disrupt the most immune signaling pathways. The Kaposi herpesvirus that is known as latency-associated nuclear antigen-2 (LANA2) serves as another immune-evasion determinant in latency **[3]**. Like nuclear importation through nuclear localization signals (NLS), and nuclear export, nucleocytoplasm-based transport protein fermented by nuclear export signals (NES), are essential to the coordination of nuclear cellular infection by herpesvirus. These proteins modulate temporally regulated viral gene expression as well as delayed development of the lytic phase. Thorough knowledge of these processes of transport has become central to understanding the pathogenesis of herpesvirus **[5].**

Figure 2: A summary of the Genome Structure of Herpesvirus **[7].**

2. The Mechanism of Nucleocytoplasmic Shuttling in Shuttling Proteins

The remaining shuttling proteins have been shown to have nucleocytoplasmic taxishm which is similar to that of the herpesvirus shuttling proteins. As further outlined below, individual steps are characterized by many protein protein interactions and influenced by complex strict regulatory systems **[8].**

2.1. Pathways of Nucleocytoplasmic Shuttling

The nuclear envelope that separates the cytoplasm and the nucleoplasm is a physical barrier that limits movement of macromolecules in between the nucleoplasm and the cytoplasm. The inner and outer nuclear membranes are penetrated and bridged by a number of supramolecular structures known as nuclear pore complexes, which are made up of nucleoporins and mediate nucleocytoplasmic transport **[9]**.

2.2. Receptors Mediating Nucleocytoplasmic Transport

Nuclear transport receptors are specialized transport proteins that regulate the nucleocytoplasmic shuttling of proteins across nuclear pore complexes (NPCs). Karyopherins are essential for transporting materials between cytoplasm and nucleus. Karyopherins are divided into two primary categories depending on their functions: exportins and importins. Usually acting as heterodimers, importins are classified into two families: importin-β and importin-α. Finding the NLS on cargo proteins is the task of importin-α. Three functional domains are present in this molecule: Importin-β1 interacts with an N-terminal importin-β-binding (IBB) domain. NLS-bearing cargo is recognized and bound by Armadillo (ARM) repetitions in a central region. C-terminal domain that promotes nucleocytoplasmic translocation by binding to nucleoporin 50 and the export factor CAS. Conversely, importin-β proteins have a role in the nuclear import of RNA and proteins **[10]**. They are distinguished by two conserved regions: an N-terminal domain unique to importin-β and a central HEAT domain (called after Huntingtin, Elongation factor 3, and protein phosphatase (2A). As shown in **Figure 3**, members of the importin-β family include exportins and bidirectional transport receptors in addition to importins.

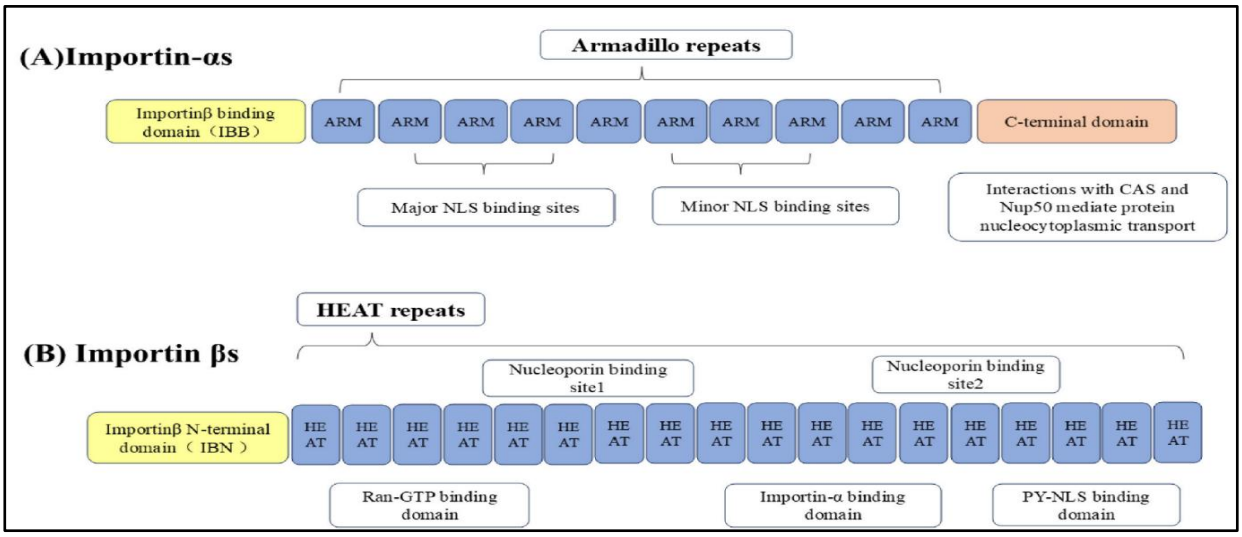


Figure 3: The two groups of importins are (B) importin-β and (A) importin-α **[11].**

