Minor Capsid Proteins of Simian Virus 40 Are Dispensable for Nucleocapsid Assembly and Cell Entry but Are Required for Nuclear Entry of the Viral Genome

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We investigated the roles of simian virus 40 capsid proteins in the viral life cycle by analyzing point mutants in Vp1 and Vp2/3, as well as a deletion mutant lacking the Vp2/3 coding sequence. The Vp1 mutants (V243E and L245E) and the Vp2/3 mutants (F157E-I158E and P164R-G165E-G166R) were previously shown to be defective in Vp1-Vp2/3 interaction and to be noninfectious or poorly infectious, respectively. Here, we show that all these point mutants form stable particles following DNA transfection into cells. The Vp2/3-mutant particles contained very low levels of Vp2/3, whereas the Vp1 mutant particles contained no detectable Vp2/3. As expected, the deletion mutant also formed particles that were noninfectious. We further characterized the two Vp1 point mutants and the deletion mutant. All three mutant particles comprised Vp1 and histone-associated viral DNA, and all were able to enter cells. However, the mutant complexes failed to associate with host importins (owing to the loss of the Vp2/3 nuclear localization signal), and the mutant viral DNAs prematurely dissociated from the Vp1s, suggesting that the nucleocapsids did not enter the nucleus. Consistently, all three mutant particles failed to express large T antigen. Together, our results demonstrate unequivocally that Vp2/3 is dispensable for the formation of nucleocapsids. Further, the nucleocapsids’ ability to enter cells implies that Vp1 contains the major determinants for cell attachment and entry. We propose that the major role of Vp2/3 in infectivity is to mediate the nuclear entry of viral DNA.
Vp2/3 are required for some or all of these processes in the context of SV40 infection of host monkey kidney cells.

Based on a structural model, we previously identified residues of SV40 Vp1 and Vp3 that, when mutated, selectively disrupt the interaction of the two proteins (20, 41). The analysis of three Vp3 mutants (F157E-I158E, P164E-G165R-G166E, and P164R-P165E-P166R) and three Vp1 mutants (V243E, L245E, and V243E-L245E) by transfection of the mutant viral genomes into host monkey kidney cells showed that all of the mutants replicate viral DNA and produce capsid proteins normally. However, the mutants are much less infectious than the wild type (41). A plausible interpretation of these data is that the capsids of these particles comprise Vp1 but little or no Vp2/3 owing to compromised Vp1-Vp3 interaction. Since Vp2/3 mediate the nuclear entry of the SV40 genome via interaction between the Vp3 NLS and importins (42), particles lacking Vp2/3 are predicted to be defective in infecting new host cells.

Here, we provide evidence for the formation of nucleocapsids by SV40 mutants in which Vp2/3 is either absent or nearly so. These particles consist of Vp1 and the encapsidated viral DNA, along with core histones. The degree of loss of Vp2/3 in the particles correlates with the reduction in viability, so that particles with no detectable Vp2/3 are essentially noninfectious. Mutant particles are able to enter new host cells but fail to associate with importins and fail to express large T antigen.

Our study thus reveals distinct roles for the capsid proteins during SV40 infection: Vp1 is sufficient for packaging of the viral DNA-histone complex, as well as attachment and entry into the new host; in contrast, Vp2/3 are not required for nucleocapsid assembly, and their major role in infectivity appears to be limited to mediating host interactions that promote the nuclear entry of viral DNA.

MATERIALS AND METHODS

Plasmids, cells, infection, and immunocytochemistry. The construction of plasmid DNAs harboring mutant viral genomes has been described, including the nonoverlapping pSV40 (pSFV40) mutants Vp3 F157E-I158E, Vp3 P164E-G166E-V156E, and Vp2/3 L245E (41) and the Vp1-only mutant pSV-Vp1 (19). Prior to use in transfections, pSF-Vp40 and pSV-Vp1 plasmids were digested with BamHI and recircularized to yield the respective viral genomes (19). The conditions for TC-7 and CV-1 cell cultures, infection, and immunocytochemistry to detect the expression of T-antigen have been described (42).

