Nuclear Import of Viral DNA-Genomes

Urs F. Greber[+)] & Ariberto Fassati[&)]

[+)] Zoologisches Institut der Universität Zürich, Winterthurerstrasse 190
CH-8057 Zürich, Switzerland

[&)] Wohl Virion Centre, Windeyer Institute, University College London Medical School, 46 Cleveland Street
London W1T, UK

[+)] corresponding author:
Telephone: +41 1 635 4841
Fax: +41 1 635 6822
Email: ufgreber@zool.unizh.ch

Keywords: virus, DNA, genome, nuclear import, nuclear pore complex

Word count: 5441 (including references)
137 (abstract)

Abstract

The genomes of many viruses traffic into the nucleus where they are either integrated into host chromosomes or maintained as episomal DNA and then transcriptionally activated or silenced. Here, we discuss the existing evidence on how the lentiviruses, adenoviruses, herpesviruses, hepadnaviruses and autonomous parvoviruses enter the nucleus. Depending on the size of the capsid enclosing the genome, three principles of viral nucleic acids import are discussed. The first principle is that the capsid disassembles in the cytosol or in a docked state at the nuclear pore complex and a subviral genomic complex is trafficked through the pore. Second, the genome is injected from a capsid that is docked to the pore complex, and third, import factors are recruited to cytosolic capsids to increase capsid affinity to the pore complex, mediate translocation and allow disassembly in the nucleoplasm.

Introduction

Many viruses have evolved the ability to replicate and store their genome in the cell nucleus. The nucleus not only provides a machinery for viral DNA and RNA polymerisation, processing and stabilisation, but it also supplies essential factors for viral replication, egress and maturation. Viruses aim to maintain their genome in the infected cells until the host organism dies, and they usually achieve this by integrating genomic DNA into the host chromosomes or segregating episomal DNA to daughter cells. The efficient delivery of the incoming genome into the nucleus is important for viruses with both large and small genomes. This is no simple task, illustrated by the fact that any transfected plasmid DNA is poorly delivered into the nuclei of cultured cells or tissue, and accordingly, plasmid-based expression systems are generally of limited efficacy. In contrast, viral assemblies are professional intracellular carriers maximizing gene delivery and also allowing for efficient assembly and protection of their genome. This requires a tight cooperation with cellular functions. Considering that the cell contains multiple pathways for importing proteins and nucleic acids into the nucleus, it is not surprising that viruses have found various ways to access the nucleoplasm. In this review, we are considering different DNA-import strategies of prototypic retroviruses and DNA-tumor viruses. For additional discussions of viral import the reader is referred to recent reviews (1-5).

The nuclear pore complex controls nucleo-cytoplasmic traffic

Ever since the discovery of pores in replica and thin section preparations of the nuclear envelope in the 1950's (6, 7), a wealth of experiments have addressed the question of the maximal pore diameter. Early on, the diameter of the pore proper was estimated to be about 70 nm and in the late 1980's it was restricted to 26 nm (8). The pores are part of the nuclear pore complex (NPC), which spans the double membrane of the nuclear envelope and controls the macromolecular transport into and from the nucleus. In a growing cell, an NPC

accommodates a considerable flux of proteins and nucleic acids. It has been estimated in permeabilized cells that several dozens of cargo-receptor complexes translocate through one NPC per second (9). Both yeast and vertebrate NPCs are large, eight-fold symmetrical structures that slightly differ in size but are thought to function similarly, although the yeast NPCs lack certain components (10). The overall mass of a vertebrate NPC is about 125 MDa and roughly corresponds to the mass of an Adenovirus (Ad) particle (11). This considerable size may be related to the diverse transport functions of the NPC.

Nuclear cargo often bears nuclear localization sequences (NLSs) that are complexed with NLS-receptors, i.e. importins or karyopherins. This complex passes through the central NPC core that contains nucleoporins rich in hydrophobic repeats, such as FxFG (12, 13). The translocation of small proteins is independent of ATP and GTP hydrolysis and the transport directionality is determined by the different concentrations of Ran-GTP in the nucleus versus the cytoplasm (12, 14). The translocation mechanism has been addressed by several models, as recently reviewed in detail (15). An affinity gradient model invokes the movement of molecules along binding sites of increasing affinity from the cytoplasmic to the nucleoplasmic side (16). Alternatively, receptor-cargo complexes may bind to NPC filaments and thus increase the probability to cross the central channel by Brownian motion (17). A third model proposes that the central channel filled with a mildly hydrophobic selective phase excludes most molecules with the exception of the specialised transport receptors (18).

