Possible Role for Cellular Karyopherins in Regulating Polyomavirus and Papillomavirus Capsid Assembly

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Polyomavirus and papillomavirus (papovavirus) capsids are composed of 72 capsomeres of their major capsid proteins, VP1 and L1, respectively. After translation in the cytoplasm, L1 and VP1 pentamerize into capsomeres and are then imported into the nucleus using the cellular α and β karyopherins. Virion assembly only occurs in the nucleus, and cellular mechanisms exist to prevent premature capsid assembly in the cytosol. We have identified the karyopherin family of nuclear import factors as possible “chaperones” in preventing the cytoplasmic assembly of papovavirus capsomeres. Recombinant murine polyomavirus (mPy) VP1 and human papillomavirus type 11 (HPV11) L1 capsomeres bound the karyopherin heterodimer α2β1 in vitro in a nuclear localization signal (NLS)-dependent manner. Because the amino acid sequence comprising the NLS of VP1 and L1 overlaps the previously identified DNA binding domain, we examined the relationship between karyopherin and DNA binding of both mPy VP1 and HPV11 L1. Capsomeres of L1, but not VP1, bound by karyopherin α2β1 or β1 alone were unable to bind DNA. VP1 and L1 capsomeres could bind both karyopherin α2 and DNA simultaneously. Both VP1 and L1 capsomeres bound by karyopherin α2β1 were unable to assemble into capsids, as shown by in vitro assembly reactions. These results support a role for karyopherins as chaperones in the in vivo regulation of viral capsid assembly.

The virus families Polyomaviridae and Papillomaviridae, collectively referred to as papovaviruses, have a nonenveloped icosahedral capsid surrounding a double-stranded circular DNA genome. The structural proteins of polyomavirus (VP1, VP2, and VP3) and papillomavirus (L1 and L2) are synthesized late in viral infection and imported into the nucleus, where they assemble around newly synthesized viral genomes. Viral capsids are constructed from 72 capsomeres (pentamers) of L1 or VP1, arranged on a T = 7 icosahedral lattice (1). The carboxyl terminus of VP1 or L1 mediates contacts between the pentamers in the assembled capsid (14, 26, 27, 32). Disulfide bonds stabilize the interpentamer contacts for L1 (25, 30, 42), while both disulfide bonds and calcium bridges stabilize these contacts for VP1 (2, 4, 21, 28, 44, 45).

The papillomavirus L1 and polyomavirus VP1 capsid proteins each have a well-defined, canonical nuclear localization signal (NLS) (3, 12, 20, 31, 35, 38, 40, 49). In the cytoplasm, NLS-containing proteins bind the adaptor protein karyopherin α, which has at its amino terminus a karyopherin β-binding domain and at its carboxyl terminus the NLS binding domain (8). Karyopherin α binds karyopherin β, which then docks the protein complex at the nuclear pore (19, 36). After translocation through the nuclear pore, RanGTP dissociates karyopherin β from the complex, resulting in the accumulation of karyopherin α plus NLS protein in the nucleus (15, 36). Once in the nucleus, factors such as nucleoporin Nup2 and the export receptor for karyopherin α, CAS, may facilitate the dissociation of karyopherin α from the NLS (24, 29). An alternative model for the dissociation of karyopherin α from an NLS involves the “NLS-like” sequences at the amino terminus of karyopherin α forming an intramolecular bond with the karyopherin α NLS binding domain, thus competing α from the VP1 or L1 NLS (17, 18).

The subcellular location of papovavirus assembly is controlled such that capsids are assembled in the cell nucleus and not in the cytoplasm (13, 33, 41). One such control mechanism may involve the hsc70 family of cellular chaperone proteins. For example, in mouse cells infected with polyomavirus, hsp70 binds polyomavirus VP1 in the cytoplasm and colocalizes with VP1 after transport into the nucleus (9). Moreover, in vitro capsid assembly of polyomavirus VP1 capsomeres is inhibited by bound hsc70 (7). We have investigated a potential role for karyopherins as chaperones of mouse polyomavirus (mPy) VP1 and human papillomavirus type 11 (HPV11) L1 capsid assembly. We show that the NLS sequences of both mPy VP1 and HPV11 L1 are required for binding karyopherin α2β1. Karyopherins affected the DNA binding properties of HPV11 L1 but not mPy VP1. In vitro, capsid assembly of both HPV11 L1 and mPy VP1 was inhibited in the presence of karyopherin α2β1. We propose that karyopherins play a chaperone function in the papovavirus life cycle by inhibiting capsid formation in the cytoplasm while importing L1 and VP1 capsomeres into the nucleus.

