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Nuclear Import of HPV11 L1 Capsid Protein Is Mediated by Karyopherin α2β1 Heterodimers

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Abstract L1 major capsid proteins of human papillomaviruses (HPVs) enter the nuclei of host cells at two times during the viral life cycle: 1) after infection and 2) later during the productive phase, when they assemble the replicated HPV genomic DNA into infectious virions. L1 proteins are stable in two oligomeric configurations: as homopentameric capsomers, and as capsids composed of 72 capsomers. We found that intact L1 capsids of HPV type 11 cannot enter the nucleus, suggesting that capsid disassembly may be required for HPV11 L1 nuclear import. We established that HPV11 L1 is imported in a receptor-mediated manner into the nuclei of digitonin-permeabilized HeLa cells. HPV11 L1 docked at the nuclear pore complexes via karyopherin α2β1 heterodimers. Anti-karyopherin-β1 and anti-karyopherin α2 antibodies specifically inhibited nuclear import of HPV11 L1. Moreover, nuclear import of HPV11 L1 could be reconstituted using karyopherin α2, β1, RanGDP and p10. In agreement with the docking and import data, we found that HPV11 L1 binds to karyopherin α2 and that this interaction is inhibited by a peptide representing the classical nuclear localization signal of SV40 T antigen. These results strongly suggest that HPV11 L1 enters the nucleus of the infected host cell via the karyopherin α2β1 pathway. J. Cell. Biochem. 74:628–637, 1999.

Key words: HPV; karyopherin

Human papillomaviruses (HPVs) are small, nonenveloped, icosahedral, DNA viruses that replicate in the nucleus of squamous epithelial cells. About 70 genotypically distinct HPV genotypes have been isolated and characterized, with roughly half infecting cutaneous skin and the other half preferentially infecting oral/anogenital mucosal epithelial tissues. Mucosal HPVs have demonstrated varying degrees of oncogenic potential, with some classified as "high risk" such as, for example, types 16, 18, 31, and 45, and others as "low risk" such as types 6 and 11. High risk HPVs are frequently detected in invasive cervical carcinomas, whereas the low risk types are more often associated with benign exophytic condylomas [zur Hausen, 1991; Galloway, 1994; Shah and Howley, 1996; Howley, 1996]. HPV replication is dependent on differentiation of the infected host cell. As a consequence, the ability to synthesize HPV virions in vitro has been hindered by the lack of a reproducible tissue culture system that would allow viral morphogenesis.

HPV virions (52–55 nm in diameter) consist of a single molecule of double-stranded circular DNA approximately 8,000 bp in size contained within a capsid composed of 72 capsomers. The capsid consists of two structural proteins. L1, the major capsid protein, represents approximately 80% of the total virion protein, whereas L2 is a minor structural component of the capsid. Results from several studies have indicated that L1 alone is capable of self-assembly in vivo and in vitro into capsid-like structures [Kirnbauer et al., 1992; Hagensee et al., 1993; Zhou et al., 1993; Rose et al., 1993; Li et al., 1997]. L1 (55 kDa) is stable in two oligomeric configurations: homopentameric capsomers, and capsids composed of 72 capsomers [Kirnbauer et al., 1992; Hagensee et al., 1993; Zhou et al., 1993; Rose et al., 1993; Hagensee et al., 1994; Belnap et al., 1996; Li et al., 1997]. Recently, it has been shown that HPV L1 capsids can be disassembled quantitatively by agents that reduce disulfide bonds [McCarthy et al., 1998; Li et al., 1998]. These observations have suggested that

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HPV entry into the reducing environment of the cytoplasm may result in disassembly of the virions into capsomers and minichromosomes.

Successful viral infection depends on the import of the viral genome and capsid proteins into the nucleus, where viral gene transcription, DNA replication, and virion maturation take place [for a review see Howley, 1996]. Results from a study on the early phase of infection of cultured cells with bovine PV virions showed that although binding to the cell membrane and uptake of virions into large cytoplasmic vesicles could be monitored by electron microscopy, complete virions were not observed in infected cell nuclei. In contrast, both L1 and L2 capsid proteins were efficiently transported into the nucleus of infected cells, suggesting that nuclear import of incoming capsid proteins may occur along with import of the viral genomic DNA [Zhou et al., 1995].

