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Minireview

Chromosomal tethering and proviral integration

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ABSTRACT

Since integration into the host cell genome is an obligatory step for their replication, retro-elements are both potent insertional mutagens and also suitable vectors for gene therapy. Many recent studies reported that the integration process is not random but, on the contrary, higly regulated at the molecular level. Many viral proteins and cellular factors play a key role in the integration step, explaining the reason why different retro-elements display distinct integration profiles. This review describes the recent highlights about integration of retro-elements with particular focus on the mechanisms underlying the specificity of integration target-site selection and the step of chromosomal tethering which preceeds insertion of the provirus.

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1. Introduction

Integration of the viral genome is a key step of retroviral infection because it ensures both expression of viral genes, and thus production of new progeny viruses, and also stable maintenance of viral genetic information in infected cells. Integration is performed by integrase (IN), a virus-encoded protein produced as part of the Gag–Pol polypeptide precursor, from which it is released following viral protease (PR)-mediated cleavage. Integration is not a random process. Each retrovirus genera displays a distinct and specific pattern of integration, which is regulated by viral and cellular factors as well as by local DNA conformation at the site of integration.

The unique property of retroviruses to integrate their genome constitutes a major advantage for retrovirus-based gene therapy, which aims at long-term correction of genetic defects. This therapeutic approach has been successfully employed to treat children suffering from X-linked severe combined immunodeficiency syndrome. Remission was obtained after re-grafting patients with their own hematopoietic stems cells transduced *ex vivo* with a murine leukemia virus (MLV)-derived vector carrying the γ-chain cytokine receptor gene [23]. However, because proviral integration near essential cellular genes leads to uncontrolled cell proliferation, leukemia-like disorders arose in some cases [62,63] representing the first description of insertional mutagenesis following a clinical trial [24]. This adverse effect raised not only issues about the safety of these viral vectors, which were already known to cause cancers in their natural hosts, but also brought an old question up to date: what dictates the integration specificity of a retro-element?

Since both viral and cellular determinants critically modulated proviral integration, understanding the precise interactions occurring between cellular and viral partners could certainly lead to the elaboration of safer therapeutic vectors, specifically targeting the corrective gene to chosen regions of the host genome, allowing an adequate level of transgene expression without altering cellular gene transcription. Moreover, this will help us to better define the mechanisms allowing long-term persistence of retro-elements into a cellular genome without causing major defects to its host.

2. Early steps of retroviral replication

2.1. Viral entry and reverse transcription

Retroviral entry into the target cell is a complex multi-step process which involves specific interactions between the viral envelope (*Env*) glycoprotein and cellular receptor/co-receptor couples, leading to the fusion between viral and cellular membranes (for a review see [106]). Next, the viral core is released into the cytoplasm and the viral reverse

Abbreviations: HIV-1, Human Immunodeficiency Virus type-1; PFV, Primate Foamy Virus; IN, Integrase; NLS, Nuclear Localization Sequence; RT, Reverse Transcriptase; LTR, Long Terminal Repeat; RTC, Reverse Transcription Complex; PIC, Pre-integration Complex; LEDGF, Lens Epithelial-Derived Growth Factor

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transcriptase (RT), contained within the core, converts the viral RNA genome into a double-stranded linear cDNA form. This reaction usually occurs upon entry into the host cell within a nucleoprotein complex termed the reverse transcription complex (RTC). However, there is evidence for limited cDNA synthesis within viral particles before infection [91,137,159]. Foamy retroviruses (FVs) constitute an exception since their reverse transcription is mainly a late event, taking place after integration. Consistently, the form of FVs genome responsible for a productive infection is the viral particle-associated cDNA [153], although FVs RNA genome seems to play also an important role during the early events of viral replication [45].

Reverse transcription and viral core disassembly are concomitant, tightly coupled processes [155]. The molecular mechanism underlying the RNA-to-DNA conversion is today well defined, while the kinetics of uncoating is still a matter of debate. Earlier studies suggested that incoming viral particles undergo uncoating soon after entry [50]. However, more recent results indicate that it might occur later near the centrosome [79] or the nuclear envelope [2], likely depending on the virus and/or the cell metabolism. In the case of FVs both viral and cellular proteases participate in this process [120].

While reverse transcription proceeds, the RTC undergoes a progressive remodeling to become a pre-integration complex (PIC), which is defined as the viral nucleoprotein complex competent for proviral integration. HIV-1 PICs consist of viral components, such as nucleocapsid (NC), matrix (MA), RT, IN and Vpr protein, as well as several cellular proteins, which are associated with the viral genome [15,50,101].

2.2. Trafficking and nuclear import

The cell cytoplasm is a highly viscous environment, containing organelles and cytoskeletal elements embedded in an extremely dense protein matrix, making passive diffusion of viral particles quite impossible. Viruses have therefore evolved many strategies to hijack cellular machineries to cross the cytoplasm and make their way to and from the nucleus [104,114]. Indeed, several retroviral proteins interact with elements of the cytoskeleton and/or components of the molecular motor complexes. For example, incoming FVs Gag engages the light chain 8 of the minus-end directed microtubule (MT) motor dynein, allowing its accumulation at the centrosome prior to nuclear translocation [111]. Similarly, incoming HIV-1 particles traffic along the MT network from the cell periphery toward the centrosome [99]. For shorter range movements, HIV-1 NC protein has been shown to engage actin [68,88].

