Nuclear targeting of SV40 and adenovirus

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Summary
Import of viral DNA into the nucleus is essential for the successful replication of DNA tumor viruses. To achieve this goal viruses have adapted strategies to efficiently traverse the barriers between the plasma membrane and the nucleus of a host cell. Recent work on simian virus 40 (SV40) and adenovirus type 2 or 5 shows that SV40 DNA enters the nucleus through nuclear pore complexes (NPCs) in association with structural proteins, and that dissociation of adenovirus particles near the NPC is essential for nuclear import of the viral DNA. Karyophilic protein components of these viruses appear to mediate the nuclear entry of the viral genomes.
Viruses utilize unique subcellular sites for their multiplication. The information necessary for targeting infectious virions to their reproductive sites in the cell is contained within the virion structural proteins. For animal DNA viruses (except for poxviruses and iridoviruses), the nucleus is the site of all viral multiplication processes - genome replication, nucleocapsid formation, and progeny virion maturation. Targeting the viral DNA to the nucleus of a host cell is therefore a key event in the virion life cycle. Transport of nucleic acids across biological membranes is a process basic to all living organisms. Transfer of bacterial DNA from a donor into an acceptor cell, e.g., a plant cell, or spread of viral nucleic acids between different plant cells is mediated by proteins escorting nucleic acids across plasma membranes and finally the nuclear membrane (for reviews see ). Likewise, transport of cellular ribonucleoproteins during cell metabolism appears to be mediated by signals in the associated proteins (for review see ).

In eucaryotic cells nuclear activities are separated from cytoplasmic processes by the double membrane of the nuclear envelope. Communication between the compartments is established by nuclear pore complexes (NPCs), large proteinaceous structures embedded in the nuclear envelope membrane (for review, see ). An NPC harbors two types of channels, an aqueous channel with a diameter of about 9 nm that allows passive diffusion of small molecules in and out of nucleus, and a gated transport channel of maximally 28 nm allowing selective passage of large signal-bearing macromolecules (for review, see ). Signal-mediated nuclear import of proteins is specified by stretches of basic amino acids, nuclear localization signals (NLSs) (for review, see ). In the cytoplasm the NLS is recognized by specific NLS receptor protein(s), and delivered to cytoplasmic elements of the NPC with the help of additional cytosolic proteins (for review, see ). Under some conditions, Hsp70, which has a role in polypeptide folding, can also be involved in nuclear targeting of NLS-containing proteins. Docking of a multimeric complex containing the NLS-protein at the NPC can occur at reduced temperature, but is inhibited by Wheat Germ Agglutinin, a plant lectin that binds to O-linked N-acetylglucosamine. Antibodies that recognize the XFXFG repeat of the nucleoporins inhibit the translocation step (for review see ). Translocation of the docked transport ligand through the NPC depends on metabolic energy and additional soluble cofactors interacting with the transport machinery.

During the life cycle of DNA tumor viruses, such as simian virus 40 (SV40) and adenovirus, the NPC is needed at least two-fold: for nuclear uptake of the incoming genome and for nuclear import of all newly synthesized structural
proteins during virion assembly late in infection. Studies on entry mechanisms of animal and insect viruses have recently shown that incoming viral genomes are targeted to the replication site by associated viral proteins (see also the other reviews in this TICB series). Whether the NLSs in the individual viral proteins are the same needed for import of newly synthesized proteins as for nuclear targeting of incoming genomes is under investigation. In this article we describe recent progress made with nuclear targeting of SV40 and adenovirus type 2 and 5 illustrating two different strategies of DNA transport to the nucleus. The circular SV40 DNA gets into the nucleus together with viral structural proteins through the NPC. In contrast, the adenovirus DNA must first be dissociated from the virion capsid by a trigger received at the cytosolic side of the nuclear membrane before it can be threaded into the nucleus.