Entry of macromolecules into the nucleus is an active process and is governed by the interactions of transport factors (karyopherins/importins) with their respective macromolecular cargoes as well as with the nuclear pore complexes (NPC). Nuclear import of classical nuclear localization signal (NLS)-proteins is mediated by karyopherin α β1 heterodimers (known also as importin α β, NLS receptor and p97, PTAC α β, Kap60, and Kap95) [for reviews see Görlich and Mattaj, 1996; Corbett and Silver, 1997; Moroianu, 1997]. Karyopherin α binds directly to the NLS of the cargo and karyopherin β1 interacts with several peptide repeat nucleoporins and docks the trimeric complex at the NPC [Adam and Adam, 1994; Görlich et al., 1995; Weis et al., 1995; Moroianu et al., 1995; Rexach and Blobel, 1995; Delphin et al., 1997; Shah et al., 1998]. Translocation of the docked NLS-protein requires the GTPase Ran, and the RanGDP-interacting protein p10/NTF2 [Moore and Blobel, 1993; Melchior et al., 1993; Moore and Blobel, 1994; Melchior et al., 1995; Schlenstedt et al., 1995; Paschal and Gerace, 1995; Palacios et al., 1996; Nehrbass and Blobel, 1996]. RanBP1 stimulates nuclear import [Chi et al., 1996]. There are several mammalian α karyopherins that bind to the same karyopherin β1 and to distinct or overlapping types of NLS; the best characterized ones are karyopherin α2 (corresponding to Rch-1, hSRP1α, NPI-3) and karyopherin α1 (corresponding to NPI-1, hSRP) [for reviews see Moroianu, 1997; Malik et al., 1997]. Recently, several other members of the karyopherin β family that mediate distinct import pathways have been identified [for reviews see Moroianu, 1998; Pemberton et al., 1998; Wozniak et al., 1998]. For example, mammalian karyopherin β2/transportin or the yeast Kap104 mediate import of a set of hnRNP proteins [Aitchison et al., 1996; Pollard et al., 1996; Bonifaci et al., 1997; Fridell et al., 1997], whereas mammalian karyopherin β3 and β4, or the yeast Kap121p and Kap123p mediate import of a set of ribosomal proteins [Rout et al., 1997; Yaseen and Blobel, 1997; Jakel and Görlich, 1998]. Interestingly, the ribosomal proteins L23a, S7 and L5 can each be imported alternatively by four β karyopherins: β1, β2/transportin, β3/RanBP5, or RanBP7 [Jakel and Görlich, 1998].

There is little information available regarding the mechanism(s) by which HPV capsid proteins are imported into the nuclei of infected cells. In this study we investigated the nuclear import pathway for HPV11 L1 capsid protein. We found that L1 capsomers (or/and monomers), but not L1 capsids, were imported in a receptor-dependent manner into the nuclei of digitonin-permeabilized HeLa cells. These findings suggest that during infection, the L1 capsids must first be disassembled in order for L1 proteins to enter the nucleus. We discovered that HPV11 L1 docks at the NPC and is imported into the nucleus via karyopherin α2β1 heterodimers. In agreement with these findings, in overlay blot assays, HPV11 L1 protein specifically bound to karyopherin α2 and this interaction was inhibited by a peptide containing the classical NLS of SV40 T antigen. Taken together, these results suggest that HPV11 L1 capsid protein is transported into the nucleus of infected host cells via the karyopherin α2β1 pathway.

MATERIALS AND METHODS

Preparation of Recombinant Transport Factors

Recombinant full length transport factors: His-tagged karyopherin α2 [Weis et al., 1995], His-tagged karyopherin β1 [Chi et al., 1996], GST-karyopherin β1 [Chi et al., 1995], GST-karyopherin β2 [Bonifaci et al., 1997], Ran [Coutavas et al., 1993], His-tagged p10 [Moroianu et al., 1995], and GST-RanG19V mutant [Lounsbury et al., 1996; Richards et al., 1997] were prepared as previously described. All the purified transport factors were dialyzed in transport

buffer [Moroianu et al., 1995] and stored in aliquots at −80°C until use.

Preparation of HPV11 L1 Capsids and Capsomers

Recombinant HPV11 L1 capsids were generated in insect cells, purified as previously described [Rose et al., 1994, 1993], and stored at 4°C until use. Purity and lack of proteolytic degradation of L1 protein was checked by SDS-PAGE, Coomassie staining and immunoblotting prior to use. L1 pentameric capsomers were generated via incubation of L1 capsids with 5% beta-mercaptoethanol overnight at 4°C, as previously described [McCarthy et al., 1998].

Antibodies

Rabbit polyclonal antiserum R-399 was raised against HPV11 L1 capsids as previously described [Rose et al., 1994, 1998; White et al., 1998]. Murine antibodies raised against karyopherin α2/Rch-1 and karyopherin β2/transportin were from Transduction Laboratories; a mouse monoclonal antibody to karyopherin β1/p97 was obtained from Affinity Bioreagents, Inc. (Golden, CO); and goat anti-GST antibody was obtained from Pharmacia Biotech (Gaithersburg, MD).

