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Association with capsid proteins promotes nuclear targeting of simian virus 40 DNA

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ABSTRACT All animal DNA viruses except pox virus utilize the cell nucleus as the site for virus reproduction. Yet, a critical viral infection process, nuclear targeting of the viral genome, is poorly understood. The role of capsid proteins in nuclear targeting of simian virus 40 (SV40) DNA, which is assessed by the nuclear accumulation of large tumor (T) antigen, the initial sign of the infectious process, was tested by two independent approaches: antibody interception experiments and reconstitution experiments. When antibody against viral capsid protein Vp1 or Vp3 was introduced into the cytoplasm, the nuclear accumulation of T antigen was not observed in cells either infected or cytoplasmically injected with virion. Nuclearly introduced anti-Vp3 IgG also showed the inhibitory effect. In the reconstitution experiments, SV40 DNA was allowed to interact with protein components of the virus, either empty particles or histones, and the resulting complexes were tested for the capability of protein components to target the DNA to the nucleus from cytoplasm as effectively as the targeting of DNA in the mature virion. In cells injected with empty particle–DNA, but not in minichromosome-injected cells, T antigen was observed as effectively as in SV40-injected cells. These results demonstrate that SV40 capsid proteins can facilitate transport of SV40 DNA into the nucleus and indicate that Vp3, one of the capsid proteins, accompanies SV40 DNA as it enters the nucleus during virus infection.

Various animal viruses, such as DNA tumor virus, orthomyxovirus, and retrovirus, utilize the cell nucleus as the reproductive site during some part of their life cycle. Ubiquitous as it is, the process by which these viruses target their genome to the nucleus is still little known. It has been reported that herpes simplex virus bearing one temperature-sensitive mutation within its capsid proteins is defective at delivering the viral DNA to the nucleus at nonpermissive temperatures (1, 2). Adenovirus appears to release the viral DNA into the nucleus together with the hexon, but not with many other viral proteins (3, 4). In retrovirus (5, 6), influenza virus (7–9), hepadnavirus (10), hepatitis delta virus (11), adenovirus (12), and papovavirus (13–18), many structural and virion-associated proteins have been found to harbor nuclear-targeting signals (NTSs). These results are consistent with the idea that the NTSs of viral structural proteins or/and virion-associated proteins are responsible for the nuclear entry of the viral genome.

Simian virus 40 (SV40), a member of the nonenveloped, icosahedrally symmetrical papovavirus family, is composed of three viral structural proteins, histones, and double-stranded circular DNA (19). We have recently shown that biologically functional SV40 virion enters the nucleus through the nuclear pore complex (NPC; refs. 20 and 21). Characteristic of NPC-mediated nuclear transport processes, the nuclear entry of SV40 is signal- and energy-dependent (22, 23) and sensitive to such inhibitors as wheat germ agglutinin and anti-nucleoporin antibody (21). During a productive infection cycle, the viral structural proteins, Vp1, Vp2, and Vp3, localize to the nucleus (24) through the NPC by virtue of their individual NTSs, which have been identified (13–15). The karyophilic histones, some of whose NTSs are known (25, 26), also enter the nucleus through the NPC (27–29). Thus, all virion protein components could in principle individually or collectively contribute to the nuclear entry of SV40 viral DNA. In this paper, we have tested whether the nuclear entry of virion DNA is accompanied by the viral structural proteins and whether it is promoted by the capsid proteins or histones.

MATERIALS AND METHODS

Cells, Microinjection, Microinfection, and Preparation of Virion and Empty Particles. The cell culture conditions for TC7 cells and procedures for microinjection, microinfection, and immunocytochemistry have been described (20, 30). For the time-course study of Vp1 nuclear accumulation, about 100 SV40 virions or an equal number of SV40 empty particles (judged by protein profiles on SDS/PAGE), along with rhodamin-conjugated bovine serum albumin (R-BSA), were cytoplasmically injected into each of the cells located in three marked areas on the same coverslip. For cytoplasmic antibody interception experiments, cells were cytoplasmically injected with anti-Vp1 IgG or anti-Vp3 IgG and then either cytoplasmically injected or microinfected with virions. For nuclear antibody interception experiments, either anti-Vp3 IgG, anti-β-galactosidase monoclonal antibody (anti-β-gal), or injection solution alone was injected into the nucleus, and then virions were injected into the cytoplasm.

