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The L1 major capsid protein of human papillomavirus type 11 interacts with Kap β2 and Kap β3 nuclear import receptors

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Abstract

We have previously shown that the L1 major capsid protein of low-risk HPV11 binds to the Kap α2 adapter and enters the nucleus via a Kap α2β1-mediated pathway. In this study, we discovered that HPV11 L1 capsomeres bind to Kap β2 import receptor, known to mediate nuclear import of hnRNP A1 via interaction with its nuclear localization signal termed M9. Significantly, binding of HPV11 L1 capsomeres to Kap β2 inhibited the nuclear import of Kap β2, and its specific M9-containing cargo. Interestingly, HPV11 L1 capsomeres also interacted with Kap β3 import receptor and inhibited Kap β3 nuclear import. Moreover, the L1 capsomeres of high-risk HPV-16 shared these activities. These data suggest that HPV L1 major capsid proteins interact with Kap β2 and Kap β3, and they may inhibit the Kap β2- and Kap β3-mediated nuclear import pathways during the productive phase of the viral life cycle when the virions are assembled and released.

Keywords: Papillomavirus; L1; Karyopherins; Kap β2; Kap β3

Introduction

Human papillomaviruses (HPVs) are nonenveloped, icosahedral DNA tumor viruses that infect squamous epithelial cells of either anogenital and oral mucosal tissues or the skin. Mucosal HPVs have demonstrated varying degrees of oncogenic potential: high-risk HPVs, types 16, 18, 31, and 45, are frequently detected in invasive cervical carcinomas, whereas the low-risk HPVs, types 6 and 11, are associated with benign exophytic condylomas (zur Hausen, 2000). The virion particles (52-55 nm in diameter) consist of a single molecule of 8-kb double-stranded circular DNA packaged into a spherical capsid composed of 72 homopentameric L1 capsomeres and 12 molecules of L2 minor capsid protein (Trus et al., 1997). During the productive, vegetative amplification phase of the viral life cycle in terminally differentiated epithelial cells, the newly synthesized L1 major capsid proteins enter the nuclei and together with the L2 minor capsid proteins package the replicated HPV genomic DNA into infectious virions. L1 major capsid proteins can self-assemble both in vivo and in vitro into capsid-like structures, referred to as virus-like particles (VLPs) (Belnap et al., 1996; Hagensee et al., 1994; Kirnbauer et al., 1992; Rose et al., 1993; Volpers et al., 1994; Zhou et al., 1993). The VLPs can be disassembled quantitatively into L1 homopentameric capsomeres by an agent that reduces disulfide bonds and can be reassembled by removing the reducing agent (Li et al., 1998; McCarthy et al., 1998).

The general mechanism for nuclear import is that a cargo interacts in the cytoplasm either directly or via an adapter with an import receptor belonging to the karyopherin β/importin β (Kap β/Imp β) superfamily, is transported through the nuclear pore complex, and is released inside the nucleus (Moroianu, 1999; Nakielny and Dreyfuss, 1999; Wente, 2000). All Kap βs shuttle between the nucleus and the cytoplasm and bind to nucleoporins at the nuclear pore complex and to the GTPase Ran in its GTP bound form.

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Binding of nuclear RanGTP to Kap βs (importins) causes the dissociation of the import complexes with release of the cargoes inside the nucleus. In vertebrates, several members of the Kap β superfamily have been identified and shown to function in nuclear import of specific cargoes. Kap β1/Importin β can function together with a Kap α/importin α adapter in nuclear import of proteins that contain classic monopartite or bipartite nuclear localization signals (NLSs), or without adapters in import of ribosomal proteins, cyclin B1, and several viral proteins, or together with Imp7 in import of histone H1 (Moroianu, 1999; Nakielny and Dreyfuss, 1999; Wente, 2000). Mammalian Kap β2/transportin mediates nuclear import of hnRNP A1 and A2 via interaction with their Gly/Asn-rich NLS termed M9, of hnRNP F (Bonifaci et al., 1997; Pollard et al., 1996; Siomi and Dreyfuss, 1995; Siomi et al., 1997), of an mRNA export factor called TAP (Truant et al., 1999), and of ribosomal proteins (Yaseen and Blobel, 1997; Jakel and Gorlich, 1998). Interestingly, the ribosomal proteins L23a, S7, and L5 can interact with four Kaps, Kap β1, Kap β2/transportin, Kap β3/Imp5, and Imp7, and be imported alternatively by these Kaps (Yaseen and Blobel, 1997; Jakel and Gorlich, 1998).