Synthetic Peptides and Conjugates

A peptide corresponding to the NLS of SV40 T antigen [Goldfarb et al., 1986] was synthesized with an N-terminal Cys added for coupling purposes: CYTPPKKKKRKVED. This peptide was conjugated to human serum albumin (HSA) as previously described [Moore and Blobel, 1992]. The conjugates were found to contain about five to ten peptides per HSA as assayed by SDS/PAGE.

In Vitro Nuclear Import Assays

Nuclear import assays in digitonin-permeabilized cells were carried out as previously described [Moore and Blobel, 1992; Moroianu et al., 1995; O'Neill et al., 1995; Moroianu et al., 1996]. Subconfluent HeLa cells grown on glass coverslips for 1 day were permeabilized with 35 μg/ml digitonin for 5 min on ice. Digitonin permeabilizes the plasma membrane but leaves the nuclear envelope intact and, as a consequence, digitonin-permeabilized cells retain intact import-competent nuclei, but are largely depleted of cytosolic transport factors [Adam and Adam, 1994]. Import reactions contained an energy regenerating system (ATP, GTP, bovine serum albumin, phosphocreatine, and creatine phosphokinase) [Moore and Blobel, 1992; Moroianu et al., 1995; O'Neill et al., 1995; Moroianu et al., 1996], plus various transport factors [0.5–1 μM for karyopherins, 3 μM RanGDP (or RanG19V mutant), 0.1 μM p10] or HeLa cytosol (Cellex Biosciences Inc., Minneapolis, MN), plus the L1 capsids/capsomers. Final reaction volume was adjusted to 20 μl with transport buffer. For inhibition experiments the import reactions also contained murine antibodies (1:10 dilution) to either karyopherin α2/Rch-1, or to karyopherin β1/p97, or to karyopherin β2/transportin. To visualize nuclear import, the HPV11 L1 protein was detected by immunofluorescence with HPV11 L1 antibody. Nuclei were identified by DAPI staining. Nuclear import was analyzed with a Nikon Eclipse TE 300 Microscope in fluorescence. Quantitation of import was done as described [Moore and Blobel, 1992; O'Neill et al., 1995; Moroianu et al., 1996], by measuring the fluorescence of 25 nuclei with IPLAB software.

Docking Assays

Docking assays were done essentially as previously described [Moore and Blobel, 1992; O'Neill et al., 1995; Moroianu et al., 1995, 1996]. Digitonin-permeabilized HeLa cells were incubated for 15 min at room temperature with the HPV11 L1 capsomers or capsids in the absence or presence of different exogenous karyopherins (β1, α2, each at 1 μM). For visualization of docking, the HPV11 L1 protein was detected by immunofluorescence with HPV11 L1 antibody.

Immunofluorescence

To detect nuclear import or docking of HPV11 L1 capsomers/capsids by immunofluorescence we used rabbit polyclonal antibody to HPV11 L1 capsids. At the end of the import/docking reactions the cells were washed with transport buffer and fixed with 3.7% formaldehyde for 15 min on ice followed by methanol for 3 min at −20°C. After blocking nonspecific binding with 2% BSA in PBS for 1 h, the cells were incubated for 1 h with HPV11 L1 antiserum (1:200 dilution). Detection was accomplished with fluorescein-labeled goat anti-rabbit IgG second antibody (1:100 dilution). Specificity controls were performed in the absence of HPV11 L1 or of the first antibody.

Fluorescence Labeling of Proteins

For in vitro nuclear import and docking assays, the classical import substrate, NLS-HSA, was labeled with fluorescein 5′ maleimide dissolved in dimethylformamide at a 1:1 molar ratio. The reaction was allowed to proceed for 2 h on ice, quenched with 50 mM mercaptoethanol and the labeled protein was dialyzed against transport buffer overnight at 4°C to remove the free fluorophore.

Overlay Blot Assays

HPV11 L1 protein (2 μg/lane) was subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The blots were stained with Ponceau and blocked overnight at 4°C with 5% non-fat milk in PBS. Then the blots were incubated for 30 min at room temperature with either karyopherin α2 alone (100–25 nM), or karyopherin α2 (25 nM) in the presence of the NLS peptide of SV40 T antigen (200x molar excess), or with GST-karyopherin β1 (200 nM), or with GST-karyopherin β2 (200 nM). Karyopherin α2 was detected by immunoblotting with a specific antibody. GST-karyopherin β1 or GST-karyopherin β2 were detected by immunoblotting with an anti-GST antibody. As immunoblotting controls, karyopherin α2, GST-karyopherin β1, and GST-karyopherin β2 were used in parallel lanes.