MATERIALS AND METHODS

Preparation of recombinant proteins. Recombinant full-length HPV11 L1, mPy VP1, truncated HPV11 L1 (CA29), and truncated mPy VP1 (NΔ6) were expressed as glutathione S-transferase (GST) fusion protein in Escherichia coli.

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RESULTS

Karyopherin α2β1 binds to the NLS sequences of both mPy VP1 and HPV11 L1. Previous studies have shown that HPV16, HPV45, HPV11 L1, and simian virus 40 (SV40) VP1 proteins are bound by a heterodimer of αβ karyopherins (31, 37–39). The NLS of mPy VP1 is located at its amino terminus, in contrast to HPV11 L1, where the NLS is situated at the carboxyl terminus (Fig. 1A). To determine whether karyopherins specifically interact with the NLS of mPy VP1 pentamers, both the full-length recombinant VP1 protein and a recombinant VP1 protein lacking the NLS (VP1NΔ6) were incubated with karyopherins α2 and GST-β1. Proteins bound to GST-β1 were detected by glutathione-Sepharose chromatography followed by SDS-PAGE. As shown in Fig. 1B, GST-β1 bound both karyopherin α2 and full-length VP1 pentamers, but did not bind VP1NΔ6 pentamers (Fig. 1B, NΔ6 lane). Recombinant full-length HPV11 L1, previously shown to bind the karyopherin heterodimer α2β1 (38), was used as a positive control for the GST-β1 interaction experiments. Glutathione-Sepharose chromatography was performed using GST-β1 incubated with full-length HPV11 L1 pentamers or recombinant HPV11 L1 pentamers lacking the NLS (L1CΔ29) and karyopherin α2 (Fig. 1C). GST-β1 bound karyopherin α2 and full-length L1, but not L1CΔ29 lacking an intact NLS (Fig. 1C, compare lanes L1 and CΔ29). These results demonstrate that the karyopherin heterodimer α2β1 binds both mPy VP1 and HPV11 L1 pentamers in an NLS-dependent manner.

Karyopherins affect HPV11 L1, but not mPy VP1, DNA binding. During translocation of classical NLS-containing proteins through the nuclear pore, RanGTP dissociates karyopherin β1 from the karyopherin α2β1 heterodimer bound to the NLS, resulting in accumulation of the karyopherin α2-cargo protein complex in the nucleoplasm (8). After import into the nucleus, VP1 or L1 capsomers assemble around the viral genome through interactions between the DNA binding domain of VP1 or L1 and the viral DNA genome. Because the NLSs of both mPy VP1 and HPV11 L1 overlap their DNA binding domains, we determined whether karyopherin α2, β1, or α2β1 bound to the NLS of VP1 or L1 would affect capsomere binding to DNA.

To determine the effect of karyopherins on the binding of VP1 or L1 to DNA, the capsid proteins were first incubated with the karyopherins. The protein complex of capsid protein and karyopherin(s) was then incubated with linearized plasmid DNA. The nucleoprotein complexes were analyzed by EMSA.
to determine whether the capsid protein bound by karyopherins maintained the ability to bind DNA. As shown in Fig. 2A, mPy VP1 capsid protein (2.6 μM) associated with DNA migrated slower than DNA alone (Fig. 2A, compare lanes 2 through 5). The order of karyopherin addition to VP1 was examined by adding karyopherin α2 to VP1 prior to karyopherin β1 (lane 5), karyopherin β1 to VP1 prior to karyopherin α2 (lane 6), or mixing karyopherins α2 and β1 together followed by the addition of VP1 to the binding reaction (lane 7). The order of karyopherin addition to VP1 did not significantly affect VP1 DNA binding (compare lane 2 with lanes 5 to 7). These data suggest that the binding of karyopherins to VP1 capsomeres does not inhibit VP1 binding to DNA.

