Nucleocytoplasmic Transport of Preintegration Complex and Viral mRNA in Retrovirus-Infected Cells

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Active transport of proteins and mRNAs between the nucleus and cytoplasm is a major process in eukaryotic cells. Recently, factors that recognize transport substrates and mediate nuclear import or export have been characterized, revealing interactions that target substrates to the nuclear pore complexes, through which translocation occurs. In the first half of the report, nuclear import of the human immunodeficiency virus 1 (HIV-1) preintegration complex (PIC) is discussed. The second half describes recent information on the mechanistic details of nuclear export of viral mRNA so-called Rev-dependent *trans*-activation of viral genes.

Keywords : Retrovirus / Viral integration / Rev-dependent *trans*-activation / Nuclear import / Nuclear export / Nucleocytoplasmic transporter

The ability of HIV-1 to transport its preintegration complex (PIC) into the nucleus of an infected cell during interphase is a unique feature (1). A basic-type nuclear localization signal (NLS) was identified in the N-terminal region of the HIV-1 matrix protein (MA) and was found to regulate viral nuclear import and infection. Genetic analysis also implicated viral protein Vpr in the process of nuclear import of the HIV-1 PIC. But this protein does not have a classical NLS, and its role in nuclear import remained unclear until recently.

Recent studies identified that Vpr controls nuclear import through an importin α-independent mechanism (1). This result was consistent with resistance of Vpr-regulated viral nuclear import to competition by NLS peptides. The binding site of Vpr on importin α does not appear to overlap with the NLS-binding sites. In fact, importin α, Vpr, and MA can assemble into a trimer. As a result of Vpr binding to importin α, the affinity of interaction between the NLS and importin α was increased approximately 10-fold. This mechanism may be instrumental in increasing the karyophilic potential of the HIV-1 PIC.

It appears, therefore, that nuclear import of the HIV-1 PIC is controlled by two viral proteins, Vpr and MA (Fig. 1). While MA contributes its NLS which connect the PIC to importin α and cellular import machinery, Vpr functions as an enhancer of the MA-importin α interaction. In addition, Vpr was found to bind nucleoporins and was suggested on these grounds to function as an importin β analog. Although this hypothesis is consistent with the role of Vpr in docking of the HIV-1 PIC to the nuclear envelope, it is hard to imagine that a small protein, such as Vpr (with a molecular mass of only 14 kDa), can perform the function of a 97-kDa importin β. We therefore favor the notion that the effects of Vpr are mediated through the importin α/β pathway. Inactivation of either partner in the HIV-1 nuclear import (i.e., MA, Vpr, or importin α/β) results in the loss of

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Scope of research

This laboratory was founded in 1994 with the aim of linking (bio)chemical research and clinical medicine. Thus, the scope of our research encompasses the structure, function and regulation of various biomolecules, the pathophysiological significance of bioreactions in relation to human diseases, and the application of molecular techniques to clinical diagnosis and therapy. Our current interest is focused on poly(ADP-ribosyl)ation, nucleocytoplasmic transport of proteins in association with leukemogenesis and apoptosis, and the molecular etiology of Alzheimer's disease and related disorders.

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import function and virus inability to establish efficient infection in primary cells. These proteins, therefore, present an attractive target for development of novel anti-HIV therapeutics.

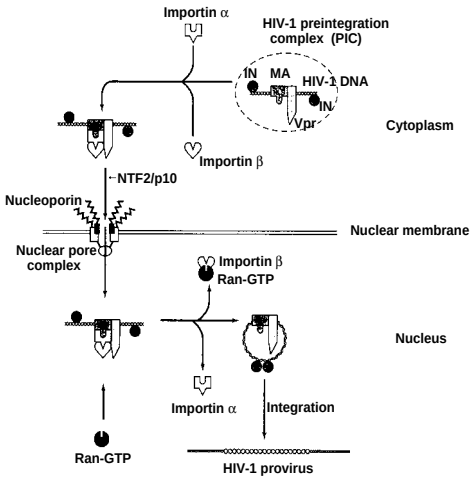

Figure 1. A model of nuclear import of the HIV-1 PIC.