**Structure of SV40**

The SV40 virion particle is icosahedral with a diameter of 50 nm (Fig. 1) and is the smallest DNA virus. It consists of a double stranded closed circular DNA of 5 kb, three structural proteins Vp1, Vp2, and Vp3, and four core histones, H2A, H2B, H3 and H4 (Fig. 2). The viral DNA is condensed by the four core histones into a minichromosome. The structure of the capsid, the protein shell of the virus, already elucidated by X-ray crystallography, is determined by the major coat protein Vp1, which forms a total of 72 pentameric capsomers (Fig. 1)\(^\text{13}\). Either of the two interiorly located minor capsid proteins, Vp2 and Vp3, is thought to contact the minichromosome and the Vp1 pentameric capsomer through the common residues of Vp2 and Vp3\(^\text{14}\).

**SV40 replication cycle**

In the course of a productive infection of monkey cells, SV40 enters cells by constitutive endocytosis\(^\text{15}\) using MHC I molecules as receptors\(^\text{16}\) (Fig 3). Although the entry of SV40 into the cell is very much simpler than adenovirus, reflecting perhaps its simpler structure, little is known about how SV40 comes out of vesicles or travels towards the nuclear envelope. Although some reports indicate that SV40 intracellular transport requires intact microtubules\(^\text{17}\), transport to the nucleus per se is microtubule-independent. When SV40 is introduced directly into the cytoplasm of cells that had been treated with a microtubule-destabilizing reagent, virion derived proteins localize efficiently to the nucleus (M. Yamada, personal communication). The majority of the virion particles have been seen to enter and remain in vesicular
compartments including ER (see references in 18, 19); however, biologically active SV40 must conceivably be released from them into the cytoplasm prior to nuclear entry 20. This question has been probed by injecting antibodies against structural proteins into the cytoplasm and observing the effect of the antibodies on the expression of the large tumor-antigen (T-antigen) — the first gene product to appear during infection, as the antibodies would not recognize virions if they were enclosed in vesicles. The cytoplasmic injection of antibodies into SV40-infected or -injected cells blocks T-antigen expression, demonstrating that the structural proteins of biologically active virions become accessible to antibodies in the cytoplasm 20.

Once the virion DNA reaches the nucleus, whose process is discussed below, it is transcribed and replicated according to a specific temporal program. About 10 to 12 hours into infection, T-antigen starts to appear in the nucleus. The transcriptional promoter for the early genes encoding T-antigen and small-tumor antigen, the latter of which alters the growth property of the infected cell, is a strong one and functions independently of the promoter for the late genes that encode three structural proteins Vp1, Vp2, and Vp3 and agnoprotein, the latter of which appears to have a yet-to-be defined role in the assembly and peri-nuclear accumulation of Vp1 (see references in 21). T-antigen acts as the replication protein of SV40 DNA, by binding to its origin, initiating its replication, and unwinding the DNA at replication forks, in addition to its role in cell growth related functions 22. The transcription of the late genes depends on the presence of T-antigen in addition to many cellular transcription factors. Following the replication of viral DNA, programmed switch in transcription from early genes to late genes takes place and leads to production of viral structural proteins and production/assembly of, progeny virion particles. All viral reproductive processes, except the synthetic process for viral DNA encoded proteins, are confined to the nucleus. Thus, for assembly of the virus, newly synthesized proteins must be imported to the nucleus. Although each viral protein can in isolation be targeted to the nucleus alone using NLS, evidence shows that heterotypic complexes of Vp1, Vp2 and Vp3 are transported into the nucleus for virus assembly 21. A Vp1-NLS mutant protein that cannot enter the nucleus on its own can do so when the wild-type Vp2 and/or Vp3 is expressed in the same cell, and similarly a Vp3-NLS mutant protein can be piggy-backed if the wild-type Vp1 is expressed in the same cell.

