CHAPTER 11

Nuclear Import and Export of Mammalian Viruses

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Viruses are intracellular parasites that commandeer cellular processes, such as RNA processing or protein synthesis, to perform virus-specific functions. For this purpose, many viral proteins shuttle between the nuclear and cytoplasmic compartments, even when the viral genome is replicated in the cytoplasm. This shuttling process is usually regulated by classical nuclear import and export signals (NLSs and NESs, respectively), which are also found in many cellular proteins and are described elsewhere in this book. In this Chapter, we will focus on viruses that replicate in the nucleus, and in particular on the mechanisms by which they transport their genomes into and out of the nucleus.

While replication in the host cell's nucleus provides clear benefits for the virus, such as ready access to cellular transcription and splicing apparatus, it imposes the barrier of the nuclear envelope that viruses have to overcome. During the early stages of infection, they have to transport their genome from the site of penetration to the nucleus and then through the nuclear membrane, while at the late stage they need to export newly made genomes or assembled viral capsids out of the nucleus to the site of virus assembly. With some notable exceptions, viruses accomplish these tasks by mimicking nuclear import or export signals used by cellular proteins, which allows them to hijack the nuclear transport machinery evolved to transport cellular proteins or RNPs in and out of the nucleus.

Nuclear envelope is disassembled during mitosis, thus providing the viruses with an opportunity for unobstructed nuclear entry and exit. Some viruses, such as most retroviruses, critically depend on mitosis for nuclear entry.[61] However, because mitosis constitutes only a small part of the cell cycle, this dependency greatly diminishes the efficiency of viral infection. Therefore, many viruses with high replicative capacity (which often correlates with high pathogenicity), including HIV and other members of the *Lentivirus* genus of the *Retroviridae* family, evolved mechanisms that ensure efficient transport of their genome into the interphase nucleus.

Viral nuclear import in most cases occurs through the nuclear pore and relies on the cellular nuclear import machinery. Given the diffusional barrier imposed by the cytoplasm to the movement of macromolecules, the step of nuclear targeting is unlikely to proceed via free diffusion. Instead, viruses use some form of active transport by engaging actin or microtubulin cytoskeleton to deliver the viral nucleoprotein complex to the nuclear membrane.[10,70,72] The next step is actual translocation of the viral genome (together with viral proteins important for initiation of the replication cycle) through the nuclear pore complex. This step is relatively unified between virus families and relies in most instances on interaction with cellular soluble import factors. Because of restrictions imposed by the nuclear pore on the size of the passing molecules, complex (and poorly characterized) steps of uncoating and capsid rearrangement

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are required before entry of the viral nucleoprotein complex can occur through the nuclear pore. Different virus families display diverse uncoating programs involving interactions between viral and cellular molecules. Uncoating either precedes or coincides with migration of the viral nucleoprotein complex towards the nucleus.

Nuclear envelope presents a barrier also during exit of viruses from the nucleus. One simple solution of this problem is lysis of the nucleus along with the cell. This mechanism is used by nonenveloped viruses, such as adeno- or papovaviruses (e.g., SV40), which mature inside the nucleus. This strategy, however, cannot be employed by enveloped viruses, which need an intact cell membrane for assembly. Enveloped viruses with small genomes (retroviruses, hepadnaviruses, orthomyxoviruses) exit through the nuclear pore, while viruses with large genomes that assemble their capsid in the nucleus, such as herpesviruses, exit the nucleus via budding through the nuclear envelope.

Despite certain differences between the virus families in the mechanisms of nuclear entry and exit, there are many common features that reflect interaction of the viral nucleoprotein complex with the cellular nuclear transport machinery. In this Chapter, we will review sequential steps of viral nuclear import and export using human immunodeficiency virus type 1 (HIV-1) as an example. Intense research by the international scientific community into the fundamental processes of HIV-1 replication has yielded knowledge that in many aspects equals or exceeds that of many other viruses. Other viruses will be used to illustrate alternative mechanisms or events that are not well characterized for HIV-1.

Transport to the Nuclear Envelope

As a member of the *Retroviridae* family, HIV-1 copies its RNA genome into a double-stranded DNA molecule that is subsequently integrated into the host chromosomal DNA. This process is mainly carried out in the cytoplasm of infected cells in the context of the viral reverse transcription complex (RTC).[74] These complexes contain viral genomic RNA associated with several proteins, including reverse transcriptase (RT), integrase (IN), matrix protein (MA), and viral protein R (Vpr).[12,49] While the function of RT and IN is well defined, the role of two other protein components of the RTC (MA and Vpr) is less clear. One likely possibility is that they facilitate nuclear import of the PIC (see below). The final product of reverse transcription reaction (termed now pre-integration complex or PIC), a blunt-ended linear duplex DNA associated with several viral proteins, is then transported to and through the nuclear pore. A specific feature of reverse transcription of HIV (and other lentiviruses as well) appears to influence subsequent nuclear import steps. The synthesis of plus-strand cDNA of these viruses is discontinuous: in addition to normal initiation at the polypurine tract proximal to the U3 region of the LTR, it can be reinitiated at the additional polypurine tract in the middle of genome. As a result, unintegrated HIV-1 DNA has a central plus-strand overlap 99 nucleotides long. This central DNA flap was shown recently to be necessary for the nuclear import of the PIC.[83] While the mechanism by which the DNA flap promotes nuclear uptake of HIV DNA is unclear, it is unlikely to perform nuclear import of the HIV PIC on its own. Most likely, it synergizes with the nuclear import function of HIV proteins (see below) by providing the optimal conformation to the PIC necessary for its translocation through the nuclear pore.

Viral reverse transcription complexes were found to associate with the host cell cytoskeleton, and in particular, with actin filaments.[10] This association appears to be mediated by the HIV-1 matrix protein (MA), which is also a component of the PIC.[12,49] It is logical to surmise, therefore, that the PICs' movement towards the nucleus might be mediated by actin cables. Consistent with this hypothesis, disruption of actin microfilaments or inhibition of myosin-mediated movement along actin microfilaments significantly impaired viral infectivity.[10] If the role of actin microfilaments in nuclear targeting of the HIV-1 PIC is confirmed by future