Immunoisolation Assays

HeLa cytosol (100 μl) was incubated first with HPV11 L1 capsomers (4 μM) for 30 min at room temperature to allow the formation of L1/karyopherin complexes and then with an antibody to HPV11 L1 for another 60 min. Complexes were recovered by incubating with Protein A-Sepharose beads for 1 h at 4°C. The beads were washed four times with transport buffer and eluted with 1.5 M $MgCl_2$. Eluted proteins were subjected to SDS-electrophoresis and transferred to nitrocellulose. Blots were probed with HPV11 L1 capsid antiserum, or anti-karyopherin α2/Rch-1 and then with corresponding secondary antibodies. Positive immunoblotting controls consisted of blots of HPV11 L1, and HeLa cytosol containing the karyopherins. Specificity controls consisted in the omission of L1 capsomers during isolation.

Immunoblotting

Blots were incubated for 1 h at room temperature with the primary antibodies (1:500–1:1,000 dilution). After washing, blots were incubated for 1 h at room temperature with corresponding secondary antibodies conjugated to horseradish peroxidase (1:1,000 dilution). Immunoblotting controls consisted in the absence of the first antibodies. The signal was detected with a Chemiluminescent Detection Kit (Amersham, Arlington Heights, IL) and exposure to Biomax film (Kodak, Rochester, NY).

RESULTS

Nuclear Import of HPV11 L1 Requires Disassembly of L1 Capsids

We investigated the nuclear import of HPV11 L1 protein either as capsids or capsomers by the use of in vitro nuclear import assays in digitonin-permeabilized cells. This technique has been used successfully to investigate distinct nuclear import pathways mediated by β karyopherins/importins. Digitonin-permeabilized HeLa cells were incubated with: A) L1 capsomers in only transport buffer; B) L1 capsomers plus HeLa cytosol; or C) L1 capsids plus HeLa cytosol. HPV11 L1 protein was detected by immunofluorescence staining with specific antibodies. We found that when the import assays were carried out with L1 capsomers, but not with L1 capsids (Fig. 1B,C), L1 was imported into the nuclei of digitonin-permeabilized cells in the presence of HeLa cytosol and energy. Nuclear import of L1 did not occur in the absence of cytosol (Fig. 1A) indicating that it is mediated by transport factors present in the cytosol.

Nuclear Import of HPV11 L1 Is Mediated by Karyopherin α2β1 Heterodimers

HPV16 L1 capsid protein contains two classical NLSs [Zhou et al., 1991] that are partially conserved in HPV11 L1 (Table I). One NLS consists of six basic amino acids at the carboxy terminal of HPV16 L1, whereas the other is a bipartite type NLS and overlaps with the first NLS [Zhou et al., 1991]. Since the potential NLSs of HPV11 L1 are similar to classical types of NLS, we first investigated if HPV11 L1 could be docked at the NPC via karyopherin α2β1 heterodimers. Digitonin-permeabilized HeLa cells were incubated for 15 min with HPV11 L1 capsomers in the presence of: A) transport buffer alone, B) karyopherin β1, or C) karyopherin α2 and β1 together. We found that HPV11 L1 efficiently docked at the NPC in the presence of both karyopherin α2 and β1 (Fig. 2C). More-

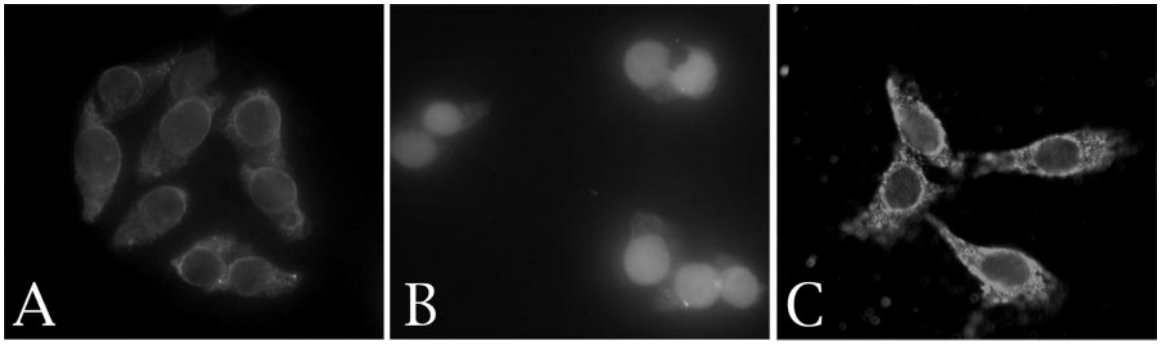

Fig. 1. Nuclear import of HPV11 L1 requires disassembly of L1 capsids. Digitonin-permeabilized HeLa cells were incubated for 30 min at room temperature with: HPV11 L1 capsomers in the presence of only transport buffer (**A**), or HeLa cytosol (**B**); or with intact L1 capsids in the presence of HeLa cytosol (**C**). The L1 capsomers and capsids were detected by immunofluorescence with specific antibodies to HPV11 L1 capsids (VLPs). Note the nuclear import of HPV11 L1 in B and the cytoplasmic background in A and C.