Preparation of mutant particles. Most mutants are noninfectious (41), so the formation of mutant particles requires cells to be transfected with mutant DNAs, which are then isolated as described previously (27). Briefly, lysates from 5 × 10^6 to 1 × 10^7 cells that had been transfected with mutant DNAs were separated by sedimentation through sucrose gradients, and the presence of particles in the fractions was determined by anti-Vp1 Western blotting of fractions. The amount of packaged viral DNA was measured by the Hirt procedure, digested with BamHI, resolved on agarose gels, and detected by Southern blotting using 32P-labeled SV40 DNA as a probe. For detecting Vp1, the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto membranes, and then probed for Vp1 using rabbit anti-Vp1 serum and the enhanced chemiluminescence detection system (GE Health Science).

Analysis of cell-internalized particles by immunoprecipitation. The immunoprecipitation procedure was as described previously (42). Briefly, TC-7 cells were infected with approximately 10^3 particles per cell, incubated for 1 h at 4°C and then at 37°C for 4 h, and harvested either by scraping or by trypsin treatment. For detecting DNA, low-molecular-weight DNAs were extracted by the Hirt procedure, digested with BamHI, resolved on agarose gels, and detected by Southern blotting using 32P-labeled SV40 DNA as a probe. For detecting Vp1, the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto membranes, and then probed for Vp1 using rabbit anti-Vp1 serum and the enhanced chemiluminescence detection system (GE Health Science).

Detection of histones in particles. Particle preparations containing 10 to 100 µg of viral DNA were diluted to a 40-µl volume with 10 mM HEPES, pH 7.5, treated with 3 mM DTT and 10 mM EDTA for 20 min at 37°C, and then treated with 10 mM N-ethylmaleimide in the dark at room temperature for 20 min. To the resulting viral chromatin samples, 160 µl of buffer B (10 mM HEPES [pH 6.8], 150 mM KOAc, 2 mM Mg(OAc) 2, 0.5 mM CaCl 2, 0.1% Tween 20, 0.1% Casamino Acids, 5 mM glutathione, 1,000 units/ml aprotinin, 1 µg/ml pepstatin, and 1 µg/ml leupeptin) and 15 µl of protein A beads (GE Healthcare; catalog no. 17-5800-01) were added, and the mixture was rotated at 4°C for 5 h. The protein A-pretreated supernatants were carefully retrieved after being centrifuged at 5,000 rpm at 4°C for at least 10 min and then incubated with rotation at 4°C for 2 to 16 h following the addition of 2 µl of either sheep anti-histone H2B (Abcam; ab9408), sheep anti-histone H3 (ab7834), rabbit anti-histone H4 (ab7311), or control rabbit anti-sheep IgG (Chemicon International; AP147) antibody. Antibody-antigen complexes were recovered with protein A beads and subjected to Western blotting for histone H2A (ab2571), as it did not recognize the histone H2A of TC-7 cells. The reaction mixtures were further incubated for 2 h after the addition of 400 µl of buffer B plus 15 µl of either protein A beads pretreated with 0.2 mg/ml bovine serum albumin and 0.3 mg/ml sheared salmon sperm DNA for rabbit antibody reactions (35) or beads prebound with rabbit anti-sheep IgG for sheep antibody reactions. The IPs were collected by centrifugation, extensively washed with buffer B, and mixed with 20 µg of NO-pSV40Acold standard DNA, and DNA was extracted by phenol-chloroform treatment and ethanol precipitation in the presence of 2 µl of Pellet Paint Coprecipitant (Novagen). The resulting DNA was resuspended in 20 µl of 10 mM Tris-HCl (pH 8.0), 1 µl of which was subjected to semiquantitative PCR as described above. The PCR products were separated by electrophoresis on 1% agarose–Tris-acetate-EDTA gels, visualized by staining them with ethidium bromide, and photographed using the FOTO/Analyst Mini Visionary system (FOTODYNE).
RESULTS