2.3. Sources of Energy Driving Nucleocytoplasmic Transport

A dynamic flow of energy headlined by Ras-GTPases and Ran GTPases has been outlined within nuclear envelope, which is necessary to the transfer of shuttling proteins to the specific nuclear transport receptors. Ran majorly serves intercapillary and cytoplasmic protein and RNA transport, is the biggest Ras-GTPase superfamily and also serves as a operation regulator of the importin-β protein. To conclude, nuclear transport receptors, nuclear pore complex, Ran GTPases, and cargo proteins spatial, and temporal interaction facilitate the nuclear pore complex in accomplishing numerous biological functions **[12]**.

2.4. Nucleo-cytoplasmic-shuttling proteins into nucleus

A particular device of the translocation of cytoplasm proteins to the nucleus is organized by a set of signaled sequences, one of which is the transport nuclear localization signal (TNLS). TNLS is a small peptide, which has a unique amino acid chain and which acts as a nuclear localization signal whose disposition coordinates in an orientation-specific translocation of proteins via the nuclear pore complex (NPC) to specified subcellular destinations. A wide range of proteins contain nuclear localization signals that have irregular structural motifs, herein referred to as noncanonical nuclear localization signals (ncNLSs). Varied range of ncNLS variants has been discovered.

These could be broadly classified as spatial epitope NLSs, proline and tyrosine-containing NLSs, and NLSs rich in arginine (IKNLSs). Importin5 recognizes the IK-NLS, whereas

transportin1 recognizes the PY-NLS, which is more complicated when compared to cNLS. A cytoplasmic cNLS−cargo is first accessible to binding to importin -a using the canonical nuclear import pathway and later it links to importin -b1 by its IBB domain to form a trimeric complex [13]. Importin -2 is then the one that assists in getting this trimer to the nucleus. GTP hydrolysis catalysed by the Ran GTPase provides the energetics needed by the trimer to traverse the nuclear pore complex. Integrations The activation of importin-2 and the cNLS cargo depends on both binding of Ran GTP to the trimer, with the aid of importin-2, i.e. release of importin-2 and subsequent conformational rearrangements of the trimer.

It is during the solid state of the internal skeleton that cargo is carried, and it is kept in the nucleus. Second to importin- 1 in mediating non-classical nuclear entry is transportin-1, which is often mediated through importin- 2 binding to non-canonical NLS signals. It seems that the selective importation of proteins that contain the PY-NLS motif is determined by Transportin-1. Conclusively, protein importation into the nucleus by NLS is an essential constituent that assures correct protein functioning **[14]**.

2.5. Nucleocytoplasmic shuttling of proteins out of the nucleus

Nucleocytoplasmic shuttling of proteins must interact with exportins to facilitate the gradual transport regarding the shuttling protein from nucleus to the cytoplasm when it has completed its function. NES is a brief motif of hydrophobic or leucine-rich amino acids. CRM1, sometimes referred to as export 1 or Xpo-1, is the most well-identified of seven exportin types. CRM1 has a key role in nuclear export mechanism of shuttling proteins. CRM1-NES cargo RanGTP ternary export complex is first formed when NES on shuttling-protein links to the CRM-1. After attaching itself to a variety of nuclear pore proteins, the ternary complex passes through NPC and enters the cytoplasm **[15]**. Lastly, for the subsequent export cycle, CRM1 makes its way back to the nucleus via NPC. In conclusion, NES is crucial for shuttling protein transport, and the dynamic mechanisms that NES and NLS mediate serve as the foundation for shuttling proteins to carry out various tasks, as illustrated in **Figure 3.**