Within the nucleus, the structural proteins arrange on the minichromosome in a sequential process to form a virion (see references in 23). As would be required for any protein that interacts with the DNA or the minichromosome, Vp2 and Vp3 can
bind DNA sequence non-specifically through a DNA binding domain that resides in the carboxyl end of both proteins \(^{14}\). Several SV40 mutants whose Vp3 mutant proteins show greatly reduced DNA binding can accumulate in the nucleus but cannot form plaques, demonstrating an important role of the binding domain in the formation of infectious particles \(^{24}\). At the end of the reproductive cycle, about \(10^5\) virions are made in a cell, and virion particles are seen in the nucleus (Fig. 1).

**Nuclear import of SV40**

How infecting SV40 or its genome enters the nucleus was not known until recently. This is partly because that many reported morphological and biochemical observations could reflect events undergone by a majority of virions that may have little to do with the virion that actually leads to an infection. One of the difficulties in studying animal virus-host interaction lies in the fact that the proportion of virion particles in a suspension that score as infections is variable from virus to virus, and for SV40 it is in the range of 0.5 to 1%. The fact that an average of one virion particle microinjected directly into the nucleus or an average of ten virion particles delivered to the cytoplasm can lead to infection \(^{25, 26}\), supports an idea that the biological activity of the virion can be lost during membrane penetration steps during infection. For example if virions dissociate into empty particles and minichromosomes in the cytoplasm during infection, minichromosomes could be left behind in the cytoplasm as empty particles have been shown to enter the nucleus \(^{20}\). Since the presence of electron dense virion particles in the nucleus has been noted in numbers of infection studies (see references in \(^{19}\)), “virions” do appear to enter the nucleus. This contrasts with adenovirus infection where no particles have not been seen in the nucleus (see below). Whether SV40 particles observed in the nucleus contain the viral genomes is another matter and cannot be deduced from these observations. In fact only a fraction of SV40 genomes enter the nucleus. When \(^{3}\)H-thymidine labeled SV40 virions were cytoplasmically injected, less than 3% of silver grains counted were found in the nucleus, and the remainder of the grains were found in the cytoplasm. In contrast to the paucity of the nuclear silver grains, those Vp1’s that had either dissociated from the DNA or still associating with the DNA efficiently accumulated in the nucleus (J. Clever, Ph.D. Thesis, 1992, University of California at Los Angeles). Therefore any functional assay system must be capable of distinguishing an infectious event occurring in a cell by one biologically active SV40 out of 100 or so noninfectious particles. How can we achieve this? If an intact virion presumably enters the nucleus and virion uncoating takes place there
how can a virion particle with a diameter of 50 nm pass through an NPC with a maximum opening up to 26 nm? The NLSs of the structural proteins have been identified. The NLS of Vp1 is a bipartite signal comprising the amino-terminal 19 residues, MAPTKRKSCPGAAPKKPK, in which two clusters of basic residues (a cluster of lysine 5, arginine 6, and lysine 7 and a cluster of lysine 16, lysine 17, and lysine 19) are independently important for nuclear localization activity. In the intact virion there are 360 Vp1 NLSs hidden near the minichromosome. The NLS of Vp2 and Vp3, GPNKKKRKL, is located close to the carboxyl end of the both proteins, and is very much like the NLS of SV40 large-T antigen (see references in ). The precise location of the Vp2 and Vp3-NLS in the interior of the virion is not known. If we assume that these internal NLSs are responsible for SV40 nuclear targeting, how can they become accessible to the NLS receptor proteins in the cytoplasm? Are these two NLSs the only signals that are necessary and sufficient for SV40 nuclear entry? What follows below is the results that form the present understanding of how infectious SV40 virion or viral DNA enters the nucleus.

The study for mechanisms of SV40 import into the nucleus was initiated by microinjection procedure in which isolated SV40 particles were directly injected into the cytoplasm in the presence of neutralizing extracellular antibodies, a method that bypasses the limiting membrane penetration steps. The same procedures, where possible, were then used to obtain comparable results in SV40 infection. Within the first 2 hours of cytoplasmic injection, the protein components of introduced virions as well as virion particles accumulate in the nucleus. Subsequently viral genome-encoded T-antigen appears in the nucleus. For the nuclear entry of the virion proteins, the intrinsic nuclear targeting properties of the proteins/or virion and the cellular transport machinery are sufficient, while the nuclear accumulation of T-antigen requires host protein synthesis. Addition of an inhibitor of protein synthesis, cyclohexamide, did not block virion protein nuclear accumulation but did block T-antigen nuclear accumulation (J. Clever, Ph.D. Thesis, 1992, University of California at Los Angeles). Important facts are that injected virion can complete lytic infection and that the temporal pattern of gene expression is the same as that in infection ( and A. Nakanishi, personal communication).