We recently reported that SV40 mutants defective in the interaction between Vp1 and Vp3 showed either several-thousand-fold-reduced viability (Vp3 F157E-I158E and Vp3 P164R-G165E-G166R) or an essentially nonviable phenotype (Vp1 V243E and Vp1 L245E). Viral DNA replication, capsid protein expression, and subcellular localization were unaffected by these mutations (41). In the current study, we further analyzed these mutants, as well as a new mutant (“Vp1-only”) that lacks the coding region for Vp2/3. Table 1 summarizes the phenotypes observed from infection of the two Vp1 point mutants and the Vp1-only mutant, along with our previously reported viabilities of the Vp1 point mutants (41).

Particle formation by mutants defective in Vp1-Vp2/3 interaction. We tested whether Vp1 and Vp2/3 mutants could package DNA and form particles. TC-7 cells were transfected with either wild-type or mutant viral DNA; the lysates were fractionated after sucrose gradient sedimentation and examined for Vp1 by Western blotting (Fig. 1A). Vp1 was mostly found in the fourth to sixth fractions for both the wild type and the mutants, suggesting that the mutants were capable of making particles. The peak fractions were collected and examined for the presence of viral DNA, Vp1, Vp2, and Vp3 (Fig. 1B). The presence of viral DNA was tested by Southern blotting (Fig. 1B, top) before and after DNase I treatment. The fractions contained amounts of mutant DNAs comparable to those of the wild type (lane 1); Vp3 mutants F157E-I158E (lane 2) and P164R-G165E-G166R (lane 3) and Vp1 mutants V243E (lane 4) and L245E (lane 5). All mutant DNAs were resistant to DNase I treatment (Fig. 1B, bottom, +; compare the non-nuclease-treated sample shown in the upper panel [−]), indicating that most were protected by the capsid. The Vp1, Vp2, and Vp3 compositions of the mutant particles were next examined by Western blotting (Fig. 1C). Whereas the Vp1 contents detected by Western blotting were comparable for all particles (Fig. 1C, top), the Vp2 and Vp3 contents of the mutant particles were severely reduced (Fig. 1C, bottom). The Vp3 F157E-I158E mutant particles contained reduced but detectable amounts of Vp3 (lane 2). Vp3 P164R-G165E-G166R particles (lane 3) contained very little mutant Vp3 (faintly visible only after long exposure) (data not shown). The F157E-I158E Vp3 migrated more slowly than wild-type Vp3 possibly

![FIG. 1. Analysis of mutant particles. (A) Particle preparations by sucrose sedimentation. Lysates prepared from cells transfected with either NO-SV40 wild type (Wt), Vp3 mutants F157E-I158E and P164R-G165E-G166R (PGG-RER), Vp1 mutants V243E and L245E, or SV-Vp1 (Vp1-Only) were treated with DNase I and sedimented through a 5 to 32% sucrose gradient. Aliquots of 10 fractions taken from the bottom of the tube were run on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Vp1 was detected by Western blotting. Vp1 peak fractions are indicated (Particles). (B and C) Viral DNA contents and capsid protein compositions of mutant particles. (B) Peak particle fractions from panel A were pooled and concentrated by pelleting them with high-speed centrifugation. Mutant particles, as well as virions, were either not treated (−) or treated (+) with 0.1 U/µl of DNase I for 20 min at 37°C; DNA was extracted, and viral DNA was detected by Southern blotting using 32P-labeled SV40 DNA as the probe. (C) Particle preparations were probed by Western blotting for the presence of either Vp1 (top) or minor capsid proteins (bottom). The positions of the bands representing the respective capsid proteins are indicated.](http://jvi.asm.org/issue/11/12/3780/supp/fig1a.png)