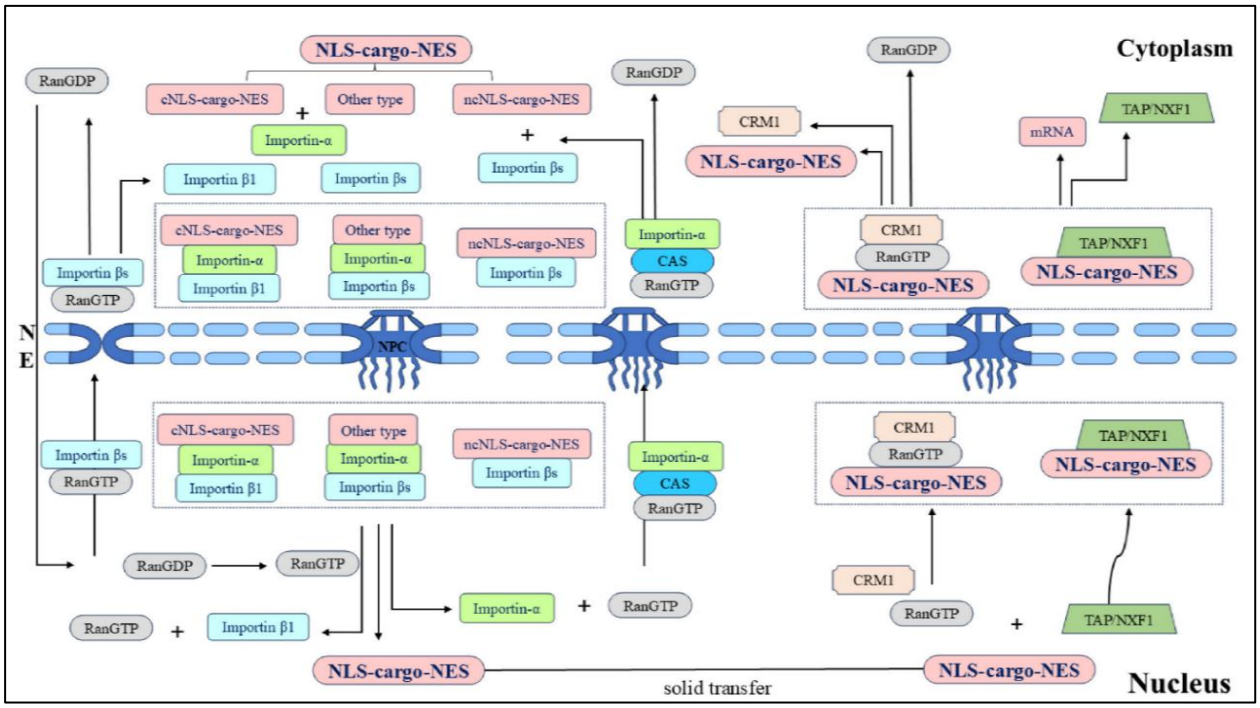


Figure 4: Diagrammatic representation of shuttling-protein NLS-cargo-NES protein structure nucleocytoplasmic transport and related protein molecular circulation **[16].**

3. Latency and reactivation mechanisms in herpesviruses

Herpesvirus DNA often persists as stable, circular episomal forms in the host cell nucleus throughout the latent phase. HHV-6 is an exception, as it integrates into the host chromosomes' telomeric regions. The expression regarding a single transcript known as latency-associated transcript (LAT) is what defines latency in HSV-1, whereas immediate-early protein ICP-0 is highly essential for starting the lytic phase and reactivation. Only VLT-ORF63 (VLT63) fusion transcript as well as the VZV latency-associated transcript exhibit latent gene expression in varicella-zoster virus (VZV) [11]. The tegument protein IE62, which is expressed by VZV, enters the nucleus after infection and activates immediate early, early, and late viral genes, causing lytic replication [17]. Different viral proteins are involved in the control regarding latency as well as reactivation in gamma and beta herpesviruses. The IE2 and IE1 proteins, which are encoded by UL122 and UL123, respectively, are crucial in HCMV. IE2 regulates the expression regarding downstream viral genes, IE1 is necessary to start lytic infection as well as reactivation. EBNA1, the first latency protein identified in EBV, serves to bind the viral episomes to host chromatin. Classified as latency I, 0, IIb, IIa, and III, EBV latency exhibits a variety of gene expression patterns. Rta (BRLF1) and Zta (BZLF1) are immediate-early transcription factors that regulate reactivation [13].

Through limited expression of viral genes, mainly LANA1, which stabilizes episomal maintenance, KSHV sustains latency. Transcriptional activator RTA, encoded by UL50, regulates the transition to lytic phase. Herpesviruses utilize several latency techniques. Alpha herpesviruses typically do not require viral anchoring proteins to establish latency in non-dividing neurons. By using viral proteins to secure genome to host chromosomes and guarantee their distribution throughout cell division, gamma and beta herpesviruses, at the same time, maintain latency in growing cells. The lifetime of herpesviruses in all three subfamilies is essentially the same, despite these variations. The shuttling proteins' function in influencing the course of reactivation and infection can be better understood by looking at the lytic phase **[18].**

3.1. Replicative Cycle of Herpesviruses

3.1.1 Mechanisms of Herpesvirus Entry into Host Cells

The entry process of alpha, beta, and gamma herpesviruses into host cells involves a complex interplay among several envelope glycoproteins, each designated by a letter preceded by the prefix "g" to indicate glycosylation. Initially, herpesviruses attach to the host cell surface using multiple glycoproteins and a range of cellular receptors; however, this attachment alone does not initiate viral entry **[19].** Depending on the type of cell, the viral envelope either enters the cell by endocytosis or directly fuses with host cell membrane after attachment. Herpesviruses share a consistent core entry mechanism including the glyco protein gB and the gH–gL heterodimer, despite the fact that they infect a variety of host cells. However, in order to contact various host receptors, each herpesvirus subfamily uses unique combinations regarding glyco proteins, enabling them to target particular cell types as well as tissues **[20]**.