TABLE I. NLSs of HPV11/16 L1 Proteins

HPV Type	NLS Sequence
HPV16 L1	**KRK**atpttsststta**KRKKRK**
HPV11 L1	**KR**pavskpstap**KRKRtKtKK**

over, we also determined that docking of HPV11 L1 via karyopherin α2β1 heterodimers was inhibited by the classical NLS peptide of SV40 T antigen (Fig. 2D).

Docking of HPV11 L1 at the NPC via karyopherin α2β1 heterodimers suggests that both karyopherin β1 and karyopherin α2 are required for nuclear import of HPV11 L1. Indeed, antibodies against either karyopherin β1 or karyopherin α2 inhibited nuclear import of HPV11 L1 when added to the import reaction (Fig. 3B,C), whereas an antibody to karyopherin β2/transportin had no effect (data not shown). Moreover, nuclear import of HPV11 L1 in digitonin-permeabilized HeLa cells could be reconstituted with only recombinant transport factors. When RanGDP and p10 were added to karyopherin α2 and β1, the HPV11 L1 protein was transported into the nuclei (Fig. 4B). It should be noted that nuclear import of HPV11 L1 in the presence of recombinant transport factors was less efficient (about 40%) than in the presence of HeLa cytosol. As expected, the RanG19V mutant (that was in the GTP form and is unable to hydrolyze GTP [Lounsbury et al., 1996; Richards et al., 1997]) inhibited nuclear import of HPV11 L1 to the control level in the absence of transport factors, both by qualitative and quantitative analysis (compare Fig. 4C with Fig. 1A, or Fig. 2A). The inhibition is probably due to binding of RanG19V to karyopherin β1 and, as a consequence, disruption of karyopherin α2β1 complex that docks the cargo at the NPC. Indeed, in the presence of RanG19V mutant there was no docking of HPV11 L1 at the NPC (Fig. 4, compare C with A).

HPV11 L1 Binds Specifically to Karyopherin α2

The docking and nuclear import data strongly suggest that HPV11 L1 can bind directly to karyopherin α2 and be carried into the nucleus as L1/karyopherin α2β1 complexes. Indeed, in overlay blot assays karyopherin α2 bound to HPV11 L1 and this interaction was efficiently inhibited by the classical NLS peptide of SV40 T antigen (Fig. 5A), but not by the mutant NLS peptide (data not shown). Neither karyopherin β1, nor karyopherin β2 bound directly to HPV11 L1 (data not shown). We also isolated the complexes that HPV11 L1 capsomers form in HeLa cytosol by incubation with specific antibodies to HPV11 L1 and then with Protein A-Sepharose beads. The bound proteins were eluted with 1.5 M $MgCl_2$ and then probed by immunoblotting with anti-karyopherin α2/Rch-1. We found that karyopherin α2 was present in the immunoisolated HPV11 L1 complexes (Fig. 5B).

These data suggest that HPV11 L1 capsid protein is able to interact with cytoplasmic karyopherin α2 and exploit this interaction to gain entry into the nuclear compartment of host cells.

HPV11 L1 in Excess Inhibits Nuclear Import of the Classic NLS-HSA Conjugate

In this experiment we sought to investigate whether HPV11 L1 in excess might inhibit karyopherin α2β1-mediated nuclear import of cellular proteins. To investigate this issue, we used the classical import substrate, the conjugate of SV40 T antigen NLS with human serum albu-