The swift appearance of virion proteins in the nucleus led to a speculation that a “virion” may have a signal(s) that allow its nuclear entry through NPC. This proved to be the case. Microinjecting into the cytoplasm anti-nucleoporin antibodies or wheat germ agglutinin, both inhibitors of the nuclear protein import machinery proteins, or chilling and energy depleting cells, which also block the
translocation step, completely block the accumulation of virion’s protein as well as T-antigen \(^{19, 28}\). The presence in the cytoplasm of anti-nucleoporin also blocks SV40 nuclear entry from the cell surface. Virion particles and Vp1 are visualized by EM and immuno-EM, respectively, around and in the NPC \(^{19}\). Therefore, infectious SV40 uses NPC, the cellular nuclear protein entry machinery, to enter the nucleus.

Cytoplasmically injected virions dissociate into DNAs and protein components and only a fraction of viral DNAs but most of virion’s proteins enter the nucleus. For those DNAs that enter the nucleus, do capsid proteins remain associated with the viral DNAs during nuclear entry? When anti-Vp1 or anti-Vp3 antibody has been injected into the cytoplasm then virions are introduced either by cytoplasmic injection or by a normal infection, either antibody, but not control antibodies, can intercept the incoming virus and quantitatively block T-antigen expression, arguing for the association of virion proteins to DNA at this stage \(^{20}\). Nuclearly injected anti-Vp3, but not anti-Vp1, antibody also blocks the nuclear accumulation of T-antigen. Since the presence \textit{per se} of any of these antibodies in the nucleus does not block T-antigen expression of SV40 DNA \(^{20}\), the inhibition observed in the nuclear anti-Vp3 interception is due to the depletion of SV40 virion or SV40 DNA-virion protein complexes that has entered the nucleus. These results demonstrate that at least Vp3 accompanies the viral DNA into the nucleus. Whether the viral DNA enters the nucleus together or separately from the major capsid protein Vp1 remains to be determined.

Reconstitution experiments, however, indicate that viral structural proteins are important for targeting of the viral DNA to the nucleus. Microinjected empty SV40 particles that are either devoid of viral DNA or associated with exogenously added viral DNA efficiently enters the nucleus, but naked SV40 DNA or histone-complexed minichromosomes are inefficiently targeted to the nucleus \(^{20}\). Why the histone NLSs, in particular H2B-NLSs of the minichromosomes, are not functional remains to be explained. Another important question to be answered is which NLS is responsible for nuclear entry of the virion/viral genome. Are the NLSs on Vp1, Vp2 and Vp3 somehow exposed in the cytoplasm? If so what are the triggers exposing these NLSs? In vitro studies have shown that conditions present in the cytoplasm of living cells - reducing milieu and low calcium concentrations - are sufficient to promote virus disassembly \(^{29}\). These conditions could induce structural alterations in the virion exposing NLSs. Alternatively, the SV40 virion could contain a yet to be identified viral NLS that is formed on its surface during assembly. In any case the virion particle with a diameter of 50 nm must be somehow compressed to
pass through the gated channel of the NPC with a diameter of 28 nm. Hypothetically, a virion could dissociate in the cytoplasm into an empty capsid and a minichromosome with a diameter of about 34 nm. If structural virion proteins then associate with the minichromosome, such a complex could enter the nucleus, provided it could change its shape for passage through the NPC.