SV40 and empty particles were obtained essentially as described (31). Empty particles were prepared either from virus-infected cells or from virion preparations following repeated freeze/thaw cycles and rebanding in $CsCl_2$ (20). Less than one plaque was detected from the equivalent of 2×10^6 physical particles, and their microinjection of empty particles did not lead to large tumor (T)-antigen expression (data not shown).

Antibodies and Immunofluorescence Microscopy. The preparation of rabbit anti-Vp1 and anti-Vp3 sera and of guinea pig anti-Vp1 as well as the condition for immunofluorescence microscopy have been described (20, 32). Affinity-purified anti-Vp1 and anti-Vp3 IgGs were prepared from polyclonal sera by using nitrocellulose membrane onto which either virion

Abbreviations: SV40, simian virus 40; T antigen, large tumor antigen; NTS, nuclear-targeting signals; NPC, nuclear pore complex; BSA, bovine serum albumin; R-BSA, rhodamine-conjugated BSA; β-gal, β-galactosidase; F-dex, fluorescein-conjugated dextran.

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Vp1 or glutathione *S*-transferase (GST)-Vp3 fusion protein, respectively, had been immobilized (33). The IgG preparations used in this study were relatively free of nuclease activity, since incubation of SV40 DNA at 50 μg/ml with various concentrations (0.15–2 mg/ml), of several IgG preparations for 1 hr at room temperature or for 6 hr at 37°C did not change either the DNA mass or the distribution of the topological forms of circular DNA (data not shown).

***In Vitro* Reconstitution of Minichromosome.** Minichromosome was reconstituted *in vitro* by salt-gradient dialysis (34) and then was analyzed either by 0.7% agarose gel as described (35) or by micrococcal nuclease digestion (34).

RESULTS

Association of Structural Proteins during Nuclear Entry of Virion DNA. During SV40 infection, a virion particle adsorbs to the cell surface, enters the cytoplasm by endocytosis, and is enclosed in a vesicle (36–38). If infecting SV40 is to enter the nucleus through NPC, the NTSs must become accessible to the nuclear transport machinery. This process requires the release of the virion particle from the membrane enclosure. When the infecting virion is exposed in the cytoplasm, cytoplasmic introduction of reagents such as antibodies against viral proteins, anti-Vp1 IgG or anti-Vp3 IgG, could interfere with the nuclear entry route, thereby leading to the lack of nuclear T-antigen detection, the first sign of the SV40 gene activity. On the other hand, the antibodies would not recognize virions if they were enclosed in vesicles. Cytoplasmically injected SV40 virion is expected to be free from membrane enclosure and thus can serve as a control in the antibody interception experiments.

We tested the effect of anti-Vp1 IgG or anti-Vp3 IgG after its cytoplasmic injection on the expression of the T-antigen gene in SV40-injected or -infected cells. IgGs, confirmed to be free of nuclease activities (*Materials and Methods*), were introduced into the cytoplasm together with R-BSA, an injection marker, prior to virion injection or infection. Injection of BSA or control antibodies into the cytoplasm has no obvious deleterious effect on the expression of the T-antigen gene (21). In the injection experiments, nuclear T-antigen staining was not detected in SV40-injected cells in which anti-Vp1 IgG was cytoplasmically injected (Fig. 1*A*, arrow), as visualized by cytoplasmic rhodamin fluorescence (Fig. 1*a*, arrow), but was observed in SV40-injected cells that received no antibodies (Fig. 1 *A* and *a*, arrowhead). Identical results were obtained in cells that received or did not receive anti-Vp3 IgG (Fig. 1 *C* and *c*, arrow and arrowhead, respectively). Simultaneous injection of antibody, R-BSA, and virions into the cytoplasm gave the same results (data not shown).