Similar DNA binding reactions were performed using HPV11 L1 capsomeres alone or in the presence of karyopherins. In contrast to VP1 capsomeres, the binding of L1 capsomeres to DNA was inhibited by karyopherin α2β1 as measured by the change in DNA migration through an agarose gel (Fig. 2B, compare lanes 2 and 5). Interestingly, L1 binding to DNA was also inhibited when L1 was preincubated with karyopherin β1 alone (Fig. 2B, compare lanes 1, 2, and 4). When L1 was incubated with karyopherin α2 and DNA, the DNA protein complex was supershifted relative to L1-bound DNA (Fig. 2B, compare lanes 2 and 3). The latter results suggest that capsomeres of L1 bound to karyopherin α2 can also bind DNA. The order of karyopherin addition to L1 was examined by adding karyopherin α2 to L1 prior to karyopherin β1 (lane 5) or karyopherin β1 to L1 prior to karyopherin α2 (lane 6), or mixing karyopherins α2 and β1 together followed by the addition of L1 to the binding reaction mixture (lane 7). The order of karyopherin addition to L1 did not significantly affect the inhibition of DNA binding by L1 (compare lane 2 with lanes 5 to 7).

Proteins can localize to the nucleus in a karyopherin α-independent, β-dependent manner (22, 34, 47). Because karyopherin β1 alone inhibited the association of HPV11 L1 capsomeres with DNA, the interaction between karyopherin β1 and L1 was investigated by immunoblotting of the proteins associated with DNA after EMSA. HPV11 L1 capsomeres alone or bound by karyopherin α2, β1, or α2β1 were subsequently incubated with plasmid DNA, and the resultant complexes were resolved by agarose gel electrophoresis. Consistent with the HPV11 L1 DNA binding experiment shown in Fig. 2B, the addition of karyopherin α2 alone to the L1-DNA complex resulted in a DNA supershift, whereas addition of karyopherin β1 alone or karyopherin α2β1 completely inhibited L1 DNA binding (Fig. 2C, top panel). Western blot analysis of proteins transferred from agarose gels shown in the top panel of Fig. 2C identified L1 and karyopherin α2 in the supershifted nucleoprotein complex (Fig. 2C, anti-L1 and anti-α2 panels). A protein complex comprised of L1 and karyopherin β1 or L1 and karyopherin α2β1 was observed in the lanes where DNA was no longer bound by L1 (Fig. 2C, anti-L1, anti-α2, and anti-β1 panels). In contrast to the formation of an L1-karyopherin α2-DNA complex, these data suggest that when karyopherin β1 or α2β1 binds L1, L1 is subsequently unable to bind DNA. While a significant amount of DNA is released from L1 with the addition of karyopherin β1 or α2β1 to the DNA binding reaction, it is possible that some DNA remains associated with L1, accounting for the L1 migration pattern shown in Fig. 2C. Western blot analysis of HPV11 L1 incubated with or without DNA followed by agarose gel electrophoresis demonstrated that the migration of L1 in native agarose gels was not altered when DNA was present in the binding reaction (not shown).

To further validate the interaction between karyopherin β1 and HPV11 L1, full-length recombinant L1 protein fused to GST was incubated with karyopherin α2, β1, or α2β1. Proteins bound to GST-L1 were detected by SDS-PAGE followed by Western blot analysis using antibodies against HPV11 L1 or karyopherin α2 or β1. As shown in Fig. 2D, GST-L1 bound karyopherin α2 and the α2β1 heterodimer. Interestingly, GST-L1 was also capable of binding karyopherin β1 independent of karyopherin α2.

FIG. 1. The karyopherin heterodimer α2β1 binds VP1 and L1 in an NLS-dependent manner. (A) Schematic representation of the mPy VP1 and HPV11 L1 proteins depicting the location of the NLS and the DNA binding domain (DBD). (B) GST-β1 or GST bound to glutathione-Sepharose incubated with VP1 or VP1Δ6 in the presence of karyopherin α2. (C) L1 or L1CΔ29 in the presence of karyopherin α2. Bound protein complexes were identified by SDS-PAGE and Coomassie staining.
mPy VP1 and HPV11 L1 capsomeres can bind karyopherin α2 and DNA simultaneously. Our initial experiments showed that karyopherin α2 (0.5 μM) did not inhibit DNA (6.9 nM) binding by either mPy VP1 or HPV11 L1 (2.6 μM) capsid proteins. Because the amino acid sequence comprising the NLS of mPy VP1 and HPV11 L1 overlaps their DNA binding domain, we tested whether binding karyopherin α2 would inhibit DNA binding when karyopherin β1 is in molar excess relative to the capsid protein concentration. Karyopherin ranging from an equal molar concentration up to a 16-fold molar excess relative to the capsid protein monomer was preincubated with either mPy VP1 or HPV11 L1 protein prior to addition of plasmid DNA. The resultant DNA-protein complexes were then resolved by agarose gel electrophoresis. A decrease in the mobility of the DNA-protein complex corresponding to the amount of karyopherin α2 added to the reaction (Fig. 3A and B). These results suggest that karyopherin α2 binding to VP1 or L1 capsomeres was insufficient to disrupt DNA binding and that mPy VP1 or HPV11 L1 capsomeres can bind karyopherin α2 and DNA simultaneously.