### TABLE 1. Summary of mutant phenotypes

<table>
<thead>
<tr>
<th>NO-SV40</th>
<th>Particle compositiona</th>
<th>Cell entryb</th>
<th>Viral DNA in immunoprecipitationc</th>
<th>% T-positive cellsd</th>
<th>Viability (PFU)e</th>
</tr>
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<tbody>
<tr>
<td>Wild type</td>
<td>+ + + +</td>
<td>Yes</td>
<td>Anti-Vp1 Anti-importins</td>
<td>46.4 ± 5.5</td>
<td>1.2 × 10³</td>
</tr>
<tr>
<td>V243E</td>
<td>+ + +</td>
<td>Yes</td>
<td>− −</td>
<td>0.02 ± 0.01</td>
<td>&lt;1</td>
</tr>
<tr>
<td>L245E</td>
<td>+ + +</td>
<td>Yes</td>
<td>− −</td>
<td>0.08 ± 0.03</td>
<td>46</td>
</tr>
<tr>
<td>Vp1-only</td>
<td>+ + +</td>
<td>Yes</td>
<td>− −</td>
<td>0.1 ± 0.03</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

a + and − represent the presence or absence, respectively, of signal in assays conducted to detect the given component for the indicated particle, based on results shown in Fig. 2B and 3.
b Yes indicates that the given particle has ability to enter cells comparable to that of the SV40 virion, based on results shown in Fig. 4A.c + and − represent the presence or absence, respectively, of viral DNA in the immunoprecipitate of the indicated antibody reaction with the cytoplasmic lysate prepared at 6 h postinfection with the given particle, based on results shown in Fig. 4C.d Positive for the expression of large T antigen as probed by immunofluorescence; summarized from results shown in Fig. 4B.e Values for the wild type and mutants V243E and L245E were reported previously (41). The value for the Vp1-only mutant is based on the number of plaques formed by infection with lysate containing 1 µg of replicated viral DNA prepared from the viral-DNA-transfected cells.
because of the alteration of two hydrophobic residues into charged residues. For the two Vp1 mutants (lanes 4 and 5), Vp3 was not detected even after a long exposure (data not shown). Viability was reduced for the Vp3 mutants or eliminated for the Vp1 mutants (41). Thus, the reduction or loss of viability correlated with the extent of incorporation of minor capsid proteins. The observation that the mutant particles contained very little mutant Vp3 for the Vp3 mutants or undetectable amounts of Vp3 for the Vp1 mutants suggests that viral DNA packaging progressed in the absence of Vp3 and that the mutant particles were formed in the nucleus. It also suggests that Vp1 alone can package viral DNA.

For simplicity, we will call NO-SV40 Vp1 point mutants V243E and L245E the “Vp3-less Vp1 mutants” to distinguish them from the SV40 derivative expressing only Vp1 but not Vp2/3 (SV-Vp1), the “Vp1-only” mutant (see below).

**Formation of particles by the Vp1-only mutant.** We used another approach to prepare particles that contained no minor capsid proteins and to verify the observation described above. We used viral mutant DNA lacking Vp2- and Vp3-coding segments, SV-Vp1 (19), to transfect cells and prepare lysate. The lysate was examined for particle formation by sedimentation through a sucrose gradient (Fig. 1A, Vp1-only, bottom). Fractions were pooled, pelleted, and examined for Vp1 by Western blotting (Fig. 2, inset). Vp1 was found in fractions 4 to 6, similar to what was seen for the wild-type particles. The particle preparation was next treated with DNase I and examined by quantitative PCR for the presence of DNase I-resistant DNA harboring the SV40 origin (Fig. 2). Wild-type and Vp1-only particles contained similar amounts of DNase I-resistant viral DNA (Fig. 2) relative to Vp1 (Fig. 2, inset). This result confirms that Vp1 alone can package DNA and form particles. However, Vp1-only particles were noninfectious (Table 1).

SV40 is known to be sensitive to treatment with chelating agents and reducing agents (2, 37), which render the viral DNA sensitive to DNase I treatment (61). Following treatment with 5 mM EGTA and 5 mM DTT, the wild-type and Vp1-only particles were equally sensitive to DNase I digestion (Fig. 2, EGTA/DTT treatment, +), indicating that the DNAs in the mutant and wild-type particles were similarly protected. In summary, these results show that Vp1 alone can package DNA and generate particles with stability similar to that of wild-type virions.