**Structure and assembly of adenovirus**

Compared to SV40, adenovirus has a more complex structure with a diameter of 90 nm. The mature adenovirus contains at least 11 different polypeptides (Fig. 2). A hierarchically organized network of interactions between capsid proteins is set up around a linear double-stranded DNA of about 34 kb, which is linked to the capsid via the internal protein VI. Individual building blocks of the major capsid protein hexon join with minor elements, such as the facet-stabilizing protein IIIa and the hexon-cementing protein IX, and the vertex proteins penton base and fiber. All these proteins with the scaffolding proteins at the inside of the virion form empty incomplete particles in the nucleus of an infected cell.

As with SV40, all the proteins needed for adenovirus assembly must enter the nucleus after synthesis in the cytoplasm. The mechanisms of nuclear transport are unknown for most adenovirus polypeptides. Of the structural proteins, only the NLSs of the fiber and the preterminal protein have been identified. Most recently, also the identification of an NLS in the hexon protein has been announced (Shaphire, A.C.S., Nemerow, G.R., Gerace, L. (1995), Molecular Biology of the Cell, 6, 313a). Other capsid proteins may be imported into the nucleus by yet to be identified NLSs or by association with a karyophilic protein. An example for the latter mechanism is described for the nonstructural DNA polymerase, which enters the nucleus in association with the NLS-bearing preterminal protein.

Unlike the sequential assembly of SV40 around the minichromosome, adenovirus DNA with condensing proteins V, VII and µ and cysteine protease L3/p23, are thought to be packaged into the preformed empty capsid. Correctly folded L3/p23 protease interacts with the precursor of protein VI (pVI) and is retained inside the capsid. In the reducing milieu of the cell nucleus L3/p23 then cleaves the N- and the C-termini of pVI inside the emerging capsid and generates an 11 amino acid long pVI C-terminal-peptide. The disulfide-linked pVI C-terminal-peptide dimer is a potent activator of the reduced L3/p23 protease by a disulfide exchange mechanism. Activated protease then efficiently removes the scaffolding proteins and processes six different capsid proteins at a common
consensus sequence. Although there are many more potential cleavage sites present in the capsid proteins, capsid proteolysis stops and mature virions containing the full length genome and about ten copies of activated cysteine protease are released into the extracellular space by cell lysis. The absence of reducing agents outside the cell and the inaccessibility of substrates in the mature capsid inactivate the L3/p23 protease and so stable virus particles emerge.

**Internalization and penetration of adenovirus**

Mature adenovirus enters cells by binding to an unknown receptor for the vertex fiber protein at the plasma membrane (see also Fig. 4). The anchor of the fiber at the vertex of the capsid, the penton base, then engages with a secondary receptor, a fibronectin-binding integrin, and the virus internalizes via coated vesicles in an integrin dependent manner\(^ {39-41}\). Before the virus is exposed to mild acid in early endosomes the fibers are quantitatively released from the capsid in preparation for penetration into the cytoplasm. Although the exact mechanism of the acid-triggered membrane penetration is unknown, recent results indicate that penetration requires acid-resistant binding of the penton base to the α\(_{v}\)β\(_{5}\) integrin perhaps increasing the affinity of the capsid for membranes\(^ {42}\). Interestingly, when added to cells in high amounts adenovirus can infect cells in the absence of a functional endosomal proton pump suggesting that the pH threshold for endosome penetration is only mildly acidic\(^ {41, 43}\). Intact capsids are able to disrupt endosomes, but not chemically broken viruses or isolated protein components suggesting that the concerted action of multiple capsid proteins or perhaps enzymatic activities, which could reside either in the virus or in the endosome, is required for penetration\(^ {42, 44, 45}\). Subsequent release of the altered capsid from the endosomal membrane may occur by shedding of penton base proteins bound to the integrin receptors\(^ {41}\).