In the infection experiments, the antibodies and R-BSA were injected into the cytoplasm, and cells were then infected with SV40. Results that were essentially the same as those derived from the virion injection approach were obtained either with anti-Vp1 IgG (Fig. 1 *B* and *b*) or with anti-Vp3 IgG (Fig. 1 *D* and *d*). These results indicate that the structural proteins of biologically active infecting virions become accessible to antibodies in the cytoplasm. These results are consistent with the interpretation that antibody against either of the capsid proteins, if it is present in the cytoplasm, can effectively block nuclear entry of viral DNA. There are, however, two alternative interpretations. One is that the cytoplasmic inclusion of antibodies led to the formation of large antibody-bound virion aggregates that precluded their nucleir entry. This possibility is difficult to test directly at this time. The other interpretation is that the virion dissociates in the cytoplasm, and viral proteins and DNAs enter the nucleus separately instead of associating with each other. Thus, the antibodies introduced in the cytoplasm could prevent the dissociation. Since minor coat proteins Vp2 and Vp3 are internal proteins in the virion structure (39), the observed anti-Vp3 effect would argue for this possibility. Even if virion dissociation takes place in the cytoplasm, would the structural proteins accompany the viral DNA when it enters the nucleus? This was tested by nuclear antibody interception experiments.

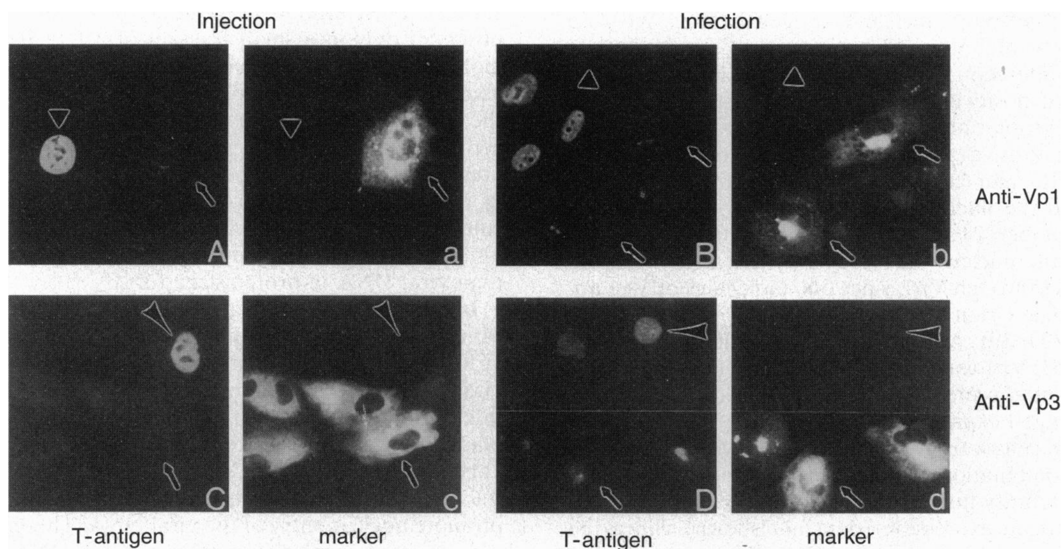

FIG. 1. Effect of cytoplasmically introduced antibodies against capsid proteins on T-antigen expression. Each pair of photographic panels (*A* and *a*, *B* and *b*, *C* and *c*, or *D* and *d*) shows the identical field of cells. Cells shown were either cytoplasmically microinjected [*Left* (*A* and *a* and *C* and *c*)] or microinfected [*Right* (*B* and *b* and *D* and *d*)] with SV40. A portion of these cells, indicated by an arrow, was cytoplasmically injected with either anti-Vp1 IgG (*Upper*) or anti-Vp3 IgG (*Lower*) at 0.5 mg/ml together with R-BSA at 1 mg/ml to mark antibody-injected cells, prior to virus injection or infection. In some cells, R-BSA was noted to accumulate at the perinuclear region. The boundary between the cytoplasm and the nucleus was ascertained by phase microscopy. All cells were harvested 6 hr after injection or 20 hr after infection and stained for T antigen. An arrowhead (the two shapes of arrowheads being equivalent) marks a virus-infected or -injected cell or group of cells that had not been injected with the antibody (plus marker) and as a result expressed T antigen. One such cell in *A*, three in *B*, one in *C*, and two in *D* can be seen to exhibit T-antigen staining and lack marker staining in *a*, *b*, *c*, and *d*. An arrow points to a virus-infected or -injected cell or group of cells that did not express T antigen as a result of having an antibody (plus marker) introduced. One such cell in *a*, two in *b*, four in *c*, and three in *d* can be seen to exhibit the cytoplasmic marker protein but do not show T-antigen staining in *A*, *B*, *C*, and *D*.