We have previously shown that the L1 capsomeres of HPV types 11, 16, and 45 can efficiently enter the nucleus in the presence of cytosol containing the karyopherins, whereas the intact capsids (50–55 nm) cannot pass through the nuclear pore complexes which have a functional diameter of 39 nm (Merle et al., 1999; Nelson et al., 2000, 2002). Moreover, we had established that the L1 capsomeres of both low-risk HPV11 and high-risk HPV-16 and -45 enter the nucleus via a classical Kap α2β1-mediated pathway (Merle et al., 1999; Nelson et al., 2000, 2002). Interestingly, we recently discovered that the L1 capsomeres of high-risk HPV-16 and -45 interact with Kap β2 import receptor and inhibit the nuclear import of Kap β2, and of a Kap β2-specific M9-containing cargo (Nelson et al., 2002). This novel interaction of high-risk HPV L1 capsid proteins with Kap β2 raised several questions: (1) do the low-risk HPV L1 proteins (e.g., type 11) share this activity, (2) do L1 proteins of both low- and high-risk HPVs interact with other members of Kap β superfamily, and 3) if they do, what are the consequences of these interactions?

In this study we found that the L1 capsomeres of low-risk HPV11 interact with Kap β2 import receptor and binding of RanGTP to Kap β2 does not dissociate the HPV11 L1–Kap β2 complex. Significantly, binding of HPV11 L1 capsomeres to Kap β2 inhibited the nuclear import of Kap β2, and of a Kap β2-specific M9-containing cargo. Interestingly, HPV11 L1 capsomeres also interacted with Kap β3 import receptor and inhibited Kap β3 nuclear import. Moreover, these activities were shared by the L1 capsomeres of high-risk HPV16. Together, these data suggest that during the productive phase of viral life cycle, when the HPV L1 major capsid proteins enter the nucleus via a classical Kap α2β1-mediated pathway to assemble the virions, they also interact with Kap β2 and Kap β3 and may thereby inhibit the Kap β2- and Kap β3-mediated nuclear import of host proteins.

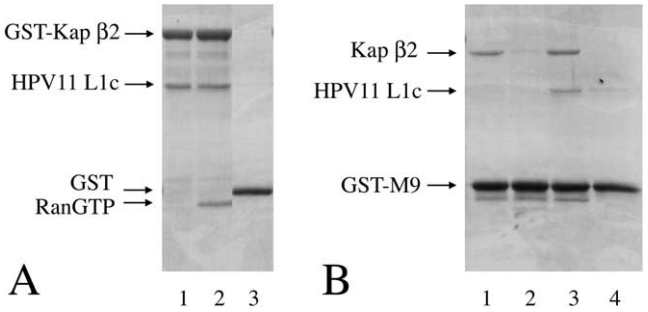

Fig. 1. HPV11 L1 capsomeres interact with Kap β2. (A) Immobilized GST-Kap β2 was incubated at RT for 30 min with either HPV11 L1 capsomeres alone (Lane 1), or the L1 capsomeres plus RanGTP (Lane 2). As a specificity control, HPV11 L1 capsomeres were incubated with immobilized GST (Lane 3). (B) Immobilized M9-GST was incubated at RT for 30 min with either Kap β2 (Lane 1), Kap β2 plus RanGTP (Lane 2), Kap β2 plus HPV11 L1 capsomeres (Lane 3), or HPV11 L1 capsomeres alone (Lane 4). Bound proteins were eluted with sample buffer and analyzed by SDS–PAGE and Coomassie blue staining.

Results

HPV11 L1 capsomeres interact with Kap β2 and inhibit the Kap β2-mediated nuclear import pathways

We have previously shown that the L1 major capsid protein of low-risk HPV11 interacts with Kap α2 adapter and enters the nucleus via a classical Kap α2β1-mediated pathway (Merle et al., 1999).

In this study, we found that HPV11 L1 capsomeres bound to the Kap β2 nuclear import receptor (Fig. 1A, Lane 1). As a control, HPV11 L1 capsomeres did not bind to glutathione-*S*-transferase (GST) alone (Fig. 1A, Lane 3). Binding of RanGTP to Kap βs inhibits their interaction with specific cargoes (Moroianu, 1999; Wente, 2000), and, as expected, RanGTP was efficient in inhibiting the interaction between Kap β2 and M9-GST (Fig. 1B, compare Lanes 1 and 2). In contrast, binding of RanGTP to Kap β2 did not inhibit the interaction between HPV11 L1 capsomeres and Kap β2 (Fig. 1A, compare Lanes 1 and 2).