Concerning the import of large protein cargo, a recent study suggested that Ran and hydrolysable GTP was required to facilitate diffusion of the large complexes through the NPC (19). From the viral point of view, the concept of a large and impermeable central channel structure is rather attractive. Intact or partially uncoated viruses that are small enough could thus cross the NPC simply by recruiting the right nuclear import receptors or by undergoing conformational changes that would allow the outer surface to interact with channel components. Larger viruses, on the other hand, would need to uncoat their nucleic acid prior to

nuclear import. Whether facilitated translocation will be efficient enough to translocate very large DNA molecules, such as the 150 kbp genome of herpes simplex viruses (HSVs) is not clear, and it may be that additional mechanisms are in place. Here we present some of the recent advances and problems in understanding nuclear import of viral DNA genomes.

Onco-retroviruses

Retroviruses are enveloped viruses with a diploid positive strand RNA genome. After entry into the cell, the viral RNA is reverse transcribed into a double stranded DNA molecule that integrates into the host chromosomes. While reverse transcription and integration are fairly well understood processes, little is known on the mechanisms that mediate nuclear import of retroviral genomes (20). Several problems limit our ability to investigate nuclear-cytoplasmic trafficking of retroviruses. First is the lack of an *in vitro* assay that allows direct detection of nuclear import of intracellular viral particles and particularly viral genomes. Tagging of viral proteins like the human immunodeficiency virus type-1 (HIV-1) Vpr with the green fluorescent protein (GFP) is a potentially fruitful strategy. Accordingly, GFP-tagged Vpr has recently been used to trace incoming HIV reverse transcription complexes (RTCs) that were shown to traffic along microtubules towards the nucleus (21). The second limitation is that we have only limited structural and biochemical information on the RTC (21, 22) and essentially no structural information on the pre-integration complexes (PICs). This makes it is difficult to follow the nuclear translocation of viral genome-protein complexes by, for example, electron microscopy techniques. The third reason limiting our understanding of retroviral nuclear import is that genetic analyses of viral proteins associated with either RTCs or PICs have so far shown little clear cut phenotypes, in particular in the case of HIV (for reviews, see 3, 4, 20, 23). Possible reasons are that the introduced mutations have pleiotropic effects, that different proteins of the PIC act cooperatively or that there are unknown cellular PIC-binding factors that have key functions in importing the viral genome into the nucleus.

Despite these limitations, there is sufficient information to conclude that the type C onco-retroviruses, like the Moloney murine leukemia virus (MoMLV), the lentiviruses, such as HIV-1, the avian retroviruses, like Rous sarcoma virus, and the foamy viruses utilize different mechanisms to enter the nucleus (24, 25). MoMLV can infect only dividing cells while HIV-1 and also HIV-1 derived vectors infect dividing and non-dividing cells, including macrophages, post-mitotic neurons and hematopoietic stem cells. MoMLV restriction in nondividing cells is very tight while Rous sarcoma virus is only partially restricted (26) and human foamy virus might have some ability to infect cells in interphase, although there is controversy on this issue (27, 28). The difference between MoMLV and HIV has been largely attributed to the assumption that the intracellular complexes of MoMLV are too large to be transported to or into the nucleus and that they lack the appropriate nuclear targeting signals to cross the NPC. Rather, they would gain access to host chromatin after breakdown of the nuclear envelope in mitosis. Yet there is no definitive evidence that this is the case. Earlier electron microscopy studies indicated that MoMLV integrase and nucleocapsid proteins, which are part of the RTC/PIC accumulate in the nuclei and nucleoli shortly after infection (29). In addition, mutations in the p12 portion of the gag protein block MoMLV replication after reverse transcription is completed (30). The mouse anti-viral gene Fv-1 can also block viral replication after completion of reverse transcription, presumably by interfering with the trafficking of the viral genome towards the nucleus (31). Together, this evidence suggests that nuclear import of MoMLV might involve more complex mechanisms than the simple breakdown of the nuclear envelope in mitosis.