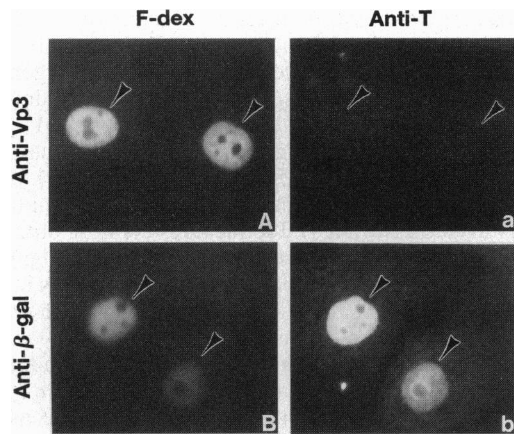

FIG. 2. Effect on T-antigen expression of nuclearly injected antibodies against structural proteins. Cells in marked areas were nuclearly injected with anti-Vp3 IgG (anti-Vp3) together with F-dex (70-kDa molecular mass) at 5 mg/ml prior to cytoplasmic injection of SV40 virions (about 10 virions per cell). As a control, monoclonal anti-β-gal was injected into cells in place of anti-Vp3 IgG. F-dex was included to mark antibody-injected cells (*A* and *B*). Cells were harvested 6 hr after virion injection, fixed in 2% paraformaldehyde as described (21), and stained for T antigen (anti-T) (*a* and *b*). An arrowhead marks a cell that had received anti-Vp3 antibody (*a*) or a cell that had received the control antibody (*b*).

When anti-Vp3 or anti-β-gal was nuclearly introduced together with the injection marker—fluorescein-dextran (F-dex)—and then ≈10 virions per cell were cytoplasmically injected, the presence of anti-Vp3 IgG in the nucleus greatly inhibited nuclear T-antigen accumulation (Fig. 2 *A* and *a*), as evidenced by only 1 of 46 and 4 of 36 injected cells (2–8%) being stained. Among 43 cells injected with anti-β-gal antibody (F-dex/anti-β-gal-positive cells, Fig. 2 *B* and *b*), 12 cells (36%) showed nuclear T-antigen staining, so the number is within the 30–40% range observed in virion-injected control cells that had received no nuclear injection treatment, in which 12 of 35 and 91 of 421 cells showed nuclear T-antigen staining. We were unable to observe inhibition of T-antigen expression by nuclear anti-Vp1 IgG interception; 15 of 56 anti-Vp1 injected cells (F-dex/anti-Vp1-positive cells) showed T-antigen staining, while 16 of 84 virion-only injected cells (F-dex-positive cells) were T-antigen positive (photographs not shown). We estimate that $\approx 1\text{–}5 \times 10^3$ and $2\text{–}10 \times 10^4$ antibody molecules were introduced into the nucleus and into the cytoplasm, respectively. The number of antibody molecules that could be introduced to the nucleus may not be sufficient to exert the inhibitory effect through Vp1, since 360 molecules of Vp1 are present in a single virion as opposed to about 72 molecules of Vp2 and Vp3 (24, 39). Alternatively, the result could suggest that, unlike Vp3, Vp1 was not associated with the DNA upon nuclear entry and therefore anti-Vp1 in the nucleus could not intercept T-antigen expression.

Finally, when cells were nuclearly injected with SV40 DNA along with a combination of F-dex and either anti-Vp3 IgG, anti-β-gal, or affinity-purified goat anti-rabbit IgG, all cells exhibited T-antigen expression (data not shown). Since the presence *per se* of any of these antibodies in the nucleus did not block T-antigen expression from SV40 DNA, the inhibition observed in the nuclear anti-Vp3 interception experiment was caused by the antibody's depletion of SV40 virion or of SV40 DNA–virion protein complexes that had entered the nucleus. Taking these results together with the results from the cytoplasmic antibody interception experiments, we conclude that biologically active viral DNA reaching the nucleus was in association with viral structural proteins.