Analysis of HPV11 L1 amino acid sequence does not reveal any M9-like motif, suggesting a different type of interaction between the Kap β2 and HPV11 L1. Indeed, we found that HPV11 L1 capsomeres can bind to Kap β2 at the same time Kap β2 interacts with M9-GST (Fig. 1B, Lane 3). As a control, HPV11 L1 capsomeres did not bind to the M9-GST alone (Fig. 1B, Lane 4). These data indicate that the two binding sites on Kap β2 for the M9 signal and HPV11 L1 are distinct and separate.

The interaction between HPV11 L1 capsomeres and Kap β2 lead us to investigate if HPV11 L1 capsomeres can enter into the nucleus via a Kap β2-mediated pathway. Therefore,

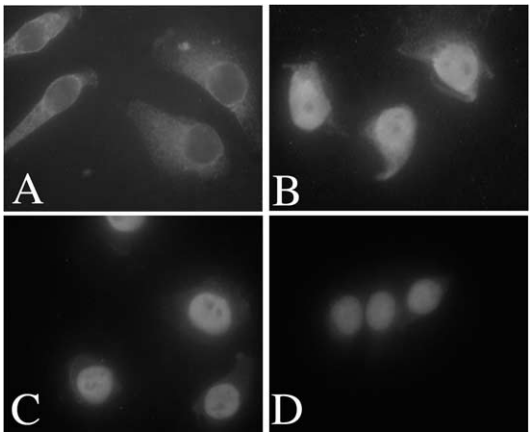

Fig. 2. Kap β2 does not mediate nuclear import of HPV11 L1 capsomeres. Digitonin-permeabilized HeLa cells were incubated for 30 min at RT with either HPV11 L1 capsomeres (A and B) or GST-M9 (C and D) in the presence of either Kap β2 plus RanGDP (A and C) or HeLa cytosol (B and D). HPV11 L1 capsomeres were detected with a specific polyclonal antibody to HPV11 L1, and M9-GST, with a polyclonal antibody to GST.

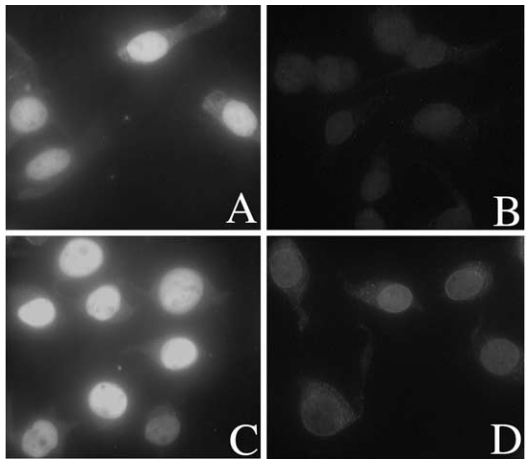

Fig. 3. HPV11 L1 capsomeres inhibit nuclear import of Kap β2- and Kap β2-mediated import of M9-GST. Digitonin-permeabilized HeLa cells were incubated for 30 min at RT with either GST-Kap β2 (A) or a mixture of GST-Kap β2 and HPV11 L1 capsomeres that had been preincubated for 30 min before import (B). Nuclear import of GST-Kap β2 was detected with a monoclonal anti-GST antibody. Digitonin-permeabilized HeLa cells were incubated for 30 min at RT with either Kap β2 + M9-GST + RanGDP (C) or with Kap β2 + M9-GST + RanGDP in the presence of HPV11 L1 capsomeres (in equimolar amount to the Kap β2) (D). Nuclear import of M9-GST was detected with a monoclonal anti-GST antibody.

digitonin-permeabilized HeLa cells were incubated with either HPV11 L1 capsomeres or M9-GST (positive control for Kap β2 pathway) in the presence of either Kap β2 plus RanGDP or HeLa cytosol. We found that Kap β2 did not mediate nuclear import of HPV11 L1 capsomeres, whereas, as expected, it did mediate the nuclear import of the M9-GST control (Fig. 2, compare A and C). In the presence of cytosol, both the HPV11 L1 capsomeres and the M9-GST entered the nucleus (Figs. 2B and 2D).