The enigmatic import of lentiviral genomes

In contrast to onco-retroviruses, lentiviruses have evolved mechanisms allowing them to infect nondividing cells. HIV-1 PICs are transported into the nuclei of infected cells by a process requiring energy (32). Although the underlying molecular mechanisms of PIC nuclear import are not yet known, there is evidence to discuss several possibilites. First, PIC import into the nucleus may

be mediated by proteins that remain associated to the viral genome after uncoating, and this may be assisted by specific cellular factors. Matrix, Vpr and integrase possess one or several NLSs, are associated to the viral nucleic acids after infection and have been implicated in nuclear import of PICs. However, matrix and Vpr are unlikely to be solely responsible for PIC import, since HIV-1 mutants lacking both of these proteins efficiently infect non-dividing cells, and the same is true for HIV-1 based vectors lacking Vpr (20, 33). There is controversy on the nature and location of integrase NLSs responsible for PIC nuclear import (34, 35). Second, the retroviral genome itself may contain nuclear targeting elements. Recently, HIV-1 mutants lacking the central polypurine tract (PPT), a second site of initiation of plus strand DNA synthesis present in all lentiviruses, appear to have a reduced ability to access the nucleus (36). This reduction is, however, rather small (two to five fold) and it seems to be strain-dependent (37, 38). A third possibility has been raised by the observation that the HIV Vpr causes blebbing of the nuclear envelope and thus might locally open the gate for nuclear entry of PIC independent of NPCs (39). Since HIV-1 is not a typical lytic virus, it is uncertain, however, if the Vpr-mediated nuclear envelope disruption is enough to allow PIC passage. It is also possible that Vpr enhances viral replication by activating the target cells prior to PIC entry into the nucleus (40). Cell signalling during or prior to entry is, in fact, used by many viral agents that infect nondividing cells, although signalling of HIV through its cell surface receptors alone is not required for DNA delivery into the nucleus (reviewed in 41). Alternatively, Vpr might have a crucial, yet redundant role in PIC import, because it is able to attach directly to NPC proteins that contain FxFG repeats (42). In this case, Vpr may be acting cooperatively with NLS-receptors to drive PIC into the nucleus. It will be important to define NLSs on PIC that are accessible to the soluble import factors.

Direct docking to the NPC

The Ty element and other long terminal repeat (LTR)-containing retrotransposons of yeast have a genomic organisation close to onco-

retroviruses and encode similar structural and catalytic proteins that assemble to form the virus-like particle (VLP) in which reverse transcription takes place. The VLPs are approximately 60 nm in diameter, too large to diffuse though the nuclear pores. There is evidence that nucleoprotein complexes directly interact with the nucleoporins. Tf1, a retrotransposon of *S. pombe* has a nuclear targeting signal in the N-terminal portion of gag that mediates nuclear entry through the recognition of the nuclear pore protein Nup124 (43, 44). Yeast cells that were mutated in Nup124 block the nuclear import of Tf1 protein and also cDNA. This import defect appears to be specific since overall protein or RNA import is not affected in this mutant. A nuclear targeting signal in integrase is involved in nuclear transport of Ty3, a retrotransposon of *S. cerevisiae* (45). It is not clear if VLPs must generally disassemble to let the retrotransposon genome cross the NPC. Perhaps, this is the case for Ty3 since integrase is believed to be located inside the VLP. The strong similarity of yeast retrotransposons to retroviruses will make it very interesting to apply yeast genetics to study LTR-retrotransposon mobility. A recent screen has in fact shown that the silencing factor Sin3, which is a histone H3 and H4 deacetylase in *S. pombe*, is required for Tf1 nuclear import (46). This implies that acetylation is directly or indirectly involved in the regulation of import or disassembly of the retrotransposon particles. It will be interesting to see if a transport or a disassembly factor is regulated by acetylation.