DNA competes for karyopherin α2 binding to VP1 and L1. When karyopherin α disassociates from the capsid proteins after nuclear import is currently unknown. One possibility is that the “NLS-like” sequences at the amino terminus of karyopherin α compete the karyopherin from the VP1 or L1 NLS (17, 18). Alternatively, because their DNA binding domain overlaps their NLS, binding DNA to VP1 or L1 may release α2 from the NLS. The results shown in Fig. 3 suggested that VP1 and L1 can form a nucleoprotein complex that includes the capsid protein, karyopherin α2, and DNA, as evidenced by the binding reaction from a 2-fold to 16-fold molar excess relative to the capsid protein concentration further decreased the mobility of the nucleoprotein complex corresponding to the amount of karyopherin α2 added to the reaction (Fig. 3A and B).
the slower-migrating complex. We tested whether increasing the DNA concentration in the DNA binding reaction mixture containing VP1 and karyopherin α2 could compete karyopherin α2 from the nucleoprotein complex. VP1 (2.6 μM) was first incubated with the karyopherin α2 (0.5 μM) and linearized plasmid DNA (6.9 nM). In an attempt to compete karyopherin α2 from the nucleoprotein complex, 60-bp duplex oligonucleotides were then added to the binding reaction mixture to a final concentration of 3, 6, 12, or 24 μM. The migration of the nucleoprotein complex containing plasmid DNA was assessed by agarose gel electrophoresis. As shown in Fig. 4A, the oligonucleotides competed VP1 and karyopherin α2 binding to plasmid DNA when the concentration of oligonucleotides was 6 μM or greater, as determined by the migration of the plasmid DNA in the agarose gel (Fig. 4A, compare lanes 3 and 5). Interestingly, the nucleoprotein complex from the reaction mixture containing 3 μM oligonucleotide migrated to the same position as the nucleoprotein complex containing VP1 and plasmid DNA (Fig. 4A, compare lanes 2 and 4). These results suggest that the addition of oligonucleotide DNA above 6 μM to the binding reaction mixture competed both VP1 and karyopherin α2 from the plasmid DNA while addition of 3 μM competed only karyopherin α2 from the VP1-DNA complex. Similar results were also observed for HPV11 L1 (data not shown). The competition between DNA and karyopherin α2 binding to VP1 capsomeres was further analyzed by titrating the concentration of oligonucleotides into the binding reaction from 3 μM to 5 μM (Fig. 4B). As shown in Fig. 4B, karyopherin α2 was competed from the nucleoprotein complex with the addition of oligonucleotide DNA ranging from 3 to 5 μM (Fig. 4B, compare lanes 2 and 4 to 7). We interpret these results to suggest that DNA is able to compete karyopherin α2 from the VP1 NLS.

Karyopherin binding inhibits in vitro capsid assembly of VP1 and L1 capsomeres. The subcellular location of papovavirus assembly is controlled such that virions are formed in the cell nucleus and not in the cytoplasm (13, 33). The hsp70 family of proteins has been shown to bind polyomavirus VP1 and has been implicated in regulating polyomavirus capsid assembly (7, 9). Karyopherin proteins associate with VP1 capsomeres in the cytoplasm and remain associated with the capsomeres as they localize to the nucleus. Therefore, a potential role for karyopherins in inhibiting mPy VP1 capsid formation has been tested. In vitro calcium-mediated assembly of polyomavirus VP1 capsomeres was used as an assay to test how factors that bind VP1 capsomeres in vivo may affect assembly (7). Assembly reactions with mPy VP1 were performed in the presence or absence of karyopherin α2, β1, or αβ1 by overnight dialysis in assembly buffer containing 0.5 mM calcium chloride. The VP1 concentration did not change during dialysis, as detected by soluble protein concentration. The samples were then analyzed by TEM. Viruslike particles were counted using six images per grid from four independent experiments. VP1 assembled into T=7 particles in the absence of karyopherins, but not in the presence of the karyopherin αβ1 heterodimer (Fig. 5A). VP1 also assembled into T=7 particles in the presence of either α2 or β1 karyopherin alone (Fig. 5B). Quantitation of assembled particles demonstrated that calcium-mediated in vitro assem-
bly of VP1 viruslike particles was reduced by more than 20-fold in the presence of karyopherin α2β1 (Fig. 5C).