As Kap β2 did not mediate nuclear import of HPV11 L1 capsomeres, we next investigated if binding of HPV11 L1 to Kap β2 inhibits nuclear import of Kap β2. Digitonin-permeabilized HeLa cells were incubated with GST-Kap β2 in the absence or presence of HPV11 L1 capsomeres. As expected, GST-Kap β2 entered the nucleus in the absence of HPV11 L1 capsomeres (Fig. 3A). In the presence of HPV11 L1 capsomeres, nuclear import of GST-Kap β2 was strongly inhibited (Fig. 3, compare A and B), indicating that binding of HPV11 L1 capsomeres to Kap β2 interferes with nuclear import of Kap β2. Moreover, we have found that in the presence of HPV11 L1 capsomeres, Kap β2-mediated nuclear import of its specific M9-GST cargo was inhibited (Fig. 3, compare C and D). These results suggest that HPV11 L1 capsomeres do not use Kap β2 to enter into the nucleus via an additional pathway, instead they inhibit Kap β2 nuclear import and consequently, the Kap β2 mediated import pathways.

Significantly, we found that in the presence of cytosol containing the karyopherins (Kap α2, Kap β1, and Kap β2), HPV11 L1 capsomeres could both efficiently enter the nucleus, via a Kap α2β1-mediated pathway (Fig. 4A), and inhibit the Kap β2-mediated nuclear import of M9-GST (Fig. 4, compare B and C).

HPV11 L1 capsomeres interact with Kap β3 and inhibit Kap β3 nuclear import

The binding of HPV11 L1 capsomeres to Kap β2 raised the possibility of interactions with additional Kap β import receptors. Indeed, we discovered that HPV11 L1 capsomeres also bound to the Kap β3 immobilized on Glutathione–Sepharose but not to the GST alone (Fig. 5, Lanes 1 and 3). Binding of RanGTP to Kap β3 did not inhibit the interaction between HPV11 L1 capsomeres and Kap β3 (Fig. 5, compare Lanes 1 and 2).

Investigation of nuclear import of HPV11 L1 capsomeres in the presence of Kap β3 plus RanGDP revealed that Kap β3 was unable to mediate nuclear import of HPV11 L1 (data not shown). These results led us to investigate if binding of HPV11 L1 capsomeres to Kap β3 inhibits its nuclear import. Therefore, digitonin-permeabilized HeLa

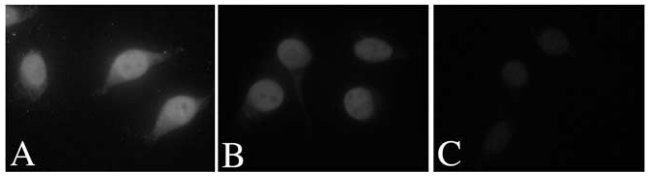

Fig. 4. In the presence of cytosol, HPV11 L1 capsomeres can both enter the nucleus via Kap α2β1-mediated pathway and inhibit Kap β2-mediated nuclear import of M9-GST. Digitonin-permeabilized cells were incubated with HPV11 L1 capsomeres + cytosol (A), M9-GST + cytosol (B), or M9-GST + cytosol + HPV11 L1 capsomeres (C). Detection was done with an anti-HPV11 L1 antibody (A) or with an anti-GST antibody (B and C).

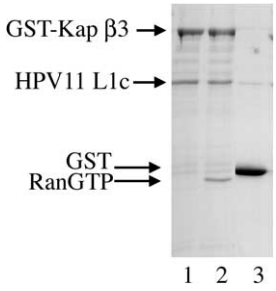

Fig. 5. HPV11 L1 capsomeres interact with Kap β3 nuclear import receptor. Immobilized GST-Kap β3 was incubated at RT for 30 min with either HPV11 L1 capsomeres alone (Lane 1) or the L1 capsomeres plus RanGTP (Lane 2). As a specificity control, HPV11 L1 capsomeres were incubated with immobilized GST (Lane 3). Bound proteins were eluted with sample buffer and analyzed by SDS–PAGE and Coomassie blue staining.

cells were incubated with GST-Kap β3 plus RanGDP in the absence or presence of HPV11 L1 capsomeres. Nuclear docking and import of GST-Kap β3 was strongly inhibited in the presence of HPV11 L1 capsomeres (Fig. 6, compare A and B), indicating that binding of HPV11 L1 capsomeres to Kap β3 interferes with these processes.