It was recently shown that the overall levels of nuclear acetylation affect the motility of the linker histone H1 (47), and histone H1 has been implicated as a key factor of Ad2 and Ad5 disassembly (48). The Ad particles directly dock to the NPC protein CAN/Nup214, located at the cytoplasmic filaments (see Fig. 2). The viral affinity to the NPC is independent of transport factors but virus particles are not imported without importins (48, 49). The NPC-docked Ad traps the nuclear histone H1, and H1 import factors together with additional cytosolic factors, such as Hsc70 (50) then trigger the disassembly of the capsid which enables DNA translocation into the nucleoplasm. The translocation of the 36 kbp double strand DNA measured appears to be a slow process as measured by

fluorescence in situ hybridisation experiments, peaking approximately 30 to 60 min after the onset of viral disassembly (48, 51). Accordingly, empty capsids, i.e. DNA-less capsids cannot be found at the NPC of Ad2 or Ad5 infected cells (K. Boucke, personal communication, and EM studies reviewed in 2).

Import by injection

In contrast to Ad, empty capsids of incoming HSV are often found docked at the NPC, suggesting an active DNA release process rather than capsid disintegration prior to DNA import (see Fig. 3, and 52). Like the Ad capsid (90 nm in diameter), the icosahedral HSV capsid with a diameter of 125 nm is far larger than the maximal estimated nuclear pore. Accordingly, capsids from purified HSV-1 were found to bind to the cytosolic face of NPCs on rat liver nuclear envelopes in the absence of cytosol, provided that the soluble importin was present (53). Viral DNA was not released under these conditions. DNA release required the addition of cytosol and energy. Earlier genetic analyses of HSV had identified a mutation of a virion protein affecting DNA release at the NPC and inhibiting the onset of viral DNA synthesis (54). This suggests that coordinated changes in the capsid are required for DNA ejection. Since the packaging of the DNA into capsids is energy-dependent (55), it can be speculated that the ejection of DNA, i.e., the reverse process of DNA-packaging occurs spontaneously, if the capsid is opened at a strategic position, e.g. at the portal of DNA entry. The factors catalysing this process are unknown.

Capsid import and disassembly in the nucleus

A recent study in Xenopus oocytes suggested that the NPC can accommodate human hepatitis B virus capsids up to about 39 nm in diameter (56). The recombinant HBV capsids devoid of DNA were found within the nuclear baskets, suggesting that the translocation process of these capsids was slow. In permeabilized cells, the association of HBV capsids with NPCs required capsid

phosphorylation, apparently to make NLSs accessible for importin and binding (57), and a classical NLS was required for the association of the duck HBV core with NPCs (58). These results show that HBV capsids increase their affinity to the NPC by recruiting importins, and that DNA-less capsids can associate with the NPCs of a non-natural host without disassembly (see Fig. 4). Whether the DNA-containing capsids can be imported into nuclei of host cells or if they dissociate at the NPC remains to be analysed.

Viral capsid proteins often carry multiple NLSs targeting them to assembly or DNA-packaging into the nucleus. Alternatively, NLSs on capsid proteins may direct the incoming particle into the nucleus. This multiple NLS strategy is elegantly used by the autonomous parvovirus minute virus of mouse (MVM). The capsid of parvoviruses is 25 nm in diameter and filled with a single-strand negative-sense DNA of about 5 kb. The MVM enters by endocytosis depending on a low endosomal pH and also the actin and microtubule cytoskeleton (59). The T=1 icosahedral MVM capsid contains 10 copies of the viral protein (VP) 1 and 50 copies of VP2. The amino acid sequence of VP1 is identical to VP2, except that it has an 146 amino acid N-terminal extension due to alternative splicing (60). The VP1 N-terminus contains four basic clusters two of which perfectly match the classical NLS of SV40 large T antigen, but it is located inside the capsid (61). In addition, VP2 has a C-terminal structural nuclear localization motive which serves to import newly synthesized VP1 into the nucleus and drives capsid formation (62), but it is also located inside the capsid. Genetic deletion studies have shown that the N-terminal basic clusters of VP1 are required for the onset of infection, as measured by Southern blots of viral DNAs and immunofluorescence stainings (63). It is thought that the N-terminus becomes exposed during capsid entry, similar to the N-terminus of the canine parvovirus, which was shown to be neutralized by cytoplasmic microinjections of anti-N-terminus antibodies (64). Possibly, a limited uncoating of MVM is facilitated by proteasomal degradation of cellular factors that normally inhibit infection, or perhaps proteasomes target the incoming capsid (59). Whether this increases the nuclear affinity of the capsids is unknown, but it is clear that the autonomous

parvoviruses are structurally dynamic during entry, similar to their larger relatives, the Ads (65). It will be interesting to see if the minor MVM capsid protein VP1 suffices for capsid traversal through the NPC or if the nuclear localization motive of VP2 needs to be exposed. If the former were true, one could argue that VP1 accompanies the viral DNA into the nucleus. If the VP2 NLSs need to be exposed for the onset of infection, this might drive the capsids through the pores prior to disassembly.