In vitro assembly of papillomavirus L1 capsomeres was used to assess the effects of karyopherins on papillomavirus capsid assembly. Assembly reactions were performed with L1 in the presence or absence of karyopherin α2, β1, or α2β1 followed by an overnight dialysis in assembly buffer that allowed formation of interpentameric disulfide bonds. As observed with VP1 capsomeres, L1 capsomeres assembled into $T=7$ particles in the absence of karyopherins, but not in the presence of the karyopherin α2β1 heterodimer (Fig. 6A). In contrast to VP1, we also observed an inhibition of L1 particle assembly in the presence of karyopherin α2 (Fig. 6B). Additionally, we observed a shift toward the formation of $T=1$ and $T=3$ particles in assembly reaction mixtures containing L1 and β1. Quantitation of assembled particles demonstrated that assembly of L1

FIG. 5. Karyopherin α2β1 inhibits in vitro assembly of VP1. Assembly reactions of VP1 with purified karyopherin α2, β1, or α2β1 were visualized by negative staining and TEM. (A) Electron micrograph of a VP1 assembly reaction (top row) or VP1 with karyopherin α2β1 (bottom row). Magnifications, ×4,800 and ×49,000. (B) Electron micrograph of a VP1-karyopherin α2 (top row) or VP1-karyopherin β1 (bottom row) assembly reaction. Magnifications, ×4,800 and ×49,000. (C) Quantitation of the average number of assembled particles per image (n = 6) from four independent assembly reactions. The scale bars are 1 μm at ×4,800 and 100 nm at ×49,000.
capsids was reduced by more than 15-fold in the presence of karyopherin α2β1 (Fig. 6C). Individually, karyopherin α2 inhibited L1 capsid assembly by threefold (Fig. 6C).

DISCUSSION

In order to determine whether karyopherin binding to papovavirus capsid protein affects capsid assembly, the interaction between karyophersins and the HPV11 L1 or mPy VP1 capsid proteins was investigated. As previously observed for the L1 capsid proteins of HPV11 and HPV45 (38), the HPV11 L1 capsid protein bound both karyopherins α2 and α2β1. mPy VP1 also interacted with karyopherins α2 and α2β1. The interaction between karyopherins and L1 or VP1 was dependent on an intact NLS (Fig. 1).

Nuclear import of proteins that contain a canonical NLS,
Karyopherin-mediated nuclear import may also be important during the initial phase of virus infection when viral nucleoprotein complexes, composed of capsid proteins bound to the viral genome, exit a membrane compartment (the endosome for papillomavirus or the endoplasmic reticulum for polyomavirus) into the cytoplasm (11, 16). In the cytoplasm, karyopherins may bind the exposed NLS(s) of capsomeres or the minor capsid proteins, which are bound to the viral DNA, and then transport the viral protein complex into the nucleus. In order for this transport pathway to function, capsomeres must be able to bind the viral genome and karyopherins simultaneously. We found that VP1 (2.6 μM) complexed with karyopherin α2 (0.5 μM) was able to bind plasmid DNA (6.9 nM). Additionally, increasing karyopherin α2 to a 16-fold molar excess over the capsid protein led to slower migration of the DNA-protein complex by EMSA (Fig. 3). We interpret these results to mean that the five DNA binding domains of a VP1 pentamer are not fully occupied by DNA, because unoccupied NLSs continued to bind karyopherin α2. However, the gel mobility of the complex also indicated that the interaction between karyopherin α2 and the NLSs of mPy VP1 was insufficient to displace DNA from the DNA binding domain. These data suggest that in vitro, a pentamer bound to plasmid DNA does not have all five DNA binding domains of the pentamer in contact with the DNA. However, during infection the polyomavirus genome is configured with nucleosomes and how the DNA binding domain of VP1 or L1 will interact with nucleosome-bound DNA is unclear.

Similar to the results of mPy VP1, we found that HPV11 L1 (2.6 μM) complexed with karyopherin α2 (0.5 μM) was able to bind plasmid DNA (6.9 nM). Additionally, increasing karyopherin α2 to a 16-fold molar excess over the capsid protein led to slower migration of the DNA-protein complex by EMSA (Fig. 3). However, DNA binding by HPV11 L1 capsomeres was inhibited by karyopherin α2β1 (Fig. 2B), and karyopherin α2 cannot alone mediate nuclear import in the absence of karyopherin β1 import receptor. These results suggest that it is unlikely that HPV11 L1 mediates import of the viral genome during the initial phase of infection as L1 binding of karyopherin α2β1 in the cytoplasm would release the viral DNA from the capsid protein prior to being transported into the nucleus. Consistent with this reasoning, L1 has not been detected in the nucleus in the initial phase of the papillomavirus infection and, instead, L2 seems to colocalize in the nucleus with the incoming viral genome (10).