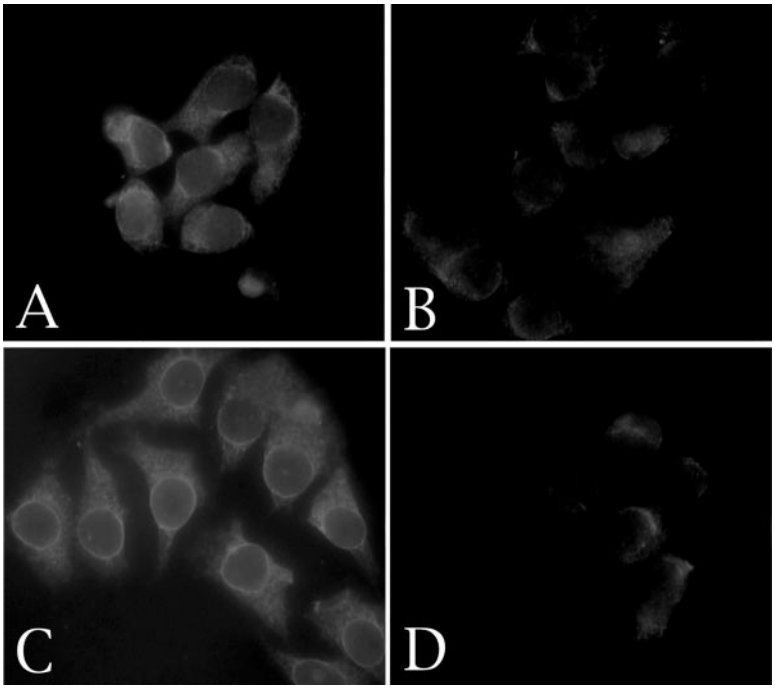

Fig. 2. Docking of HPV11 L1 is mediated by karyopherin α2β1 heterodimers. Digitonin-permeabilized HeLa cells were incubated for 15 min with HPV11 L1 capsomers (0.5 μM) in the presence of only transport buffer (**A**), or karyopherin β1 (**B**), or karyopherin β1 together with karyopherin α2 (**C**), or karyopherin β1 together with karyopherin α2 plus the NLS peptide of SV40 T antigen (×200 times molar excess; **D**). L1 protein was detected as described. The clear nuclear rim staining in C indicates docking of HPV11 L1 at the NPC.

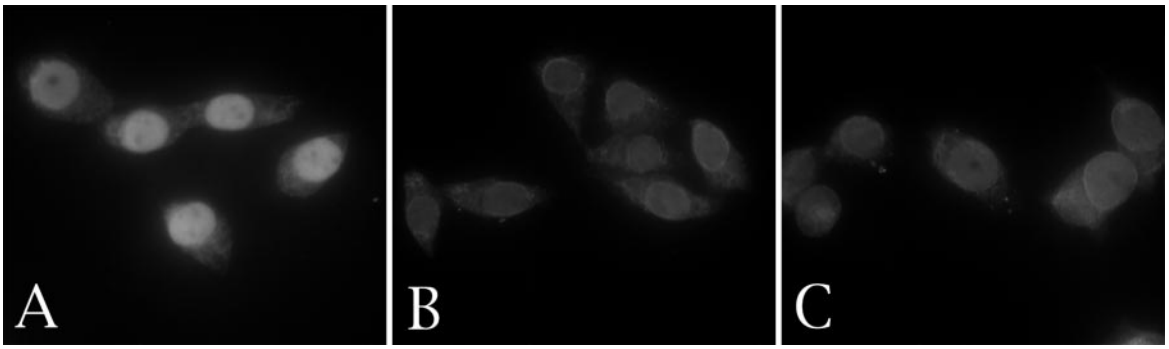

Fig. 3. Nuclear import of HPV11 L1 is inhibited by antibodies to karyopherin α2 and karyopherin β1. Digitonin-permeabilized HeLa cells were incubated for 30 min at room temperature with HPV11 L1 capsomers (0.5 μM) in the presence of either HeLa cytosol (**A**), or HeLa cytosol plus anti-karyopherin α2 (**B**), or HeLa cytosol plus anti-karyopherin β1 (**C**). L1 protein was detected as described. The nuclear import of HPV11 L1 in the presence of HeLa cytosol (A) was partially inhibited by antibodies to either karyopherin α2 (B), or karyopherin β1 (C).

min (NLS-HSA), which was previously shown to enter the nucleus via the karyopherin α2β1 pathway [Moroianu et al., 1995; Weis et al., 1995]. Digitonin-permeabilized HeLa cells were incubated in an import reaction with the NLS-HSA conjugate in the presence of HeLa cytosol and in the absence or presence of increasing amounts of HPV11 L1 capsomers. The NLS-HSA substrate was imported into the nucleus in the presence of HeLa cytosol and its import was reduced to approximately 40% when HPV11 L1 capsomers were present in excess (Fig. 6, compare A and D).

DISCUSSION

Capsid Disassembly Is Required for Nuclear Import of HPV11 L1 Protein

The L1 capsid proteins are thought to enter the nucleus at two times during the virus life cycle: immediately after the virions infect the cells [Zhou et al., 1995], and during the late