Conclusions

The nuclear pore complexes are morphologically homogeneous and functionally equivalent, but viruses have found multiple strategies to abuse them. Complex viruses, such as the lentiviruses, reverse transcribe their RNA genome into DNA and uncoat the genome in the cytoplasm. They recoat the DNA and drive it into the nucleus by a cooperative enhancement of multiple factors, including redundant NLSs, import receptors, conformational changes and unknown cellular factors and possibly also nonconventional mechanisms of cell signalling from within the infected cell. Larger DNA-viruses, such as Ad and HSV dissociate the genome from the capsid prior to nuclear import. Small DNA-viruses are thought to maintain their genome in an encapsidated state until they arrive in the nucleus. The denouement of viral import strategies will no doubt shed new light on the complexity of nuclear import of cellular macromolecules and open the way to improved viral vectors for gene therapy. We are sure that increasingly thorough analyses of the cell biology of viral infections will teach us more of the intricate relationship between viral pathogens and their hosts.

Acknowledgement

We apologize to those collegues whose work could not be cited due to space constraints. Thanks to C. Burckhardt, H. Levin, M. Kann and U. Kutay for discussions or comments to the manuscript. The authors were supported by

grants from the Swiss National Science Foundation (ufg) and The Wellcome Trust (af).

References

1. Kasamatsu H, Nakanishi A. How do animal DNA viruses get to the nucleus? Annu Rev Microbiol 1998;52:627-86.

2. Greber UF. Delivery of animal virus DNA into the nucleus. In: Seymour L, Kabanov A, Felgner P, editors. Self-assembling complexes for gene delivery: from chemistry to clinical trial. Sussex, UK: John Wiley & Sons, Ltd; 1998. p. 89-114.

3. Whittaker GR, Kann M, Helenius A. Viral entry into the nucleus. Annu Rev Cell Dev Biol 2000;16:627-51.

4. Cullen BR. Journey to the center of the cell. Cell 2001;105(6):697-700.

5. Poranen MM, Daugelavicius R, Bamford DH. Common principles in viral entry. Annu Rev Microbiol 2002;56:521-38.

6. Callan HG, Tomlin SG. Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nuclear membrane by means of the electron microscope. Proc Roy Soc London 1950;137 B:367-378.

7. Afzelius BA. The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope. Exp. Cell Res. 1955;8:147-158.

8. Dworetzky SI, Lanford RE, Feldherr CM. The effects of variations in the number and sequence of targeting signals on nuclear uptake. Journal of Cell Biology 1988;107(4):1279-1287.

9. Ribbeck K, Gorlich D. Kinetic analysis of translocation through nuclear pore complexes. Embo J 2001;20(6):1320-30.

10. Cronshaw JM, Krutchinsky AN, Zhang W, Chait BT, Matunis MJ. Proteomic analysis of the mammalian nuclear pore complex. J Cell Biol 2002;158(5):915-27.

11. Reichelt R, Holzenburg A, Buhle EJ, Jarnik M, Engel A, Aebi U. Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. J. Cell Biol. 1990;110(4):883-894.

12. Gorlich D, Kutay U. Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol 1999;15:607-60.

13. Chook YM, Blobel G. Karyopherins and nuclear import. Curr Opin Struct Biol 2001;11(6):703-15.

14. Nachury MV, Weis K. The direction of transport through the nuclear pore can be inverted. Proc Natl Acad Sci U S A 1999;96(17):9622-7.

15. Bednenko J, Cingolani G, Gerace L. Nucleocytoplasmic transport: navigating the channel. Traffic 2003;in press.

16. Ben-Efraim I, Gerace L. Gradient of Increasing Affinity of Importin beta for Nucleoporins along the Pathway of Nuclear Import. J Cell Biol 2001;152(2):411-8.

17. Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J. Cell Biol. 2000;148(4):635-51.

18. Ribbeck K, Gorlich D. The permeability barrier of nuclear pore complexes appears to operate via hydrophobic exclusion. Embo J 2002;21(11):2664-71.

19. Lyman SK, Guan T, Bednenko J, Wodrich H, Gerace L. Influence of cargo size on Ran and energy requirements for nuclear protein import. J Cell Biol 2002;159(1):55-67.

20. Goff SP. Intracellular trafficking of retroviral genomes during the early phase of infection: viral exploitation of cellular pathways. J Gene Med 2001;3(6):517-28.

21. McDonald D, Vodicka MA, Lucero G, Svitkina TM, Borisy GG, Emerman M, et al. Visualization of the intracellular behavior of HIV in living cells. J Cell Biol 2002;159(3):441-52.

22. Fassati A, Goff SP. Characterization of intracellular reverse transcription complexes of human immunodeficiency virus type 1. J Virol 2001;75(8):3626-35.

23. Fouchier RA, Malim MH. Nuclear import of human immunodeficiency virus type-1 preintegration complexes. Adv Virus Res 1999;52:275-99.

24. Lewis P, Hensel M, Emerman M. Human immunodeficiency virus infection of cells arrested in the cell cycle. Embo J 1992;11(8):3053-8.

25. Roe TY, Reynolds TC, Yu G, Brown PO. Integration of murine leukemia virus dna depends on mitosis. Embo Journal 1993;12:2099-2108.

26. Hatziioannou T, Goff SP. Infection of nondividing cells by Rous sarcoma virus. J Virol 2001;75(19):9526-31.

27. Bieniasz PD, Weiss RA, McClure MO. Cell cycle dependence of foamy retrovirus infection. J Virol 1995;69(11):7295-9.

28. Saib A, Puvion-Dutilleul F, Schmid M, Peries J, de The H. Nuclear targeting of incoming human foamy virus Gag proteins involves a centriolar step. J Virol 1997;71(2):1155-61.

29. Risco C, Menendezarias L, Copeland TD, Dasilva PP, Oroszlan S. Intracellular Transport Of the Murine Leukemia Virus During Acute Infection Of Nih 3t3 Cells - Nuclear Import Of Nucleocapsid Protein and Integrase. Journal of Cell Science 1995;108(Part 9):3039-3050.

30. Yuan B, Fassati A, Yueh A, Goff SP. Characterization of Moloney Murine Leukemia Virus p12 Mutants Blocked during Early Events of Infection. J Virol 2002;76(21):10801-10.

31. Goff SP. Operating under a Gag order: a block against incoming virus by the Fv1 gene. Cell 1996;86(5):691-3.

32. Bukrinsky MJ, Sharova N, Dempsey MP, Stanwick TL, Bukrinskaya AG, Haggerty S, et al. Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc. Natl. Acad. Sci. USA 1992;89:6580-6584.

33. Neil S, Martin F, Ikeda Y, Collins M. Postentry restriction to human immunodeficiency virus-based vector transduction in human monocytes. J Virol 2001;75(12):5448-56.

34. Bouyac-Bertoia M, Dvorin JD, Fouchier RA, Jenkins Y, Meyer BE, Wu LI, et al. HIV-1 infection requires a functional integrase NLS. Mol Cell 2001;7(5):1025-35.

35. Limon A, Devroe E, Lu R, Ghory HZ, Silver PA, Engelman A. Nuclear Localization of Human Immunodeficiency Virus Type 1 Preintegration Complexes (PICs): V165A and R166A Are Pleiotropic Integrase Mutants Primarily Defective for Integration, Not PIC Nuclear Import. J Virol 2002;76(21):10598-607.

36. Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P. HIV-1 genome nuclear import is mediated by a central DNA flap. Cell 2000;101(2):173-85.

37. Dvorin JD, Bell P, Maul GG, Yamashita M, Emerman M, Malim MH. Reassessment of the Roles of Integrase and the Central DNA Flap in Human Immunodeficiency Virus Type 1 Nuclear Import. J Virol 2002;76(23):12087-12096.