**Mutant particles contain histones.** We next tested by chromatin immunoprecipitation whether mutant particles contain histones. Particles purified by sucrose sedimentation were disrupted with EGTA and DTT and then treated with N-ethylmaleimide. The resulting components were incubated with individual anti-histone antibodies and bound to protein A or G beads. The IPs were washed, and viral DNA was extracted (along with externally added control NO-SV40 NcoI standard DNA) and subjected to semiquantitative PCR. The primer sets were designed to amplify relatively long product DNA fragments: 2.2 kbp for the 5.2-kbp wild type and point mutants and 1.8 kbp for the 4.2-kbp Vp1-only DNA. In all cases (wild type and mutant), samples immunoprecipitated by antibodies to H2B, H3, and H4 contained viral DNA (Fig. 3, right lane of each panel), while control antibodies pulled down little viral DNA (Fig. 3, left lane of each panel). Although the H2A content could not be tested because of the poor activity of the commercial anti-H2A antibody, we have no reason to believe that H2A is absent from the mutant particles. These results show that Vp1 alone is capable of packaging viral DNA and histones.

**Mutant particles are defective in DNA nuclear entry.** The Vp3-less Vp1 mutants are nonviable (41), as is the Vp1-only mutant (Table 1). We first asked if the Vp3-less Vp1 or the Vp1-only particles were able to enter cells. TC-7 cells were infected for 1 h with mutant particles, and particles that had entered the cell were distinguished from those that were bound to the cell surface, as described previously (27). We next examined the DNA and Vp1 contents of cell-associated versus internalized particles. For the Vp3-less Vp1 mutants, Southern blotting was used to detect DNA, and for the Vp1-only mutant, Western blotting was used to detect Vp1 (Fig. 4A). In the cells

**FIG. 2.** Analysis of Vp1-only particles. NO-SV40 (Wt) and SV-Vp1 (Vp1-Only) particles were purified and pelleted as for Fig. 1B. The Vp1 content in each preparation was examined by Western blotting (inset). The particles were incubated either in the absence (−) or in the presence (+) of EGTA and DTT (EGTA/DTT Treatment) and digested with DNase I. Viral DNA was extracted after the DNase I digestion and measured by quantitative PCR using the iCycler thermal-cycler system (Bio-Rad) and is indicated as copy numbers.

**FIG. 3.** Histone contents of mutant particles. Aliquots of EGTA/DTT-disrupted particles of NO-SV40 (WT; row A), of mutant V243E (row B) or L245E (row C), or of SV-Vp1 (Vp1-only; row D) were immunoprecipitated with rabbit anti-sheep IgG (Cont), anti-histone H2B (H2B), anti-histone H3 (H3), or anti-histone H4 (H4) antibody. The respective IPs were mixed with the NO-pSV40 NcoI standard DNA, and their DNAs were extracted and subjected to semiquantitative PCR for viral DNA as described in Materials and Methods. A 2.2-kbp fragment (solid arrow; vDNA) and a 1.7-kbp fragment (solid arrowhead; con DNA) were generated from the amplification of the NO-SV40 genome and coextracted standard DNAs, respectively. A 1.8-kbp fragment (empty arrow) and a 2.3-kbp fragment (empty arrowhead) were generated from the amplification of Vp1-only and coextracted standard DNAs, respectively.
infected with either V243E (lane 2) or L245E (lane 3) particles, the amount of cell-associated viral DNA (middle) or of internalized viral DNA (bottom) was similar to that seen in the wild-type-infected sample (lane 1). The levels of Vp1 were also similar for all infected cells (data not shown). When the Vp1-only particles were used for infection, similar amounts of Vp1 were found in both cell-associated and internalized fractions (Fig. 4A, lanes 4 and 5). These results show that the mutant particles could attach to and enter cells.