Nuclear Targeting of *in Vitro* Reconstituted Protein–Viral DNA Complexes. If SV40 virion proteins promote DNA nuclear entry, protein-free viral DNA would be expected to be inefficient at nuclear targeting. This difference, if any, can be followed by observing the time course of nuclear accumulation of T antigen. When SV40 virus was injected into the cytoplasm, the percentage of T-antigen-positive cells among virion-injected cells that were Vp1 positive (see Fig. 3 legend) increased steadily with increasing time after injection (Fig. 3), confirming our earlier results (20). The steady increase in the percentage of T-antigen-positive cells as well as the effectiveness of T-antigen expression allowed virion-injected cells to serve as a positive control (see below). By contrast, when the protein-free DNA was injected into the cytoplasm together with F-dex, T antigen was detected first 4 hr postmicroinjection, but only in a minute fraction of the injected cells (not shown). Even after 6 hr postmicroinjection, T antigen was observed only in a small fraction of cells (Fig. 3). As a few molecules of cytoplasmically injected SV40 DNA can bring about T-antigen expression after a long incubation time (40), the possibility that there might be selectively high nuclease activity in the cytoplasmic compartment and that this contributes to the observed low percentage of T-antigen-positive cells in the cytoplasm is unlikely, although it cannot be entirely ruled out. It is reasonable to conclude that viral DNAs in virions are more rapidly and efficiently targeted to the nucleus than viral DNA in protein-free form.

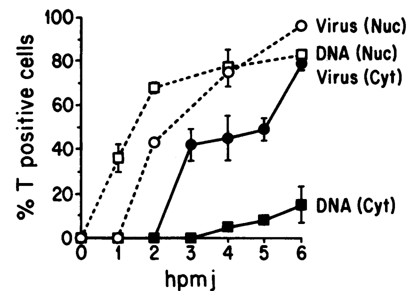

FIG. 3. Time course of nuclear accumulation of T antigen. Cells into which about 100 virions (○ and ●) or 100 SV40 DNA molecules (□ and ■) had been injected into the nucleus (Nuc) (---) or into the cytoplasm (Cyt) (—) were cultured for the indicated hours postmicroinjection (hpmj), harvested, and stained for T antigen as described (20). Cells injected with virions were doubly stained with anti-Vp1 and anti-T-antigen antibodies. The percentage of T-antigen-positive cells is the proportion of cells expressing detectable levels of T antigen among Vp1-positive cells. For DNA injected cells, the percentage of T-antigen-positive cells represents the proportion of the cells that expressed detectable T antigen among those marked by a fluorescent marker, F-dex or R-BSA. Vertical bars represent standard deviations of multiple experiments.

Inefficient Nuclear Targeting of *in Vitro* Reconstituted Minichromosomes. Histones are a component of the SV40 virion and form a minichromosome with the viral DNA. The viral minichromosome interacts with Vp2 and Vp3 to form an internal core that the Vp1 shell encloses (39, 41). Our cytoplasmic anti-Vp3 IgG interception results could suggest that karyophilic histones (27, 28) could also become exposed upon the alteration of virion structure in the cytoplasm and could promote nuclear entry of the viral DNA. Therefore, the effect of histones on nuclear targeting of SV40 DNA was examined next. To ensure that SV40 minichromosomes contained only DNA and histones and were free from capsid proteins, the minichromosomes were reconstituted *in vitro* with SV40 DNA and purified histones (34). *In vitro* reconstituted minichromosomes have been shown to retain identical properties as those formed *in vivo* (42). Upon incubation with histones, most of the SV40 DNA became a slowly migrating species, indicating that the majority of the DNA is in nucleoprotein complex (Fig. 4*A Left Inset*, compare lanes D and C). When this reconstituted sample was digested with micrococcal nuclease, a major pro-