38. Limon A, Nakajima N, Lu R, Ghory HZ, Engelman A. Wild-type levels of nuclear localization and human immunodeficiency virus type 1 replication in the absence of the central DNA flap. J Virol 2002;76(23):12078-86.

39. de Noronha CM, Sherman MP, Lin HW, Cavrois MV, Moir RD, Goldman RD, et al. Dynamic disruptions in nuclear envelope architecture and integrity induced by HIV-1 Vpr. Science 2001;294(5544):1105-8.

40. Subbramanian RA, Kessous-Elbaz A, Lodge R, Forget J, Yao XJ, Bergeron D, et al. Human immunodeficiency virus type 1 Vpr is a positive regulator of viral transcription and infectivity in primary human macrophages. J Exp Med 1998;187(7):1103-11.

41. Greber UF. Signalling in viral entry. Cell Mol Life Sci 2002;59:608-626.

42. Fouchier RA, Meyer BE, Simon JH, Fischer U, Albright AV, Gonzalez-Scarano F, et al. Interaction of the human immunodeficiency virus type 1 Vpr protein with the nuclear pore complex. J Virol 1998;72(7):6004-13.

43. Balasundaram D, Benedik MJ, Morphew M, Dang VD, Levin HL. Nup124p is a nuclear pore factor of Schizosaccharomyces pombe that is important for nuclear import and activity of retrotransposon Tf1. Mol Cell Biol 1999;19(8):5768-84.

44. Dang VD, Levin HL. Nuclear import of the retrotransposon Tf1 is governed by a nuclear localization signal that possesses a unique requirement for the FXFG nuclear pore factor Nup124p. Mol Cell Biol 2000;20(20):7798-812.

45. Lin SS, Nymark-McMahon MH, Yieh L, Sandmeyer SB. Integrase mediates nuclear localization of Ty3. Mol Cell Biol 2001;21(22):7826-38.

46. Dang VD, Benedik MJ, Ekwall K, Choi J, Allshire RC, Levin HL. A new member of the Sin3 family of corepressors is essential for cell viability and required for retroelement propagation in fission yeast. Mol Cell Biol 1999;19(3):2351-65.

47. Misteli T, Gunjan A, Hock R, Bustin M, Brown DT. Dynamic binding of histone H1 to chromatin in living cells. Nature 2000;408(6814):877-81.

48. Trotman LC, Mosberger N, Fornerod M, Stidwill RP, Greber UF. Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. Nature Cell Biology 2001;3:1092-1100.

49. Wisnivesky JP, Leopold PL, Crystal RG. Specific binding of the adenovirus capsid to the nuclear envelope. Hum Gene Ther 1999;10(13):2187-95.

50. Saphire ACS, Guan TL, Schirmer EC, Nemerow GR, Gerace L. Nuclear import of adenovirus DNA in vitro involves the nuclear protein import pathway and hsc70. J. Biol. Chem. 2000;275(6):4298-4304.

51. Greber UF, Suomalainen M, Stidwill RP, Boucke K, Ebersold M, Helenius A. The role of the nuclear pore complex in adenovirus DNA entry. EMBO J. 1997;16:5998-6007.

52. Roizman B, Sears AE. Herpes simplex viruses and their replication. In: B. Roizman RJW, C. Lopez, editor. The human herpes viruses. New York: Raven Press; 1993. p. 11-68.

53. Ojala PM, Sodeik B, Ebersold MW, Kutay U, Helenius A. Herpes simplex virus type 1 entry into host cells: reconstitution of capsid binding and uncoating at the nuclear pore complex in vitro. Mol Cell Biol 2000;20(13):4922-4931.

54. Batterson W, Furlong D, Roizman B. Molecular genetics of Herpes simplex virus. VIII. Further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. J. Virol. 1983;45:397-407.

55. Dasgupta A, Wilson DW. ATP depletion blocks herpes simplex virus DNA packaging and capsid maturation. J Virol 1999;73(3):2006-15.

56. Pante N, Kann M. Nuclear pore complex is able to transport macromolecules with diameters of similar to 39 nm. Molecular Biology of the Cell 2002;13(2):425-434.

57. Kann M, Sodeik B, Vlachou A, Gerlich WH, Helenius A. Phosphorylation-dependent binding of hepatitis B virus core particles to the nuclear pore complex. J Cell Biol 1999;145(1):45-55.