The infectivities of the three mutant particles were next examined by determining large-T-antigen expression 20 h following infection. Very few cells were T antigen positive for any of the mutants, whereas about 50% of the wild-type-infected cells were positive for T antigen (Fig. 4B). Thus, the mutant particles, though able to enter cells, were defective in the steps leading to T-antigen expression.

We have previously shown that following entry into cells, the viral DNA of wild-type particles remains associated with capsid proteins and that a small fraction of the DNA becomes associated with importins (40, 42). A region of the capsid that coordinates one of the two calcium ions (site 2) has been postulated to facilitate the exposure of minor capsid proteins and their NLSs on the virion surface (27). To see how the mutant particles devoid of Vp2/3 fared after cell entry, we examined the association of viral DNAs with capsid proteins and with importins in the cytoplasm. We assessed the ability of viral DNA to coimmunoprecipitate with Vp1 and importins using anti-Vp1 or anti-importin antibodies. The cytoplasmic fractions of cells infected with particles for 6 h were incubated with anti-Vp1 (Fig. 4C, lane 3), a mixture of anti-importins (lane 4), and a control antibody (lane 2) and then with protein A beads. Viral DNAs in the IPs were detected by semiquantitative PCR as described previously (42). The cytoplasmic fraction from wild-type-infected cells contained viral DNA in association with Vp1 and the importins (Fig. 4C, WT), confirming previously reported results (42). In contrast, the anti-Vp1 IPs prepared from cells infected with Vp1 mutants, V243E or L245E, or Vp1-only particles contained little DNA (Fig. 4C, lane 3, V243E, L245E, and Vp1-only). These results show that viral DNAs were dissociated from mutant Vp1s within 6 h following cell entry. As Vp3 was absent from these mutant particles, it is not surprising that importin IPs contained little viral DNA (Fig. 4C, lane 4, lower gels).

The results described above are consistent with the interpretation that, despite the successful cell entry of all the mutant particles, they were unable to promote the nuclear entry of viral DNAs. Consequently, T-antigen expression was not observed in the infected cells. Our study thus points to the presence of separate and distinct functions for SV40 capsid proteins: a role for major capsid protein Vp1 in packaging, particle formation, and cell entry and a role for minor capsid proteins Vp2/3 in nuclear entry of viral DNA.

**DISCUSSION**

While the minor capsid proteins of SV40 and other polyomaviruses are essential gene products and are components of the mature virion, the roles of individual capsid proteins in virus infection has not been clearly delineated. Here, through the analysis of two groups of Vp2/3-free particles produced in
monkey host cells, we showed that SV40 Vp1 is necessary and sufficient to package the viral genome-host histone complex and is sufficient for successful cell entry. However, these Vp2/3-free particles failed to associate with host importins or to initiate large-T-antigen expression in the nucleus. In fact, the viral DNAs prematurely dissociate from the Vp1s in the cytoplasm. These results demonstrate unequivocally that Vp2/3 are dispensable for the accurate assembly of nucleocapsids that closely resemble SV40 virions, contrary to previous proposals (17, 63). Furthermore, we showed that Vp1 contains the major determinants for cell attachment and entry. We conclude that the major role of Vp2/3 in infectivity lies in their ability to promote the nuclear delivery of the infecting viral DNA.

**Role of Vp1: nuclear capsid assembly and cell entry.** Even though both SV40 Vp1 and Vp2/3 harbor DNA-binding domains (17, 26), our results show that Vp1 alone can encapsidate the viral minichromosome and form particles in monkey cell nuclei. The biochemical and cell biological behavior of the Vp3-less Vp1 mutant particles and the Vp1-only particle gives us strong reason to believe that they have structural features similar to those of the SV40 virion. Although we have not characterized these particles structurally by electron microscopy, all previously tested Vp1s from the polyomavirus family, expressed in either bacteria or insect cells (16, 22, 28, 36, 45, 47, 59), have been found to spontaneously form pseudocapsids with sizes (45 to 50 nm in diameter) and shapes similar to those of wild-type virions.