HIV-1 encodes the regulatory protein Rev, which is absolutely required for viral replication (2,3). Rev promotes the nuclear export of incompletely spliced HIV-1 mRNA species containing the Rev response element (RRE). Rev interacts directly with a stem-loop within the RRE. Rev is a 116-amino acids phosphoprotein that has been shown to continuously shuttle between the nucleus and cytoplasm. Nuclear import of Rev is mediated by NLS embedded in the the RRE-binding domain. The NLS of Rev has been reported to associate with the conventional importin α/β heterodimer as well as a nucleolar shuttle protein B23. Nuclear export of Rev is mediated by the nuclear export signal (NES), which contains a conserved stretch of characteristically spaced leucine residues.

Recently, the highly conserved CRM1 protein was identified as a functional nuclear receptor for Rev NES and hence the protein was also renamed exportin 1 (4,5). Independent experiments showed that overexpression of CRM1 increased the export of nuclear-injected Rev protein. Moreover, the cytotoxin leptomycin B (LMB) inhibited *in vivo* export of both Rev protein and Rev-dependent RRE RNA export. This effect by LMB is direct because LMB binds to CRM1, and , in yeast, resistance to LMB maps to *crm 1* gene. Further support for CRM1 being an export receptor for NES-containing proteins is based on the observation that N-terminal region of CRM1 is homologous to the RanGTP-binding domain of importin β, placing CRM1 in the family of RanGTP-binding proteins that includes other known and putative import and export receptors. CRM1 has also been found in complex with at least two proteins associated with the human nuclear pore complex, namely the nucleoporins CAN/Nup214 and Nup88, and yeast two-hybrid analysis have demonstrated interactions between CRM1 and several nucleoporins as well as Rev and Ran (6).

The molecular details behind CRM1-mediated Rev export are still ambiguous. Our studies have been focused on the assembly of the RRE RNA-Rev-CRM1-RanGTP complex in greater detail *in vitro*. CRM1 interacts directly with full-length Rev independently of the conserved leucine residues within the core of the NES and the presence of RanGTP. This Ran-independent interaction is LMB insensitive. In contrast, formation of the ternary Rev-CRM1-RanGTP complex is highly sensitive to NES mutations and is readily dissociated *in vitro* by LMB or by hydrolysis of Ran GTP.

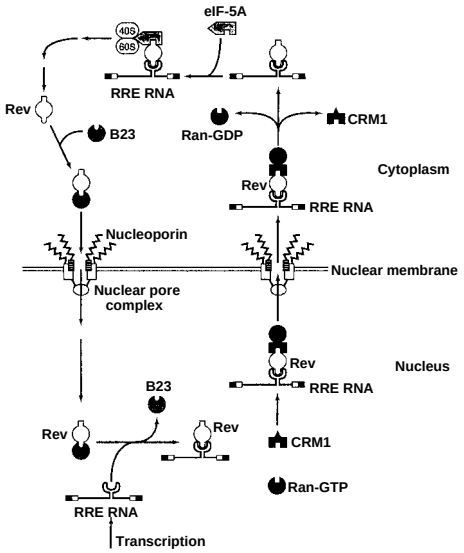

Figure 2. Mechanism of Rev-dependent nuclear export of viral mRNA.

As shown in Figure 2, Rev utilizes the cellular shuttle service to be imported into the nucleus as the Rev-B23 complex via the NLS/RRE-binding domain. As the RRE RNA has higher affinity for Rev than B23, the nuclear-imported complex is specifically dissociated by the RRE RNA. The RRE RNA-Rev complex binds to CRM1 via NES cooperatively with RanGTP and is subsequently translocated through the nuclear pore complex. In the cytoplasm, the RRE RNA-Rev-CRM1-RanGTP complex dissociates when Ran hydrolyzes GTP under the influence of RanBP or RanGAP.

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