Kap α2 competes with Kap β2 and Kap β3 for binding to HPV11 L1 capsomeres

In competition assays, we found that Kap α2 can reduce the binding of HPV11 capsomeres to either Kap β2 or Kap β3 (Figs. 7A and 7B, compare Lanes 1 and 2). Note that Kap α2 also bound to other available molecules in the homopentameric L1 capsomeres (unbound to either Kap β2 or Kap β3) (Figs. 7A and 7B, Lane 2). The competition data suggest that the affinity of HPV11 L1 capsomeres for Kap α2 is higher than for Kap β2 or Kap β3. This is in agreement with the efficient nuclear import of HPV11 L1 capsomeres in the presence of cytosol containing all the Kaps (Fig. 4).

The L1 capsomeres of high-risk HPV16 also interact with Kap β3 and inhibit Kap β3 nuclear import

We had previously found that HPV16 L1 capsomeres interact with Kap β2 and inhibit nuclear import of Kap β2

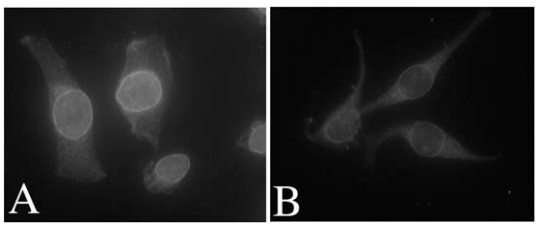

Fig. 6. HPV11 L1 capsomeres inhibit nuclear import of Kap β3. Digitonin-permeabilized HeLa cells were incubated with GST-Kap β3 plus RanGDP in the absence (A) or presence of HPV11 L1 capsomeres (B). Detection was with anti-GST polyclonal antibody.

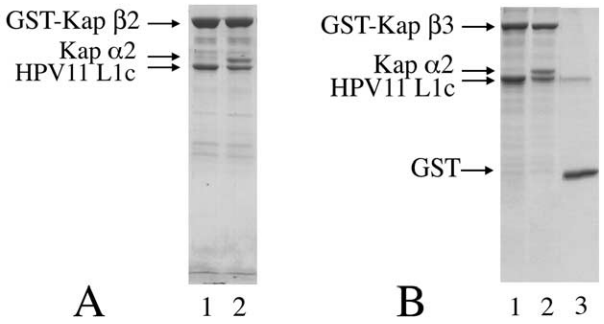

Fig. 7. Kap α2 competes with Kap β2 and Kap β3 for binding to HPV11 L1 capsomeres. (A) Immobilized GST-Kap β2 was incubated at RT for 30 min with either HPV11 L1 capsomeres alone (Lane 1) or the L1 capsomeres plus Kap α2 (Lane 2). (B) Immobilized GST-Kap β3 was incubated at RT for 30 min with either HPV11 L1 capsomeres alone (Lane 1) or the L1 capsomeres plus Kap α2 (Lane 2). As a specificity control, HPV11 L1 capsomeres were incubated with immobilized GST (Lane 3). Bound proteins were eluted with sample buffer and analyzed by SDS–PAGE and Coomassie blue staining.

(Nelson et al., 2002). The interaction between the L1 capsomeres of low-risk HPV11 and Kap β3 raised the question if this is true also for HPV16. In solution, binding assays demonstrated that HPV16 L1 capsomeres interacted with GST-Kap β3 (Fig. 8, Lane 1) and not with GST alone (Fig. 8, Lane 3). Incubation of GST-Kap β3 with HPV16 L1 capsomeres in the presence of RanGTP did not reduce the binding of L1 capsomeres to Kap β3 (Fig. 8, Lane 2).

Investigation of nuclear import of HPV16 L1 capsomeres in digitonin-permeabilized cells in the presence of Kap β3 plus RanGDP revealed that Kap β3 did not mediate nuclear import of HPV16 L1 capsomeres (data not shown). As Kap β3 did not mediate nuclear import of HPV16 L1 capsomeres, we next investigated if binding of HPV16 L1 capsomeres to Kap β3 inhibits nuclear import of Kap β3. Digitonin-permeabilized cells were incubated with GST-Kap β3 plus RanGDP in the absence or presence of HPV16 L1 capsomeres. We found that indeed HPV16 L1 capsomeres inhibited nuclear docking and import of GST-Kap β3

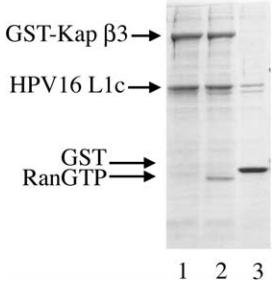

Fig. 8. HPV16 L1 capsomeres interact with Kap β3 nuclear import receptor. Immobilized GST-Kap β3 was incubated at RT for 30 min with either HPV16 L1 capsomeres alone (Lane 1) or the L1 capsomeres plus RanGTP (Lane 2). As a specificity control, HPV16 L1 capsomeres were incubated with immobilized GST (Lane 3). Bound proteins were eluted with sample buffer and analyzed by SDS–PAGE and Coomassie blue staining.