3.1.2. Dissociation of Tegument Proteins and Capsid Transport

As soon as herpesviruses penetrate a host cell, tegument associated capsid virions and their proteins are transported into cytoplasm. One component of the tegument proteins dissociates rapidly from the capsid, where the rest of the components stay attached and interact with micro-tubules network to facilitate efficient capsid transportation to the nucleus. Upon reaching the nuclear envelope, an opening forms at one capsid vertex. Certain capsid proteins then engage with NPC components, particularly Nup214 and Nup358, ensuring proper docking and triggering genome release **[21]**.

The densely packed, negatively charged viral DNA within the capsid generates substantial internal pressure, which has been hypothesized to be several magnitudes greater than regarding atmospheric pressure. This force pushes the DNA through the NPC, overpowering the selective blocking of the pore and allowing the genome to enter the nucleus. For alpha herpesvirus, e.g., HSV-1, proper function requires a stepwise dissociation of the tegument proteins. Initially, outer tegument proteins are released, followed by inner ones. VP16 is the first outer tegument protein to dissociate. It is followed by VP13/14, encoded by UL-47 gene, which serves as a shuttling protein as well. The next protein in this sequence is VP22. These proteins play roles in the viral replication process, with UL47 and UL49 representing genes uniquely associated with alpha herpesviruses. Conversely, the dynamics of dissociation in tegument proteins in varicella -zoster virus (VZV), 2 -herpesviruses, and 3 00 -herpes viruses are relatively ill-defined **[22].**

3.1.3. Transcription, replication, and capsid assembly of viral genomes

After viral DNA reaches the nucleus, linear genome replicates by a rolling circle process and goes through circularization, producing concatemeric DNA. The basic assembly unit, which eventually assembles into a mature nucleocapsid, is formed by the capsid proteins of transport into the nucleus after translation, transcription, and DNA replication are finished. Within the nucleus, transcription of viral genome takes place in a cascade-like pattern. Depending on their transcriptional timing, herpesvirus genes are divided into 3 classes, which are: immediate-early (IE), early (E), and late (L) genes. Prior to DNA replication, transcriptional activators initiate the transcription of IE genes. After being exported to cytoplasm, the resultant IE mRNA is translated into IE proteins. After returning to nucleus, such proteins trigger the transcription of E genes. The expression and transcription of L genes are then started by the E gene products. IE proteins use synchronized nuclear export and import mechanisms to promote the expression of E and L genes during this cycle **[23]**.

As will be shown in more detail in the following sections. The members regarding to the ICP27 protein family are essential for the export of viral mRNA. It is thought that most of the capsid assembly pathway is shared by all herpesviruses. Similarly, there is a great degree of similarity in the function and structure of the main capsid proteins in Herpesviridae family. For instance, VP5 and VP23 are important capsid proteins in HSV-1. Following cytoplasmic synthesis, VP5 and UL26.5 combine to create a complex, and VP23 associates with VP19C to form a second

complex. These parts are brought into the nucleus by NLS located in VP5 and VP19C, where they come together to form the capsid structure.

The terminase complex recognizes specific cis-acting "pac" motifs on the concatemeric viral DNA, performing two cleavage events to generate a unit-length genome. This genome is then loaded into the preformed capsid through an interaction with the UL6 portal protein. Amongst those components, HSV1 VP19-C functions as a shuttling protein and will be discussed in greater detail in the following section [24].

3.1.4. Nuclear egress, secondary envelopment and release.

Following assembly, the nucleocapsid engages in interactions with the nuclear envelope and acquires a temporary envelope through budding through the inner nuclear membrane. This process is referred to as primary envelopment. Thus, perinuclear virions are created and placed in the perinuclear area. Following de-envelopment at outer nuclear membrane, such virions release naked, or non-enveloped, capsids into cytoplasm [25]. All of the herpesvirus subfamilies' nucleocapsids successively attach to tegument proteins once they are in the cytoplasm. After budding into trans-Golgi network, they acquire a secondary envelope and develop into mature virions. Depending on the type of herpesvirus, secondary envelopment usually occurs in Golgi apparatus, although it can occur in early endosomes or autophagosomes. Most people agree that the Golgi is the primary location for envelope acquisition. The intercellular transmission cycle is then completed when mature virions are encapsulated in cytoplasmic vesicles and transferred to the plasma membrane by exocytosis. Two conserved regulatory proteins are necessary for herpesviruses to mediate nuclear egress. UL31's Epstein-Barr virus homolog, BFLF2, is these among one; it functions as a shuttling protein and will be covered in more detail in a later section [26].