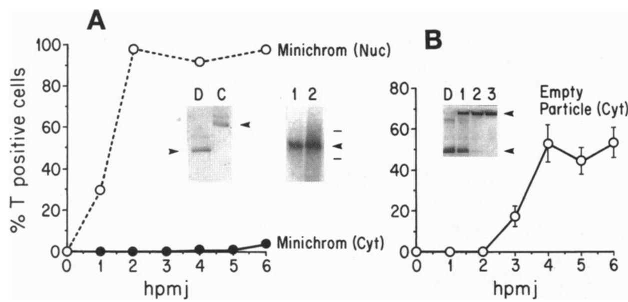

FIG. 4. Reconstitution experiments. (*A*) Effect of histones on DNA nuclear targeting. Minichromosomes were reconstituted by incubating 150 μg of SV40 DNA with 165 μg of whole histone components (Boehringer Mannheim) in 0.6 ml of 1 M NaCl/20 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM 2-mercaptoethanol and then serially dialyzing with decreasing concentrations of NaCl as described (34). The minichromosomes were injected at the DNA concentration of 50 ng/μl into either the cytoplasm (●) or the nucleus (○) of 50–100 cells per time point. The appearance of nuclear T antigen was subsequently examined as described in Fig. 3. (*A Left Inset*) Mobilities in agarose gel of protein-free covalently closed circular DNA (lane D, arrowhead) and of reconstituted minichromosomes (lane C, arrowhead; 0.5 μg per lane) are shown. One-microgram DNA samples of assembled minichromosomes were digested with 20 ng of micrococcal nuclease at 37°C for the designated period and immediately transferred to stop solution (0.2% SDS/20 mM EDTA). (*A Right Inset*) Mobility of the resulting DNA fragments in 2.5% agarose gel, after either a 30-sec digestion (lane 1) or a 15-sec digestion (lane 2) is shown. Bars at the right indicate the positions of marker fragments of 201 bp (upper bar) and 154 bp (lower bar). (*B*) Effect of empty particles on DNA targeting to the nucleus. About 1×10^{11} (0.5 μg) SV40 DNA molecules were incubated with empty particles in 15 μl of Dulbecco's PBS (Ca^{2+} and Mg^{2+} free) for 30 min at room temperature. (*Inset*) Mobility in agarose gel of 0.5 μg of SV40 DNA (lane D, bottom arrowhead, covalently closed circular DNA spieces), as well as that following incubation with 1×10^{11} (equal number) (lane 1), 1×10^{12} (lane 2), or 1×10^{13} (lane 3) empty particles is shown. The formation of high molecular weight aggregates (top arrowhead) was observed. The mixture of an equal number of DNA and empty particles, in which about half of the DNA was in protein-free DNA species (lane 1) was injected at the DNA concentration of 50 ng/μl into the cytoplasm of 50–100 cells per time point. T-antigen expression was subsequently examined as described in Fig. 3. The steady increase of the cell population expressing T antigen seen here is comparable to that seen in the cells cytoplasmically injected with virions (Fig. 3, filled circle with solid line). Abbreviations are as in Fig. 3.

tected DNA species visualized was ≈200 bp in size (Fig. 4*A Right Inset*, lanes 1 and 2), in agreement with the above observation for nucleosome formation. The reconstituted minichromosomes, when injected directly into the nucleus, were as effective as protein-free DNA or virion DNA in T-antigen expression (Figs. 3 and 4*A*). However, the minichromosomal form of SV40 DNA appeared to be insufficient for DNA nuclear targeting from the cytoplasm; T antigen was detected at 4 hr postmicroinjection, but only in a minor proportion (1%) of the cytoplasmically injected cells (Fig. 4*A*).

Empty Particles Can Promote the Nuclear Targeting of SV40 DNA. To confirm a role for capsid proteins in the nuclear targeting of viral DNA, nuclear accumulation of SV40 empty particles as well as the empty particles' effect on DNA nuclear targeting was examined. When empty particles from two different sources (*Materials and Methods*) were introduced into the cytoplasm and the subcellular distribution of Vp1 was examined, Vp1 was detected in all injected cells. When the concentration of the empty particles was raised 10–50 times, Vp3 staining was observed and was identical to Vp1 staining (data not shown). The kinetics of nuclear uptake of structural proteins from intact virions and from empty particles was indistinguishable.

When the viral DNA was incubated with empty particles and mobility alteration in agarose matrix was examined, most of the SV40 DNA was found just below the loading wells (Fig. 4*B*, compare lane D with lanes 1, 2, and 3), presumably indicating the formation of high molecular weight aggregates. When the incubation mixture (1:1) was injected into the cytoplasm and the subcellular localization of empty particle-derived Vp1 and T-antigen expression was followed, the Vp1 staining was found primarily in the nucleus (data not shown). Nuclear T-antigen staining began to appear 3 hr postmicroinjection among Vp1-positive cells, and the fraction of T-positive cells increased thereafter (Fig. 4*B*). The time course of T-antigen expression was essentially the same as that observed for virion-injected cells (Fig. 3). Thus, empty particles were effective in targeting DNA into the nucleus from cytoplasm.