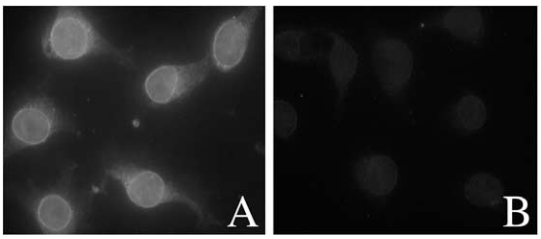

Fig. 9. HPV16 L1 capsomeres inhibit nuclear import of Kap β3. Digitonin-permeabilized HeLa cells were incubated with GST-Kap β3 plus RanGDP in the absence (A) or presence of HPV16 L1 capsomeres (B). Detection was with an anti-GST polyclonal antibody.

(Fig. 9, compare A and B). In the same conditions, HPV16 L1 capsomeres had no effect on nuclear docking and import of GST-Kap β1 (data not shown). This is in agreement with our previous data that HPV16 L1 capsomeres enter the nucleus via a classical Kap α2β1-mediated pathway (Nelson et al., 2002).

Discussion

We had previously established that the L1 capsomeres of low-risk HPV11 and high-risk HPV16 interact with Kap α2 adapter and enter the nucleus via a classical Kap α2β1-mediated pathway (Merle et al., 1999; Nelson et al., 2002). In the present study, we have discovered that HPV11 L1 capsomeres can also interact with Kap β2 and Kap β3 nuclear import receptors. Moreover, the L1 capsomeres of high-risk HPV16 interact with Kap β2 (Nelson et al., 2002) and Kap β3 (this study). This suggests that the interaction of L1 capsomeres with both Kap β2 and Kap β3 is a general property of both low- and high-risk HPVs. Interestingly, binding of RanGTP to Kap β2 or Kap β3 did not inhibit the interaction of L1 capsomeres with these Kaps, in contrast with the role of RanGTP in dissociating import complexes (Moroianu, 1999; Nakielny and Dreyfuss, 1999). Moreover, neither Kap β2 nor Kap β3 mediated nuclear import of L1 capsomeres. Recently, Ran binding to Kap β2 has been uncoupled from cargo dissociation using a TL-Kap β2 mutant, which has a truncated acidic loop (Chook et al., 2002). Approximately 75% of nuclear import of a specific cargo was lost with TL-Kap β2 mutant in comparison with the wild-type Kap β2. These data strongly suggested that at least 75% of Kap β2-mediated import is dependent on Ran-mediated cargo dissociation (Chook et al., 2002).

Significantly, the interactions of L1 capsomeres with Kap β2 and Kap β3 cause inhibition of Kap β2 and Kap β3 nuclear import, and consequently, their nuclear import pathways. The main cargoes transported by Kap β2 are hnRNP A1 and A2 via interaction with their M9 sequence (Bonifaci et al., 1997; Pollard et al., 1996; Siomi and Dreyfuss, 1995; Siomi et al., 1997), and a mRNA export factor called TAP (Truant et al., 1999). hnRNP A1 is an abundant nuclear RNA-binding protein involved in the processing of pre-mRNA (Dreyfuss et al., 1993) and its nucleocytoplasmic shuttling is strongly correlated with nuclear export of mature mRNAs (Izaurralde et al., 1997). Interestingly, the stress-induced decrease of nuclear abundance of hnRNP A/B proteins and increase of their cytoplasmic levels cause changes in the alternative splicing pattern of an adenovirus E1A pre-mRNA splicing reporter (van der Houven van Oordt et al., 2000). It can be speculated that in the productive, vegetative amplification phase of the viral life cycle, the interaction of newly synthesized L1 proteins with Kap β2 may inhibit Kap β2-mediated nuclear import of hnRNP A1 and TAP, and, as a consequence, affect pre-mRNA splicing and mRNA nuclear export.