58. Mabit H, Breiner KM, Knaust A, Zachmann-Brand B, Schaller H. Signals for bidirectional nucleocytoplasmic transport in the duck hepatitis B virus capsid protein. J. Virol. 2001;75(4):1968-1977.

59. Ros C, Burckhardt CJ, Kempf C. Cytoplasmic Trafficking of Minute Virus of Mice: Low-pH Requirement, Routing to Late Endosomes, and Proteasome Interaction. J Virol 2002;76(24):12634-45.

60. Muzyczka N, Berns KI. Parvoviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. Fundamental Virology. 4 ed. Philadelphia: Lippincott-Raven; 2001. p. 1089-1121.

61. Agbandje-McKenna M, Llamas-Saiz AL, Wang F, Tattersall P, Rossmann MG. Functional implications of the structure of the murine parvovirus, minute virus of mice. Structure 1998;6(11):1369-81.

62. Lombardo E, Ramirez JC, Agbandje-McKenna M, Almendral JM. A beta-stranded motif drives capsid protein oligomers of the parvovirus minute virus of mice into the nucleus for viral assembly. J. Virol. 2000;74(8):3804-3814.

63. Lombardo E, Ramirez JC, Garcia J, Almendral JM. Complementary roles of multiple nuclear targeting signals in the capsid proteins of the parvovirus minute virus of mice during assembly and onset of infection. J Virol 2002;76(14):7049-59.

64. Vihinen-Ranta M, Wang D, Weichert WS, Parrish CR. The VP1N-terminal sequence of canine parvovirus affects nuclear transport of capsids and efficient cell infection. Journal of Virology 2002;76(4):1884-1891.

65. Greber UF, Willetts M, Webster P, Helenius A. Stepwise dismantling of adenovirus 2 during entry into cells. Cell 1993;75:477-486.

Figures

Fig. 1. Major steps of HIV-1 entry into post-mitotic cells.

Shortly after fusion of the viral envelope with the plasma membrane, the viral core containing a diploid viral RNA-genome disassembles in the cytosol. The RNA is reverse transcribed into complementary DNA in a reverse transcription complex (RTC) to yield the preintegration complex (PIC) consisting of proteins and DNA. The PIC is imported into the nucleus using as yet undefined mechanisms that may involve various nuclear localization signals (NLSs) of viral and cellular proteins, and perhaps the central polypurine tract (PPT). The role of Vpr is controversial. Vpr has been proposed to assist in disrupting the nuclear envelope although there is no evidence yet that PICs pass through such localised lesions. Vpr also directly interacts with FxFG repeats of nuclear pore complex (NPC) components similar to cellular import receptors.

Fig. 2. Direct docking and disassembly of Ad particles at the NPC.

After a stepwise uncoating process, a fiber-less Ad capsid is delivered to the cytosol and transported to the NPC where it directly docks to the FxFG containing filament protein Nup214/CAN. At this position, a series of disassembly factors are recruited, including the nuclear histone H1, the H1 import factors importin and importin 7 and also the heat shock protein Hsc70. These factors facilitate the spacially controlled capsid disassembly at the NPC and are required for import of the viral deoxyribonucleo-protein core.

Fig. 3. Importin β-mediated NPC docking of HSV-1 and cytosol-dependent DNA injection into the nucleus.

Herpes simplex virus type 1 fuses with the plasma membrane and releases the tegument proteins in a stepwise manner from the capsid which encloses a linear double-strand DNA genome of about 150 kbp. An essentially tegument-free capsid then docks via importin β to cytoplasmic NPC proteins. Upon recruitment of unknown cytosolic factors and energy, one particular vertex region of the icosahedral capsid is thought to open up and enable the ejection of the viral DNA into the nucleus.

Fig. 4. Importin α and β-dependent nuclear import of parvoviruses and HBV capsids.

Parvoviruses and HBV capsids are smaller than about 35 nm in diameter. In the case of MVM and human HBV, the cytosolic capsids undergo conformational changes that expose NLSs and enable capsid interactions with importins. This increases the affinity to the NPC and it is suggested that the viral capsids translocate through the NPC. The mechanisms of capsid disassembly in the nucleoplasm are unknown.