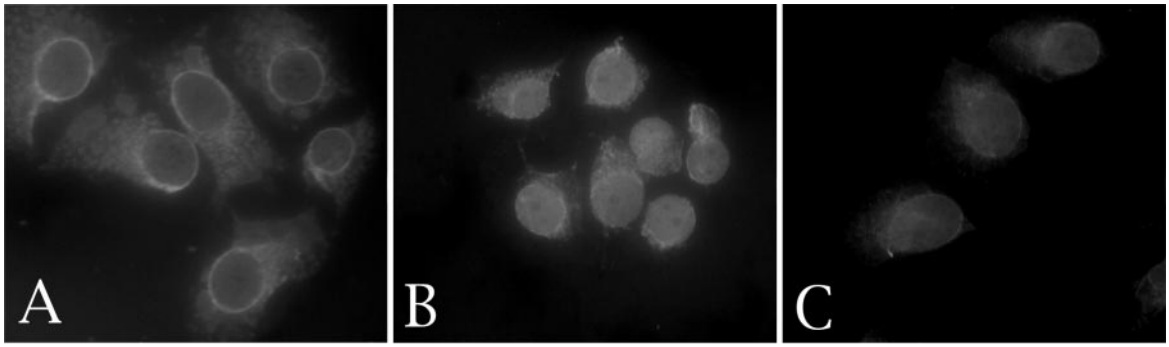

Fig. 4. HPV11 L1 can enter the nucleus in the presence of karyopherin α2, karyopherin β1, RanGDP and p10. Digitonin-permeabilized HeLa cells were incubated for 30 min at room temperature with HPV11 L1 capsomers (0.5 μM) in the presence of karyopherin α2 and β1 (**A**), or karyopherin α2 and β1 plus either RanGDP and p10 (**B**), or plus RanG19V mutant and p10 (**C**). L1 protein was detected as described. Nuclear import of HPV11 L1 was inhibited in the presence of RanG19V mutant (C).

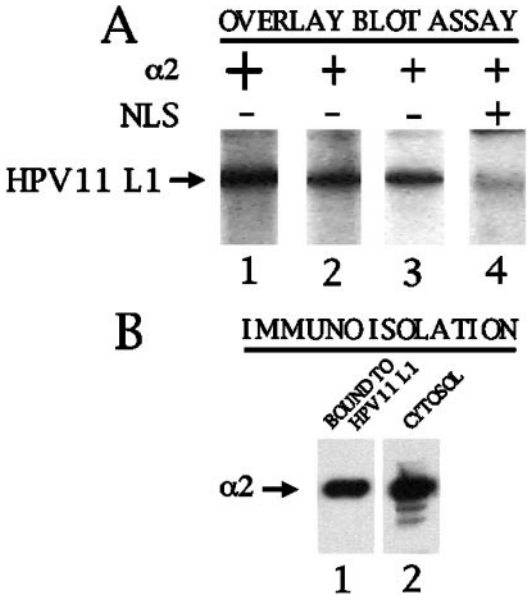

Fig. 5. **A:** HPV11 L1 binds to karyopherin α2 and the interaction is inhibited by the SV40 T antigen NLS. HPV11 L1 protein was subjected to SDS-PAGE and transferred on nitrocellulose membrane. Blots were probed with karyopherin α2 in different concentrations: 100 nM (1), 50 nM (2), and 25 nM (3). In (4) the blot was incubated with karyopherin α2 (25 nM) in the presence of a peptide containing SV40 T antigen NLS (×200 times molar excess). Bound karyopherin α2 was detected with specific anti-karyopherin α2/Rch-1 antibody. **B:** HPV11 L1 capsomers were incubated with HeLa cytosol and the complexes were immunoisolated using a polyclonal antiserum raised against HPV11 L1 VLPs (see Materials and Methods). The isolated complexes (1) and HeLa cytosol (2) were subjected to SDS-PAGE and transferred on nitrocellulose membrane. The blots were probed with anti-karyopherin α2/Rch-1.

productive phase when the newly synthesized L1 and L2 proteins co-assemble with the replicated HPV genomic DNA into infectious virions. The physiological role of nuclear import of L1 protein during the early phase of viral life cycle is not known. We investigated the nuclear import pathway for the L1 capsid protein of low risk HPV11. We found that when the import reactions were carried out with L1 capsomers, but not with L1 capsids, L1 was imported into the nuclei of digitonin-permeabilized cells in a receptor-dependent manner (Fig. 1). It is important to note that during the import reactions in digitonin-permeabilized cells in the presence of cytosol, the L1 capsomers could further be disassembled into monomers. Hence, we do not know if L1 protein was transported into the nucleus as a monomer, or as a capsomer, or both. These findings suggest that capsid disassembly (at least to capsomers) may be required for nuclear import of incoming HPV11 L1 protein during viral infection. We obtained comparable results with the L1 capsid proteins of HPV types 18 and 45 (manuscript in preparation). As the functional diameter of the vertebrate NPC is 25 nm [Dworetziki and Feldherr, 1988], disassembly of the capsids (50–55 nm) prior to nuclear import of HPV11 L1 would be expected. It was established that disulfide bonds are involved in capsomer-capsomer interactions and stability of the capsids, and that reducing agents can break these bonds [McCarthy et al., 1998; Li et al., 1998]. This would suggest that during the early stages of infection, exposure of HPV capsids to the reducing environment of the cytoplasm would break some of the stabilizing disulfide bonds leading to capsid disassembly [McCarthy et al., 1998; Li et al., 1998]. The same reducing environment could perhaps prevent capsid assembly in the cytoplasm during the late productive phase of viral life cycle when the L1 protein is synthesized and transported into the nucleus.