As HPV L1 capsomeres are efficiently imported into the nuclei of digitonin-permeabilized cells in the presence of HeLa cytosol containing all the Kaps (Merle et al., 1999; Nelson et al., 2002; this study), the higher affinity interaction of L1 capsomeres is with Kap α2β1 heterodimers leading to nuclear import. This is in agreement with the fact that Kap α2 competes with either Kap β2 or Kap β3 for binding to HPV L1 capsomeres.

Significantly, HPV11 L1 capsomeres can both efficiently enter the nucleus via a Kap α2β1-mediated pathway (Fig. 4A) and inhibit the Kap β2-mediated nuclear import of its specific M9-GST cargo (Fig. 4, compare B and C). These data suggest that L1 capsomeres can inhibit Kap β2-mediated nuclear import of cargoes under conditions expected for infections, when they are efficiently imported into the nucleus via Kap α2β1 heterodimers.

The cargoes known so far to be transported by Kap β3 are the ribosomal proteins L23a, S7, and L5 (Jakel and Gorlich, 1998), and core histones (Muhlhausser et al., 2001). Both the ribosomal proteins and the core histones can also enter the nucleus also via Kap β1, Kap β2, and Imp 7 (Jakel and Gorlich, 1998; Muhlhausser et al., 2001). Inhibition of nuclear import of both Kap β2 and Kap β3 by L1 proteins may reduce nuclear import of ribosomal proteins and/or core histones. However, it cannot be speculated at this time if this would happen during the productive, vegetative amplification phase of the viral life cycle, as both classes of proteins can enter the nucleus via alternative pathways (Jakel and Gorlich, 1998; Muhlhausser et al., 2001) and the stoichiometry of L1 protein levels vs Kap β2 and Kap β3 during viral infection is unknown. Demonstration of inhibition of Kap β2- and Kap β3-mediated nuclear import of specific cargoes by L1 capsomeres in vivo under conditions similar to viral infection would strongly support the current interpretation of our results. Future studies in vivo will bring more insight into the role(s) these interactions of L1 capsomeres with Kap β2 and Kap β3 play during the productive, vegetative amplification phase of the viral life cycle.

Intact L1 capsids can interact with both Kap β2 and Kap β3 (data not shown), suggesting that the binding sites for these Kaps are exposed at the capsid surface. Kap α2 in-

teracts with the C-terminus located classical monopartite or bipartite NLSs of HPV L1 proteins (Nelson et al., 2000, 2002), whereas Kap β2 and Kap β3 do not (data not shown). The fact that Kap α2 competes with both Kap β2 and Kap β3 for binding to L1 suggests the possibility that the binding sites for Kap α2 and Kap β2/Kap β3 may have some partial overlap. Further studies are required to map the binding domains between HPV L1 proteins and Kap β2/Kap β3 and determine the mechanism of inhibition of Kap β2 and Kap β3 nuclear import.

In conclusion, HPV L1 major capsid proteins seem to have evolved a double strategy: (1) they enter the nucleus via a classical Kap α2β1-mediated pathway and together with the L2 minor capsid proteins package the replicated viral DNA into virions, and (2) they interact with Kap β2 and Kap β3 import receptors and instead of exploiting these interactions to enter the nucleus via two additional pathways, they may block their nuclear import. Further studies in vivo are required to understand the role(s) of the inhibition of Kap β2- and Kap β3-mediated nuclear import pathways during the productive phase of viral life cycle when the virions are formed and released.

Materials and methods

Preparation of recombinant human nuclear import factors

His-tagged Kap α2 (Weis et al., 1995) and His-tagged Kap β1 (Chi et al., 1996) were expressed in *Escherichia coli* BL21(DE3) (3 h induction with 2 mM IPTG at 30°C), and the soluble His-tagged proteins were purified in their native state on Talon beads using a standard procedure. GST-Kap β1 (Chi et al., 1996), GST-Kap β2 (Chook and Blobel, 1999), and GST-Kap β3 (Yaseen and Blobel, 1997) were expressed in *E. coli* BL21(DE3) (3 h induction with 1 mM IPTG at 30°C), and the soluble GST-fusion proteins were purified in their native state on Glutathione–Sepharose beads using a standard procedure. Kap β2 was obtained by cleaving the GST-Kap β2 fusion protein, as described (Nelson et al., 2002). To obtain Kap β3, the GST-Kap β3 was incubated for 2 h at RT with biotinylated thrombin, and after cleavage the GST was removed by binding to Glutathione–Sepharose beads and the thrombin by binding to Streptavidin-containing beads, according to the manufacturer's procedures. Human Ran (Coutavas et al., 1993) was prepared as described (Floer and Blobel, 1996). All proteins were checked for purity and lack of proteolytic degradation by SDS–PAGE and Coomassie blue staining. The purified proteins were dialyzed in transport buffer (20 mM HEPES–KOH, pH 7.3, 110 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, 2 mM dithiothreitol, plus protease inhibitors) and stored in aliquots at −80°C until use. HeLa cytosol from Cellex Biosciences Inc. was centrifuged and stored in aliquots at −80°C.