4. Herpesvirus Shuttling Proteins and Their Functional Roles Throughout the Viral Lifecycle

Herpesviruses from different subfamilies use slightly distinct mechanisms to infect host cells. Over time, they have developed specific strategies to manipulate nucleocytoplasmic transport pathways, optimizing conditions for viral replication. Nuclear export sequence elements are designed specifically to move the proteins to the cytoplasm. Host cell trafficking systems were essential for viral parasitism, and viral proteins took advantage of the NLS, NES, and other functional motifs to exploit nuclear transport receptors to enhance viral propagation. The herpesvirus lifecycle is nuclear-centered, with key processes such as transcription, genome

replication, and capsid assembly occurring in the nucleus. Regulating the movement of viral factors such as DNA polymerase, capsid protein monomers, transpeptidation factors, and other proteins into the nucleus becomes crucial. After the newly formed capsids have entirely assembled, the capsids get rid of the nucleus, and the tegument and glycoprotein coating complete their assembly. The NES elements regulate the translocation of viral proteins from the nucleus to other parts of the host cell to maintain its lifecycle.

In conclusion, herpesvirus-encoded shuttling proteins are indispensable in guaranteeing the precise subcellular localization of viral factors through the interplay between NLS, NES, and adequate transport machinery provided by the host. Any mutation in the pattern of NLS and NES variants disrupts the dynamic transport process and, as a result, completely impairs viral reproduction. A comprehensive summary of the role of shuttling proteins in the herpesvirus life cycle (Figure 4).

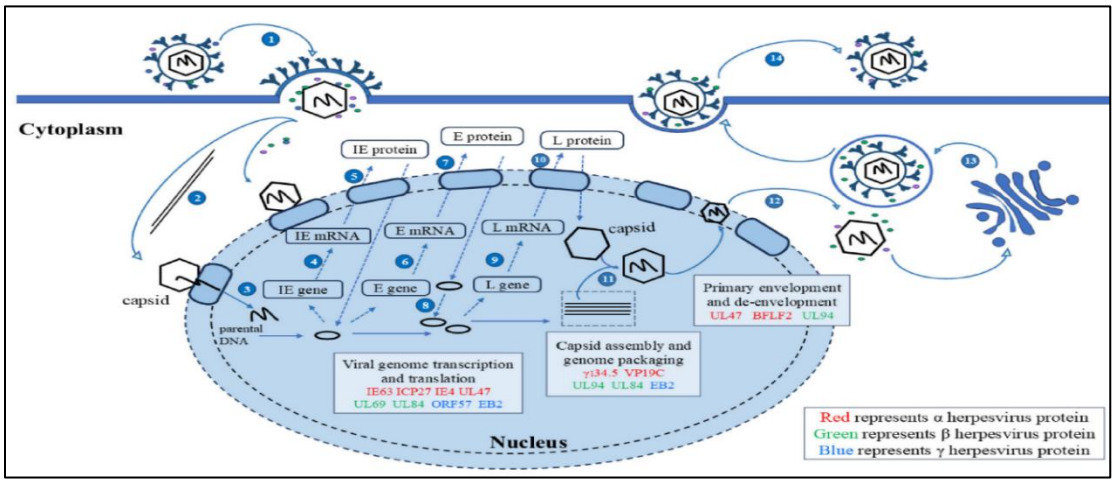


Figure 5: Herpesvirus shuttling proteins contribute to several stages of the viral life cycle [28].

4.1. Regulation of mRNA Transport by Herpesvirus Shuttling Proteins

mRNA transport in herpesviruses is a regulated process intimately tied to transcription, splicing, post-transcriptional modification and translation. These events are independent and interrelated steps in the host cell. The lack of introns in most herpesvirus genes requires them to utilize other means for nuclear export because export factor recruitment here cannot depend on splicing [29].