Once the virus has left the endosome and entered the cytoplasm the virion structure undergoes further alterations. The internal DNA stabilizing protein VI located underneath the peripentonal hexons is degraded by the reactivated protease L3/p23 and the majority of the capsid cementing protein IX is removed shortly thereafter\(^ {41}\). While the signal for protein IX removal is unknown, the engagement of the penton base with the integrin receptor and reducing agents in endosomes or the cytoplasm are needed for degradation of protein VI by L3/p23 protease\(^ {46}\). Conceivably, conformational changes in the peripentonal region of the capsid could expose previously hidden protease cleavage sites in protein VI.
While the triggers for adenovirus protein VI degradation do not include acidic endosomes, low endosomal pH is known to induce conformational changes in the lipid-embedded fusion proteins of acid-dependent enveloped viruses \(^{47}\). In addition, low endosomal pH triggers an early uncoating reaction in the influenza virus ribonucleoproteins which is needed for subsequent nuclear import of the viral genome (for review see \(^{48}\)).

**Nuclear transport of adenovirus**

The mechanism of nuclear transport of incoming adenovirus are not well understood. It is possible that adenovirus nuclear targeting involves the NLS of hexon and/or a putative NLS of another coat protein, which is still associated with the cytosolic virion. Adenovirus targeting to the nuclear membrane may involve cytosolic hsp70, which was found associated with hexon during infection \(^{49}\). Conceivably, hsp70 may help to expose an NLS present in one of the coat proteins. It can, however, not be ruled out that mechanical forces, such as motor proteins, are needed to drive the virions through the cytoplasm to the nuclear membrane. Passive diffusion of globular particles as big as SV40 or adenovirus particles in the cytoplasm is expected to be insufficient for transport to the nucleus, even if the virions were released from the endosomes near the nuclear membrane \(^{50}\). Based on early experiments using microtubule depolymerizing agents (for review see \(^{51}\)) one possible mechanism of adenovirus targeting to the nucleus in a living cell is by microtubule-mediated retrograde movement. Likewise, transport of adenovirus capsids from the cytoplasm to the nucleus does not occur at reduced temperature, conditions which depolymerize microtubules (Greber, unpublished observation). Interestingly, nuclear transport of influenza virus ribonucleoproteins, large elongated structures, or NLS-containing proteins does not require intact microtubules (for review see \(^{48}\)). In contrast, movement of herpes virus capsids in nerve cells strictly depends on intact microtubules suggesting that perhaps large globular structures, such virus capsids \(^{52}\), need microtubule guidance for their movement in the cytoplasm. Additional studies aiming at the identification of functional motor proteins are now required to test the hypothesis of microtubule involvement in adenovirus targeting to the nuclear membrane.

Structurally altered adenovirus reaches the nuclear membrane and localizes to the cytoplasmic side of the nuclear pore complex (see also Fig. 4). Quantitative analysis of viruses at the nuclear envelope revealed that every fourth particle is associated with a nuclear pore complex during the first hour of infection \(^{46}\). In
agreement with previous studies (for review see \textsuperscript{51}), no capsid-like particles have ever been found inside the nucleus. Disassembly-incompetent virus penetrates into the cytoplasm and localizes to the NPC, but cannot import DNA and associated protein VII into the nucleus \textsuperscript{45, 46} indicating that targeting of the adenovirus to the nuclear membrane occurs via capsid proteins. Disassembly of the labile cytoplasmic capsids may be triggered by fibrillar cytoplasmic elements of the nuclear pore complex, which can be involved in the docking step of NLS-containing proteins together with the NLS receptor subunits alpha and beta and the small GTP binding protein RanTC4 \textsuperscript{5, 8, 53}. Nuclear import of the DNA and associated proteins, in turn, requires dissociation from the majority of the capsid and could in principle occur via the NLS-mediated pathway involving the covalently attached terminal protein \textsuperscript{33}.