Preparation of recombinant HPV L1 capsids and capsomeres

The VLPs of HPV11 and HPV16 were generated in insect cells, purified as described (Rose et al., 1994a,b), and stored at 4°C until use. Care is taken during the purification procedure to isolate primarily intact VLPs. Purity and lack of proteolytic degradation of L1 proteins were always checked by SDS–PAGE, Coomassie blue staining, and immunoblotting. L1 capsomeres were obtained by incubating the L1 capsids with 5% mercaptoethanol overnight at 4°C (McCarthy et al., 1998).

Antibodies

Rabbit polyclonal antisera were raised against HPV L1 capsids as described (Rose et al., 1994a,b). The goat anti-GST polyclonal antibody was acquired from Amersham Pharmacia Biotech.

Preparation of M9-GST fusion protein

The M9-GST construct was a gift from Dr. Gideon Dreyfuss. For protein expression, the construct was used to transform *E. coli* BL21 (DE3) bacteria. After induction of *E. coli* BL21(DE3) with 1 mM IPTG for 3 h at 37°C, the M9-GST fusion protein was purified in its native state on Glutathione–Sepharose using a standard procedure. The purified protein was checked by SDS–PAGE and Coomassie blue staining and then dialyzed in buffer A and stored in aliquots at −80°C until use.

In vitro nuclear import assays

Digitonin-permeabilized HeLa cells have been used by many labs including ours to investigate different nuclear import pathways mediated by mammalian Kap βs/importins (Adam and Adam, 1994; Chi et al., 1995; Gorlich et al., 1995; Kataoka et al., 1999; Merle et al., 1999; Nelson et al., 2000, 2002; Paschal and Gerace, 1995; Pollard et al., 1996; Ribbeck et al., 1998, 1999; Schwoebel et al., 1998; Weis et al., 1995). The nuclear import assays were carried out as previously described (Nelson et al., 2002). Briefly, subconfluent HeLa cells, grown on poly-l-lysine-coated glass coverslips for 24 h, were permeabilized with 70 μg/ml digitonin for 5 min on ice and washed with transport buffer. Unless otherwise specified, all import reactions contained an energy-regenerating system (0.5 mM GTP, 5 mM phosphocreatine, and 0.4 U creatine phosphokinase), plus various transport factors (0.5 μM Kap β1; 0.5 μM Kap β2; 0.5 μM Kap β3; 3 μM RanGDP), plus the L1 capsomeres (0.25-0.5 μM) or the GST fusion proteins (0.5 μM). We also used Kap β2 and Kap β3 as GST-fusion proteins. The presence of GST in the GST-Kap β2 and GST-Kap β3 fusion proteins does not interfere with nuclear import of either Kap β2 or Kap β3. Final import reaction volume was adjusted to 20 μl with

transport buffer. For visualization of nuclear import, the HPV L1 proteins and the GST-fusion proteins were detected by immunofluorescence with specific antibodies, as previously described (Merle et al., 1999; Nelson et al., 2000, 2002). The nuclei were identified by DAPI staining. Nuclear import was analyzed with a Nikon Eclipse TE 300 microscope that has a fluorescence attachment and a Sony DKC-5000 CCD camera.

In-solution binding assays

GST-Kap β2 or GST-Kap β3 immobilized on Glutathione–Sepharose beads (2 μg protein/10 μl beads) were incubated for 30 min under rotation at RT with the HPV L1 capsomeres (2 μg protein) in transport buffer containing 0.25% Tween 20 (binding buffer). In some experiments, 2 or 4 μg of either Kap α2 or RanGTP were added to the incubation mixture, as indicated in the figure legends. Control experiments for binding specificity consisted of incubating GST immobilized on Glutathione–Sepharose beads with the L1 capsomeres. The bound proteins were eluted with SDS–PAGE sample buffer and analyzed by SDS–PAGE followed by Coomassie blue staining.

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