For that reason, the helter-skelter regulation of mRNA export plays significant roles in viral replication and pathogenicity. Various herpesviruses have evolved to foil multiple host export pathways, including Aly/REF, CRM-1, and TAP/NXF1, and encode regulatory proteins

that assist the export of viral transcripts from the nucleus. These are exigency viral export elements, which ensure the transfer of the transcripts from the nucleus to the cytoplasm. HSV-1 is a representative example of these proteins. ICP27, and its shuttling behavior, obliges functionality to the NLS, NES, and other useful zones. This viral protein recruits cellular terms, providing conveyance through TAP/NXF1. ICP27 straightforwardly ingenuity the trade of viral transcripts, including UL15, UL17, UL48, UL30, UL29, UL42, it can be. UL5. ICP27 mutation analysis supports that it is functional. Mutant analysis involving 16 ICP27 mutants uncovered its critical functioning. M11, M15, and M16 mutations are decisive, and they have substitutions of amino acid differences at locations 340/341, 465/466, and 488 [30].

Disruption of the M15 mutation in ICP27's worldly binding to Nup62 results in defective re-export and continuous shuttling. These viruses cannot induce late gene expression and replicate adequately. Similarly, a nuclear export signal NES-defective ICP27 mutant results in reduced shuttling and viral replication and reduced affinity for RNA binding, indicating the involvement of protein in export and gene expression. Finally, the VZV IE4 protein, another cyclical molecule with the world receptor TAP/NXF1, contains binding sites for cellular SR proteins. The three internal regions, Ra, Rb, and Rc, control its binding to RNA, and the host transcription factors p50 and TFIIB and the nuclear localization signal transmembrane, which is directed to the nucleus, are in the Rb domain [25].

As with RNA binding, when IE4 binds to RNA, the main fraction of is confined within the cytoplasm, precluding a question of spatiotemporal control. With the alpha herpesvirus UL47 protein family, HSV-1 and BHV-1 UL47 proteins use an importin β-dependent shuttling mechanism. UL47 proteins exhibit a conserved motif responsible for RNA interaction and nuclear delivery. At early times post-infection, HSV-1 UL47 forms nuclear domains adjacent to the transcription site. Thus, the protein is intimately involved in post-transcriptional processes. With the beta relative HCMV UL69, this means it functions as a posttranscriptional activator or RNA sign export factor [31].

It binds helicases UAP56 and URH49 through overlapping motifs, controlling nuclear targeting and RNA interactions. Mutations disrupting binding by either UAP56 or NES abolish virus shuttling, reducing replication and viral gene expression. Diverse homologs of UL69 across species, such as C69 in chimpanzees or Rh69 in rhesus cytomegalovirus, retain shuttling and homodimerization, but only cytomegaloviruses heterodimerize with host factors. Another related protein is HCMV UL84, which promotes cytoplasmic accumulation of early transcripts.

It enters the nucleus via interaction with importin-α and associates with UL44 in viral replication compartments. UL84 also functions as an RNA-binding protein, and NES-defective mutants show reduced export of IRS1 mRNA, a transcript critical for suppressing host protein synthesis via eIF2 dephosphorylation [32].

Predicted to belong to the DExD/H RNA helicase family, UL84 supports its involvement in nuclear export. Gamma herpesvirus proteins such as EBV EB2 utilize the DN domain to recruit REF, facilitating mRNA export. Additionally, the HVS ORF57 protein targets mRNA for export by localizing to the nucleolus. Mutations in its NLS prevent this localization and abrogate its function in mRNA export and gene activation, reinforcing the importance of nucleolar transit in viral transcript, processing as show in **Figure 5.**

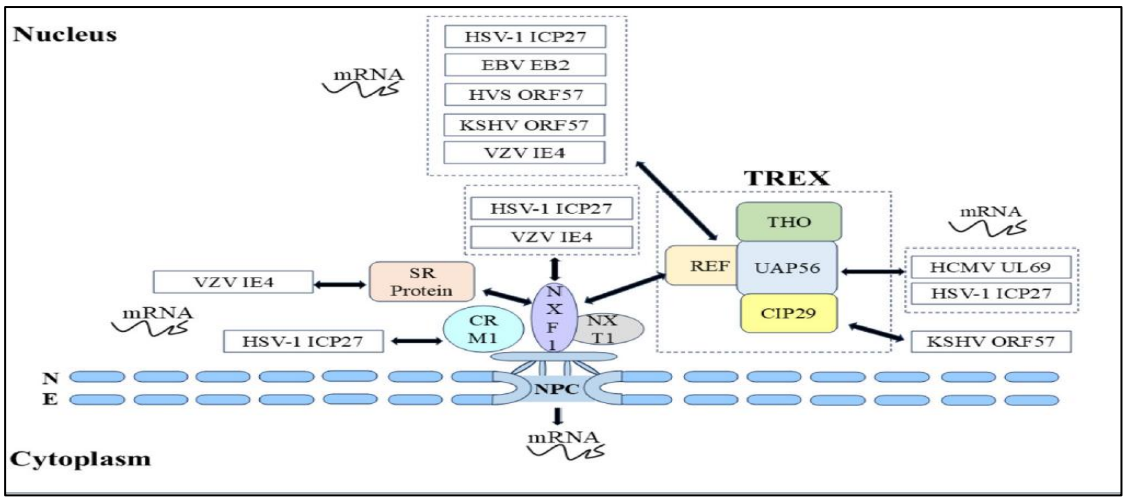


Figure 6: Mechanism of viral mRNA export by herpesvirus shuttling proteins [33].