\textbf{Perspectives}

The mechanisms by which viruses transport their genome into the nucleus are of central interest, since viruses are not only pathogens but are increasingly being harnessed for gene transfer purposes in molecular medicine. We are beginning to understand common and different mechanistic principles used by SV40 and adenovirus for their nuclear DNA deliveries. Common to both viruses is that they are in some ways altered when they have reached the cytoplasm after entering cells by receptor-mediated endocytosis. Cytoplasmic SV40 particles display on their surface NLSs for nuclear targeting, in contrast to the extracellular virions. Likewise, endosomal and cytoplasmic adenovirus particles shed and degrade certain structural proteins enabling them to overcome the endosomal and nuclear membrane on their way to the nucleoplasm. SV40 and adenovirus, however, use different nuclear import strategies. SV40 particles are transported through the NPC and the particles disassemble in the nucleoplasm before onset of viral gene expression, while adenovirus disassembles at the nuclear membrane before its DNA is transported into the nucleus. Together with what we know from bacterial DNA transport in plant cells the theme emerges that nuclear entry of DNA may be determined by NLSs in proteins directly or indirectly associated with the DNA. The question how the DNA-protein complexes are ferried through the narrow opening of the NPC is now of central interest. The combination of biochemical, cell biological and genetic studies with substrates or mutants carrying defined functional signals may soon give us a more accurate picture of how SV40 and adenovirus target to and into the nucleus.
**Figure legends**

**Fig. 1.** Electron micrographs of SV40 virions  
A) Negatively stained whole virions. The icosahedral capsid is approximately 50 nm in diameter.  B) An SV40 virion that has “collapsed” onto the substrate, revealing the individual capsomers comprising the virion capsid. C) View of an infected TC7 AGMK cell harvested at 48 hr post-infection. The cell nucleus, the site of virion assembly, is filled with small round virus particles.

**Fig. 2.** Schematic diagrams of SV40 and adenovirus  
The simian virus 40 (SV40) virion is 50 nm in diameter, and contains three capsid proteins, Vp1, Vp2 and Vp3 and four cellular histones H2A, H2B, H3 and H4. The genome is a double stranded circular DNA molecule which is condensed with histones into a minichromosome.  
The adenovirus capsid, 90 nm in diameter, is composed of the major hexon protein, the stabilizing proteins IIIa, VI, VIII and IX and the DNA-associated proteins V, VII, µ and L3/p23 cysteine protease. Covalently bound terminal protein is present at the 5’ end of the DNA molecule.

**Fig. 3.** Cell entry and nuclear transport of SV40  
Virus attachment to the cell surface is mediated by MHC class I molecules (Step 1) and uptake into cells occurs by constitutive endocytosis (Step 2). By an unknown mechanism biologically active virus penetrates into the cytoplasm (Step 3) and is targeted to the nucleus after a few NLSs of capsid protein(s) become exposed (Steps 4 and 5). Virion import into the nucleus occurs through the nuclear pore complex. This process requires that capsid proteins remain associated with the viral DNA, though the virion structure is assumed to be altered somehow (Step 6). Inside the nucleus, the capsid disassemble into its components and viral DNA is expressed (Step 7).

**Fig. 4.** Cell entry and nuclear transport of adenovirus  
Adenovirus enters cells by a stepwise destabilization program, removing or degrading selective capsid proteins in order to proceed from one barrier to the next on the way to the nucleus. After binding to an unidentified high affinity receptor at the cell surface the virus is transferred to the secondary receptor, fibronectin/vitronectin binding integrin (Step 1). The virus is internalized through coated pits by integrin-mediated endocytosis and passes from the early endosome to
the cytoplasm by a concerted action of different capsid proteins in an integrin- and acid-dependent manner (Steps 2 and 3). The cytoplasmic capsid is destabilized by the loss of several minor proteins and the vertex modified by shedding of the fibers during internalization. During or shortly after penetration into the cytoplasm the internal stabilizing protein VI is degraded by reactivated virus cysteine protease further weakening the capsid. The modified virus is then targeted to the nucleus by an NLS-mediated process and/or microtubules (Step 4). A trigger from the nuclear membrane liberates the viral DNA from the shell (Step 5) allowing DNA import into the nucleus. In a possible scenario, DNA together with condensing proteins would then be imported into the nucleus via the NLSs of the covalently bound terminal proteins (Step 6).

References