The basic amino acids that make up the NLSs for both papillomavirus and polyomavirus also function in DNA binding. The DNA binding constant for recombinant VP1 has been determined by an immunoprecipitation assay to be \(1 \times 10^{-11}\) M to \(2 \times 10^{-11}\) M (35). The DNA binding affinity of L1 has not been quantitated, but L1 has been shown to bind DNA by Western blotting and EMSA gel assay (26, 46). Karyopherin α2β1 competed DNA from HPV11 L1, but not mPy VP1. These data suggest that the DNA binding affinity of L1 may be less than that of VP1. The relatively tight binding affinity of VP1 may serve a functional role during capsid assembly, with the DNA serving as scaffolding around which VP1 capsomeres assemble. The weaker binding affinity of L1 for DNA may reflect a greater dependence on the viral L2 protein to bind the viral genome and serve as an interface for encapsidation.

As the capsomeres enter the nucleus, RanGTP dissociates karyopherin β from the cargo protein complex. However, what mediates the dissociation of karyopherin α from the capsid proteins is unknown. One possibility is that DNA binding to VP1 or L1 releases karyopherin α from the NLS. We observed that addition of oligonucleotide DNA ranging from 3 to 6 μM to a nucleoprotein complex composed of capsid protein (2.6 μM), karyopherin α2 (0.5 μM), and plasmid DNA (6.9 nM) was able to compete karyopherin α2 from the NLS of mPy VP1 (Fig. 4B). These data raise the possibility that in the nucleus, the viral DNA may displace karyopherin α2 from the capsid protein, allowing for capsid assembly around the viral genome.

The mPy VP1 and HPV11 L1 capsid proteins have the intrinsic ability to self-assemble in vitro and in vivo. Given these intrinsic self-assembly properties, there must be biological controls in vivo that regulate the quality and subcellular location of viral capsid assembly. The hsp70 family of chaperones previously has been shown to modulate assembly and disassembly of polyomavirus and papillomavirus capsid protein (6, 7). Our data suggest that another potential mechanism for modulating capsid assembly may involve the karyopherin proteins that bind the NLS of HPV11 L1 and mPy VP1 and that karyopherin proteins may have “chaperone” functions. The karyopherin heterodimer α2β1 inhibited in vitro assembly of both HPV11 L1 and mPy VP1. Karyopherin α2β1 heterodimers (157.5-kDa complex) bind HPV11 L1 at the NLS located at the carboxyl terminus of the protein. Binding to the carboxyl terminus may inhibit assembly through steric inhibition, because the L1 carboxyl terminus is also used for interpentamer bonding (25, 32). Karyopherin α2 also had an inhibitory effect on L1 assembly, although to a lesser extent than karyopherin α2β1. This finding supports the idea that steric hindrance may inhibit assembly and that by recruiting karyopherin β1 to the L1-karyopherin α2 complex assembly is further inhibited. Interestingly, karyopherin β1 appeared to shift assembly in favor of the T=3 and T=1 products. These data suggest that karyopherin β1 may interact with the carboxyl terminus of L1 and alter the interpentamer contacts mediated by the carboxyl-terminal domain. The NLS of VP1 is located at its amino terminus, which for SV40 is involved in interpentamer contacts through the formation of disulfide bonds (43).
Binding of karyopherin α2β1 to the VP1 amino terminus may prevent these interpentameric disulfide bonds between VP1 molecules as well as prevent the carboxy-terminal domains of VP1 from forming interpentameric bonds through an indirect steric effect. Inhibition of in vitro assembly of mPy VP1 and HPV11 L1 capsids by karyopherin α2β1 supports the hypothesis that karyopherins may play a role in chaperoning VP1 and L1 capsid assembly in vivo. Karyopherins are abundant in the cytosol, ranging from 1 to 2 μM of each karyopherin (23). This high level of karyopherins would allow them to function as chaperones in addition to their nuclear import function. Because the nuclear localization of many proteins is mediated by karyopherins, the chaperone function observed with karyopherins in modulating viral protein complex assembly and DNA binding may be applicable to proteins other than the capsid proteins investigated in this study.

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