5. Regulation of Apoptosis by Herpesvirus Shuttling Proteins

Apoptosis can be a defined as a programmed cell death form that is characterized by specific bio-chemical and morphological changes. During the early phase of viral infection, apoptosis can limit viral propagation by eliminating infected cells, while in later stages, certain viruses harness apoptotic processes to facilitate replication and release. In bovine herpesvirus 1 (BHV-1), the UL47 protein selectively suppresses, the phosphorylation of structural maintenance of chromosomes 1(SMC1) in the nuclei compartment, but has no effect on the processes that take place in the cytoplasm. This excessively activates the ATM/NBS1/SMC1 signaling cascade, a critical DNA damage response pathway and this drives the impairment of DNA repair. Research has shown that reduced phosphorylation of SMC1 compromises cellular survival following genotoxic stress [34].

In cells expressing the UL47 protein, apoptosis induced by DNA damage is significantly increased, suggesting that UL47 contributes to apoptosis by hindering SMC1 function and DNA repair. Another protein, VP22—encoded by the UL49 gene of BHV-1—is a tegument component with a NES at its C-terminal region that overlaps with a mitochondrial targeting sequence. Overexpression of VP22 in HeLa cells has shown to induce apoptosis, which was possibly due to the transfer of VP22 location from the cytoplasm towords the mitochondria; the mitochondrial pathways may thus play a role in the apoptosis induced by VP22 In gamma herpesviruses, the latency-associated nuclear antigen 2 Actively transcribed only in viral-infected B cells, has been identified from KSHV and is responsible for initiating the apoptotic resistance; LANA2 inhibits the p53-dependent transcriptional activation of the protein kinase R pathway. A form of NLS, the nuclear localization signal, is critical for the apoptotic resistance by LANA2, whose mutants relocate this complex from the nucleus to the cytoplasm, thereby inhibiting the capability to suppress p53-mediated transcription. [31].

Subsequent research shows that LANA2 phosphorylation disrupts NES function and reduces its anti-apoptotic potential [35]. These findings underscore the importance of LANA2 nuclear retention in suppressing apoptosis and highlight the significance of its shuttling behavior in sustaining viral replication. Overall, herpesvirus shuttling proteins modulate apoptosis-related signaling nodes by relocating between the nucleus and cytoplasm. Through this dynamic movement, they can either promote or suppress apoptotic processes, depending on the stage of infection and the needs of the virus.

5.1. Herpesvirus Shuttling Proteins and Their Role in Immune Evasion

The significant capacity of Herpesviruses to induce lifelong infection and a strong level of host selectivity have distinguished Herpesviruses. Recognition of viral nucleic acids by pattern recognition receptors, in particular MDA5 and RIG-I which sense pathogen associated molecular patterns leads to activation of host cellular innate immune response, and communication of such signals by adaptor proteins, including MAVS and STING. Events of subsequent signaling lead to nuclear translocation of major transcription factors, such as NF-κB, IRF-3, IRF-7 transcription factors, and results in the transcription of type I interferon (IFN-I) and pro-inflammatory cytokines. Subsequently after this, the JAK-STAT cascade is activated that augments the expression of interferon stimulated genes (ISGs) and therefore establishes an appropriate antiviral state. Conversely, the viruses in herpes have a synthesis amount of anti-host defense mechanisms [36].

The genomes of herpesviruses encode diverse sets of proteins that activate active immune evasion through inhibition of type I interferon synthesis, interference with downstream interferon -signaling cascades, and regulation of interferon -stimulated gene expression as well as blockage of essential factors of the innate immune responses, etc.. As an example, nuclear localization of the bovine herpesvirus 1 (BHV-1) transcriptional regulator ICP27 is essential in silencing the IFNβ promoter in cells transfected; mutations in its N-terminal nuclear localization domains disrupt this silencing capability. Also, ICP27 can disrupt the maturation of host cellular mRNAs, by disrupting the 3′-end processing of transcripts required to express IFN-β, although this potential mechanism remains under study. The HSV-1 homolog ICP27 also activates innate immune signaling; it causes the relocalization of the cellular protein Daxx out of the nucleus into the cytoplasm, thus leading to its association with the subunit of NF-κB p65 and a suppression of NF-κB transcriptional activity **[25].**