Our results imply that Vp1 contains the principal determinants for cell attachment and entry. We have previously proposed that the SV40 “site 1” calcium-binding site may function in the proper exposure of such cell-binding determinants. A site 1 mutation, E330K, preserves assembly and packaging of particles but renders the mutant particles unable to attach to or enter cells (27). The substituted lysine side chain of mutant E330K is predicted to exclude the Ca\(^{2+}\) ion and to replace its interactions with salt bridges to acidic residues from an interlocking Vp1 pentamer. The mutant particles reported in the present work, being capable of cell entry, appear not to interfere with site 1 Ca\(^{2+}\) binding and release. A comparative structural and biochemical analysis of our newly characterized mutants may further the understanding of how SV40 alters its structure for cell attachment and binding.

**Role of Vp3: infectivity and cytoplasmic-nuclear trafficking of infecting viral DNA.** We showed that the mutant particles lacking minor capsid proteins are nonviable (Table 1). Consistent with our results, others have reported that mutants of polyomaviruses defective in expressing Vp2/3 or harboring certain Vp2/3 mutations are largely nonviable (13, 15, 34, 52). The minor capsid protein L2 of papillomavirus is also required for virus cell entry (62). However, unlike SV40 Vp2/3, L2 is necessary for the efficient packaging of the papillomavirus genome (44, 56, 64) and is required for virus cell entry (62). The fact that L2 is partly exposed on the surface of the virion (21) and not cryptic like SV40 Vp2/3 (12) may help explain this functional difference with respect to cell entry.

We have recently shown that the cell-internalized virion undergoes an architectural alteration in the cytoplasm, exposing Vp2/3 and allowing the Vp3 NLS to mediate the nuclear entry of the Vp1-Vp2/3-viral DNA complex via the cellular importins (42). The small fraction of cell-internalized SV40 that becomes complexed with the importins, and hence “nuclear-entry competent” (7), is identified as the viral DNAs in the IPs of Vp1, Vp3, and host importins, complexes that sediment more slowly than the virion but have a similar Vp1-to-Vp3 ratio (40). In contrast, the internalized mutant DNAs of the Vp2/3-free particles were not found in the Vp1 or importin IPs (Fig. 4), suggesting that the mutant particles dissociate into viral genomes and Vp1s soon after cell entry. Minor capsid proteins of polyomaviruses have been postulated to be involved in the modification of the capsid (10, 11, 23, 52, 58). In polyomavirus, coexpression of Vp2/3 with Vp1 increases the phosphorylation level of Vp1, which can affect viral infectivity (11, 23, 24). A change in these modification profiles in our Vp1-only mutants might contribute to the expedited dissociation of the particles.

The phenotype of the Vp3-less and Vp1-only mutants is similar to that of two “site 2” Vp1 calcium-binding site mutants, E157A/E160A and E216K (27). These site 2 mutant particles have a normal complement of Vp2/3, whereas the Vp3-less mutants and the Vp1-only mutant carry only mutant or wild-type Vp1s, respectively. All of these mutants form nucleocapsids that are able to bind and enter cells, but the particles prematurely dissociate upon entry into the cytoplasm (reference 27 and this study). Since Vp2/3 lie immediately behind calcium-binding site 2, the absence of Vp2/3 may compromise the integrity of this site, leading to similar phenotypes.

SV40 uses endocytic pathways, both caveola-dependent and independent, to reach the ER, where many particles accumulate (8, 43, 48–50). Polyomavirus pseudovirions (i.e., Vp1 empty capsids) have the same intracellular trafficking pattern as normal virions (33, 51). It has been reported, however, that a subset of pseudovirions containing a small fragment of Vp3 fused to green fluorescent protein accumulates in lysosomes (1), although such localization has not been observed in virion-infected cells. During SV40 infection, Vp3 epitopes that are not exposed in endocytic vesicles become exposed in the ER (43). It remains unclear whether these alterations in the cytoplasmic compartment(s) are those that are necessary for recognizing importins in the cytoplasm. Further studies are clearly needed to establish how the presence of Vp2/3 influences postentry infection steps.

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