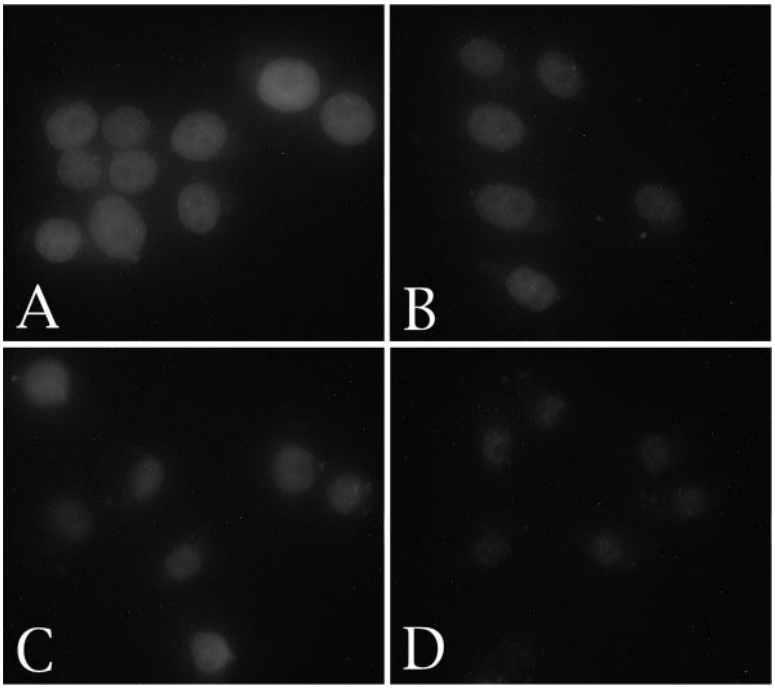

Fig. 6. HPV11 L1 in excess can inhibit the nuclear import of a classical NLS-HSA conjugate. Digitonin-permeabilized HeLa cells were incubated in an import reaction for 30 min at room temperature with fluorescein-labeled NLS-HSA (0.4 μM) in the presence of either HeLa cytosol (**A**), or HeLa cytosol plus increasing concentrations of HPV11 L1 capsomers: 1 μM (**B**), 2 μM (**C**), and 4 μM (**D**).

Other unknown factors could participate in these processes. The precise molecular mechanisms of HPV capsid disassembly and prevention of capsid assembly in the cytoplasm of host cells remain to be established.

HPV11 L1 Protein Enters the Nucleus of Host Cells via Karyopherin α2β1 Heterodimers

We investigated which karyopherins might be involved in nuclear import of HPV11 L1 capsid protein. We discovered that HPV11 L1 docks at the NPC via karyopherin α2β1 heterodimers (Figs. 2 and 4) and is translocated into the nucleus in the presence of additional RanGDP and p10 (Fig. 4). In agreement with the docking and nuclear import data we found that HPV11 L1 binds karyopherin α2 and the NLS peptide of SV40 T antigen inhibits this interaction (Fig. 5A). Binding of HPV11 L1 to karyopherin α1/hSRP was not tested. In immunoisolation assays performed with HeLa cytosol and HPV11 L1 capsomers we found that L1 interacts with karyopherin α2 (Fig. 5B). Together these data indicate that HPV11 L1 protein binds to cytoplasmic karyopherin α2 via its classical NLS and can enter the nuclei of host cells via karyopherin α2β1 heterodimers.

In competition assays we found that HPV11 L1 in excess can inhibit the nuclear import of a classical NLS-HSA conjugate (Fig. 6). These data suggest that in the late phase of the virus life cycle when the L1 protein is massively synthesized and transported into the nucleus to assemble the replicated HPV-DNA into virions, the karyopherin α β1-mediated nuclear import of cellular proteins could be affected.

Other viral proteins, such as the Influenza virus nucleoprotein and the matrix protein of HIV-1 bind to α karyopherins and can enter the nuclei of host cells via karyopherin α β1 heterodimers [O'Neill et al., 1995; O'Neill and Palese, 1994; Gallay et al., 1996]. Interestingly, the L1 capsid protein of the high risk HPV45 binds directly to karyopherin β1 (manuscript in preparation). These data are in agreement with the fact that the NLSs of HPV11 L1 and HPV45 L1 differ: HPV11 L1 NLSs resemble classical types of NLSs [Dingwall and Laskey, 1991; Garcia-Bustos et al., 1991] whereas HPV45 L1 has a potential NLS that is more complex and rich in arginines (manuscript in preparation). These results indicate that the L1 proteins of different types of HPV may exploit different nuclear import pathways of host cells.

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