This modulation of Daxx localization contributes to viral evasion of intrinsic immune responses. STAT1, a key mediator in IFN-I signaling, is regulated by BHV-1 UL47. Lack of a functional NLS in UL47 prevents STAT1 from accumulating in the nucleus following interferon stimulation, thereby attenuating antiviral signaling. HSV-1 ICP27 also inhibits IFN-I responses by repressing the phosphorylation and nuclear translocation of STAT1. ICP27 mutants that lack either NES or NLS, disrupting the specific localization to cytoplasm after nuclear export, have been tested and found unable to effectively block expression of IFN. Some viral proteins also interfere with upstream immune signaling. UL46 protein of pseudorabies virus (PRV) modulates STING activity in the cytoplasm, undermining innate immune sensing **[11]-[13]**.

Similarly, HSV-1 g134.5 inhibits the trafficking of RIG-I to mitochondria and STING to migrate from the endoplasmic reticulum to the Golgi. It also prevents IRF3 from being activated and entering the nucleus, resulting in the inhibition of IFN induction. In beta herpesviruses, the HCMV UL94 inhibits antiviral signaling by restraining STING. It blocks the drawing of TBK1 to STING composites, which is required to hasten IRF3 filial and IFN-I creation subsequently. It selectively interferes with later signal transmission, thwarting immune activators. In gamma herpesviruses, KSHV LANA2 regulates the NF-kB by prompting its nuclear accretion, improving transcriptional productions. ORF45, another KSHV-made protein, prevents IFN-I assembly by confining IRF7 and averting its filial and nuclear localization. Altogether, these examples show how herpesvirus shuttling proteins orchestrate host immune processes by

altering the cytoplasmic subcellular localizations of signaling molecules and transcriptional agents. In doing so, they weaken the interferon reaction and help with viral endurance, displaying a suave viral strategy of immune repeal. algorithm: [37]

6. Conclusion and Future Trends

The review aims to explore the role of nucleocytoplasmic shuttling proteins in herpesvirus replication and pathogenesis. These proteins regulate mRNA transport, capsid formation, and early envelopment by moving between the nucleus and cytoplasm. Viruses use host nuclear export pathways in order to enable viral protein production and in order to avoid host defense mechanisms. Without interactions with nuclear localization and export signals (NLS/NES), genome replication and immune evasion are not achievable. In-depth knowledge of these pathways can be used to focus antiviral interventions on the shuttling pathways.

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Abbreviations list

Abbreviation	Description
HSV-1	Herpes Simplex Virus Type 1
HSV-2	Herpes Simplex Virus Type 2
PRV	Pseudorabies Virus
VZV	Varicella-Zoster Virus
BHV-1	Bovine Herpesvirus Type 1
HHV-6	Human Herpesvirus Type 6
HHV-7	Human Herpesvirus Type 7
HCMV	Human Cytomegalovirus
KSHV	Kaposi Sarcoma-Associated Herpesvirus
EBV	Epstein-Barr Virus
UL	Unique Long Segment
TRL	Terminal Repeat Long
IRL	Internal Repeat Long
US	Unique Short Segment
U segments	Heterogeneous Genomic Regions in HHV-6 and HHV-7
Importin-α	Alpha Importin (Group A)

Abbreviation	Description
Importin-β	Beta Importin (Group B)
IBB	Importin-β Binding Domain
CAS	Cellular Apoptosis Susceptibility Protein (Export Factor)
Nup50	Nucleoporin 50
ARM	Armadillo Repeat Motif
NLS	Nuclear Localization Signal
IBN	Importin-β-Specific N-terminal Domain
HEAT	HEAT Repeat Domain (Conserved Structural Domain)
LAT	Latency-Associated Transcript (defines latency in HSV-1)
ICP-0	Immediate-Early Protein 0 (triggers HSV-1 lytic phase and reactivation)
VLT63	VLT-ORF63 Fusion Transcript (latent expression in VZV)
IE62	Immediate-Early Tegument Protein (activates VZV gene expression for lytic replication)
IE1	Immediate-Early Protein 1 (encoded by UL123 in HCMV; initiates lytic infection)
IE2	Immediate-Early Protein 2 (encoded by UL122 in HCMV; regulates downstream viral genes)
EBNA1	Epstein-Barr Nuclear Antigen 1 (anchors EBV episomes to host chromatin)
Rta (BRLF1)	Replication Transactivator (EBV transcription factor for reactivation)
Zta (BZLF1)	Z Transactivator (EBV transcription factor for reactivation)
LANA1	Latency-Associated Nuclear Antigen 1 (maintains episomal stability in KSHV)
RTA (UL50)	Replication Transactivator (KSHV protein regulating lytic switch)

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