The results presented above suggest that the structural proteins of SV40 are in association with viral DNA as it enters the nucleus during normal SV40 infection. Furthermore, the virion proteins promote nuclear targeting of the viral DNA.

DISCUSSION

We demonstrated that one of the capsid proteins of SV40 is important for the nuclear targeting of the viral DNA, using two independent approaches—antibody interception experiments and reconstitution experiments. The results presented here and reported previously (21) indicate that the DNA of infecting SV40 remains associated at least with Vp3 as it enters the nucleus through the NPC and that the viral structural proteins are the major promoter for the nuclear entry of the viral genome. These results also provide evidence for a release of virions from endocytotic vesicles during infection or an equivalent phenomenon that exposes virion structural proteins to the cytoplasm prior to virion nuclear entry. This result is the first demonstration of protein-directed nuclear targeting of double stranded DNA in mammalian cells.

What are the important domains in the virion that direct DNA to the nucleus? The viral proteins require independent karyophilic property and DNA interactive capability that are represented as an NTS and a DNA-binding domain, respectively. All SV40 structural proteins are sequence-nonspecific DNA-binding proteins (33, 43) and carry an individual NTS (13–15). The observation that some mutants where Vp2 and Vp3 are defective in DNA binding are nonviable (44) emphasizes the importance of the binding domain in virus infection. Although the DNA-binding domain of SV40 Vp1 is not mapped, that of polyomavirus Vp1 is within the Vp1-NTS itself (17, 45), and therefore Vp1–DNA binding could potentially mask the functioning of the NTS. Unlike SV40 Vp2 and Vp3 (33), the polyomavirus counterpart proteins do not bind DNA (46), implying that different structural proteins play a role in the DNA nuclear targeting of the two related viruses. How the antibodies introduced into the cytoplasm act to intercept T-antigen expression is not known at this time. The mechanisms of how structural proteins play roles in DNA nuclear targeting, as well as which of the SV40 structural proteins effectively targets DNA, could be determined by using wild-type or mutant proteins defective in nuclear targeting and/or in DNA binding when these two signals of SV40 Vp1 are precisely mapped.

In the SV40 virion, the NTSs of the viral proteins do not appear to be exposed at the virion surface. The Vp1 NTSs are inside the virion structure, proximal to the central core structure formed by the interaction of an SV40 DNA minichromosome with the minor coat proteins Vp2 and Vp3 (39). How the Vp2 and Vp3 molecules and their NTSs and DNA-binding domains, all of which are within the virion, are situated in the virion is not known. Based on the presence of electron-dense, virion-like structures seen in the nucleus during the infection, virus uncoating has been suggested to take place in the nucleus

(36, 47). When an intact virus passes through the NPC, the interior signals will not be recognized. Our result that nuclear T antigen was not detected when anti-Vp3 IgG was present in the cytoplasm argues for the idea that structurally altered, rather than intact, SV40 enters the nucleus and that some of the signals become accessible to the transport machinery.

Why histones that are karyophilic and can bind DNA could not target DNA in a minichromosomal form to the nucleus is intriguing. The NTS of H2B, the one defined signal of the four histones (26), lies in amino acids 20–33 of the protein but is not within residues visualized by the x-ray crystallography of a nucleosome core (48). The result could suggest that the targeting signals became inaccessible when minichromosomes were formed. In regard to the interaction of the empty particle with DNA, the observation that multiple empty particles were required to form DNA-empty aggregates (Fig. 4*B*) suggests that only a few binding domains of a particle are available for the interaction. The interaction of DNA with capsid proteins in the empty particle might be different from that in the virion. The fact that the capsid proteins were able to target DNA to the nucleus indicates that it is not necessary to maintain an intact structure to observe capsid protein-assisted nuclear targeting of DNA. Since capsid proteins of SV40 bind DNA in a sequence-nonspecific manner (33), the result could also imply that the capsid is potentially capable of targeting heterologous DNA to the nucleus. The importance of NTS and DNA-binding activities in the formation of plant crown gall tumor, which is caused by the transfer of single-stranded T-DNA from *Agrobacterium* into plant cell nucleus, have recently been shown (49–51). Conceivably, the nuclear entry of nucleic acids in general is facilitated by interaction of the nucleic acids with karyophilic proteins. For nucleus-utilizing viruses, the association of NTS-harboring virion protein components with the viral genome could be a general mechanism for targeting the genome to the nucleus.

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