The last obstacle to the penetration of the viral genome into the nucleus is the nuclear membrane. PICs from numerous retroviruses are unable to cross this physical barrier when intact. For example, γ-retroviruses such as MLV cannot replicate in interphasic cells and require nuclear envelope breakdown during mitosis to gain access to host cell DNA. However, it has been recently suggested that active nuclear import of MLV PICs may occur in differentiated cells [59]. Lentiviruses, such as HIV-1, can productively infect non-dividing cells, since their PICs can be actively imported into the nucleus [14]. Other retroviruses, such as Rous Sarcoma virus (RSV) and FVs, display intermediate dependence on the cell cycle status of the target cell [65,110].

The determinants that allow HIV-1 replication in non-dividing cells have been mapped to the viral IN, MA and Vpr proteins as well as to a cis-acting structure of the viral DNA (named FLAP). These elements have been proposed to contribute to the active nuclear import of HIV-1 PICs, either directly or indirectly. However, the exact role of each of these factors is controversial since recombinant HIV-1 particles lacking one or several of these elements retain significant ability to infect non-dividing cells [38,127]. The HIV-1 IN protein is a particularly attractive candidate to mediate nuclear import of the viral genome. Nuclear translocation of HIV-1 IN is required for productive infection of both non-dividing and dividing cells [9]. Moreover, IN harbors a non-classical Nuclear Localization Sequence (NLS) which has been shown to be both necessary and sufficient to promote nuclear import of PICs [9,54]. However, mutation of its putative NLS does not fully abolish IN nuclear entry [48,86]. More recently, three novels IN partners, the nuclear transport receptor transportin-SR2 (TRN-SR2), importin 7 and the nuclear pore component NUP153, have been proposed to be important players controlling nuclear access of HIV-1 PICs [33,143,154].

Another line of evidence points out that uncoating might be the rate-limiting step that determines nuclear import of the viral genome [82]. In support of this hypothesis, analysis of MLV–HIV-1 chimeras revealed that CA is the essential viral factor that dictates the ability of a retrovirus to replicate in interphasic cells [147].

2.3. Retroviral integration

After entry into the nucleus, the viral genome has to be integrated into the host cell genome to ensure expression of viral information. However, integration is a rare event. Estimations indicate that most viral particles are non infectious, likely due to the fact that a majority of viral genomes are defective [72,113]. Nevertheless, a recent report showed that 1 of every 8 viral particles beginning reverse transcription leads to the formation of a provirus [134]. Cellular restriction factors also contribute to limit the efficiency of viral replication [132]. Consequently, the integrated viral genome represents only 5 to 10% of the total viral DNA content of an infected cell [13]. Both extrachromosomal linear molecules and circular molecules, containing 1 or 2 Long Terminal Repeats (LTR), represent the most abundant forms of the viral DNA genome. The full-length linear cDNA, which is considered the direct precursor of the provirus, decays rapidly if not integrated (estimated half life of 1–2 days) [158], while 1- and 2-LTR circles accumulate in the nucleus at later times of infection. These circular forms, which are considered dead-end products of aborted integration events [12], can also lead to viral gene expression [57,70]. Contribution of unintegrated forms of viral genome to replication is supported also by a recent work demonstrating release of new progeny virions from HIV-1-infected quiescent lymphocytes reactivated in the presence of integrase inhibitors [112].

3. The retroviral integrase

3.1. Integrase structure and functions

Retroviral IN proteins share a similar structural arrangement, being composed of three independent domains (see for a review, [41]). The N-terminal region carries an HHCC motif analogous to a zinc finger, most likely involved in protein multimerization, a key event for IN functions [77,156]. The central region, known as the catalytic core domain, harbours the catalytic active site, encompassing the D,D-35,E motif, and is highly conserved between viral IN and transposases. Consistently, inhibitors targeting Mos-1 transposase are also efficient against HIV-1 IN [8]. All integrase functions strictly require the presence of a metallic cationic co-factor which is coordinated by two residues of the catalytic triad (D64 and D116 for HIV-1 IN) [58,92]. The C-terminal domain displays a non-specific DNA binding activity and is therefore mainly involved in the stability of the complex with nucleic acids.

IN exhibits four enzymatic activities (for a review [41]). It catalyzes the 3′-processing and strand transfer reactions leading to concerted integration of the viral DNA into the host cell genome. The 3′-processing reaction, which corresponds to a nucleophilic attack by a water molecule, involves the removal of two nucleotides, adjacent to the highly conserved CA dinucleotide, from the 3′ strand of both U3 and U5 viral LTR termini. Mutations within these sequences completely abolish IN-mediated cleavage, whereas the integrity of

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