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Roles of DNA Damage and its Cellular Response in the Transduction of Human Immunodeficiency Virus-1

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Abstract

Infection by Human Immunodeficiency Virus-1 (HIV-1) is modulated by the interaction of host cell, viral proteins, and environmental stimuli. Critically involved cellular factors are apolipoprotein B mRNA-editing enzyme-catalytic, polypeptide-like 3 protein family (APOBEC3), tetherin, and SAM and HD domain-containing protein 1 (SAMHD1). The functions of these restriction factors are overcome by viral mediators that include Viral Infectivity Factor (VIF) for APOBEC3, viral protein U (vpU) for tetherin, and Vpx (in HIV-2) for SAMHD1. In addition, DNA damage, the most common environmental stimulus, influences the multiple steps of the viral infection. Notably, DNA repair proteins play critical roles in the integration steps of viral DNA. The activated DNA damage response by viral infection leads to apoptotic cell death and prevents the viral propagation, suggesting that it is an intrinsic viral restriction mechanism. In contrast, the DNA damage responses can increases the frequency of viral infection when the integrase activity is defective or inhibited by integrase inhibitors, implying that DNA damage and its cellular response function like dual sides of the same coin. In this review, we first summarize our current understanding of the restriction factors, and then focus on the roles of HIV-1-associated DNA damage response on the transduction of viral DNA. In particular, the functional association of viral integration with the DNA repair pathways will be discussed.

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Overview of HIV-1 infection

After the entry of a virus into target cells, the viral RNA is reverse-transcribed in the cytoplasm to a linear double-stranded DNA [1,2]. The newly synthesized viral genomic DNA is wrapped with some viral proteins to form a Pre-Integration Complex (PIC), translocated to the nucleus and, subsequently, integrated into the host chromosome.

The integration of the viral DNA into the host chromosome is catalyzed by Integrase (IN), being an essential step in retroviral replication cycles. The proviral DNA serves as a template for the transcription of viral genes and viral genomic RNA, and is propagated by host cell division. It has been proposed that viral DNA integration preferentially occurs in the active transcription sites, so that the efficient transcription of viral RNA would be guaranteed [3].

Restriction factors and viral counteracting mechanis

The infection by HIV-1 is suppressed at several steps by cellular restriction factors that mainly inhibit the viral propagation through the inhibition of the viral genomic DNA synthesis and the release of progeny viral particles from the infected cells [4,5] (Table 1). The former type of factors comprises APOBEC3 and SAMHD1. APOBEC3 catalyzes the C-to-U deamination of the minus strand DNA during the reverse-transcription step and, contributing to the hypermutation from G to A in the viral genome [6]. In the presence of APOBEC3, the precise replication of the viral DNA is inhibited and a non-functional copy of cDNA is produced. Additionally, APOBEC3G inhibits the viral transduction at dual steps of the reverse transcription and integration in a manner independent of enzymatic activity [7-9]. To overcome the suppressive activity of APOBEC3, HIV-1 Vif ubiquitinates and degrades APOBEC3 through the 26S proteasome pathway. For the degradation of APOBEC3, Vif associates with the E3 ligase complex consisting of multiple components (i.e., cullin 5/RING box protein 2-elongin C/elongin B/core-binding factor subunit beta, Cul5/RBX2 -ELOC/ELOB/CBFβ). While APOBEC3 functions as a restriction factor, its activity would render the force to drive the genetic diversity, which enables the virus to escape from the host immune system [10]. Of note, APOBEC3B is considered as an endogenous mutagen in breast cancer development [11, 12].

SAMHD1, most recently identified as an HIV restriction factor, is expressed in myeloid lineage cells and has a deoxynucleoside triphosphohydrolase activity [13,14]. It is thought to inhibit the reverse-transcription by depleting the pool of intracellular deoxynucleotide triphosphates. SAMHD1 is counteracted by HIV-2 Vpx by ubiquitin-meditated degradation, but not by Vpr, an accessory gene product of HIV-1 that is a supposed functional counterpart of HIV-1 to Vpx. To degrade SAMHD1, Vpx utilizes the cullin 4-RING ubiquitin ligase CRL4 complex, which consists of damage-specific DNA binding protein 1 (DDB1), RBX1, Cul4A, and DCAF1. In addition to its phosphohydrolase activity, the exonuclease and nucleic acid binding activities are also considered to function during the viral restriction [15,16], the finding of which was further supported by recent two reports [17,18]. In these experiments, it was shown that phosphorylation of SAMHD1 at Thr592 by cyclinA2/CDK1 reduced restriction activity of SAMHD1. However, such phosphorylation of SAMHD1 did not affect its phosphohydrolase activity.

Tetherin (also called bone marrow stromal cell antigen 2, BST-2), the third restriction factor, inhibits the viral release, by tethering the virion on the cell surface [19]. The tethered virions are susceptible to endocytosis followed by degradation. The association of tetherin with viral particle triggers the nuclear factor kappa B (NFκB)-mediated proinflammatory response [20]. Additionally, tetherin induces the endocytotic degradation of viral particles that activates the

pattern recognition receptor pathway and the following type I interferon response [20]. It is noteworthy that a similar cellular response is induced by tripartite motif 5α (TRIM5α), a restriction factor against simian immunodeficiency virus [21]. Tetherin is counteracted by Vpu through both sequestration and degradation mechanisms via direct association with the transmembrane domains of each molecule [21].

Recently, it has been implicated that Dicer and miRNA pathways are also involved in viral restriction [22]. These factors could play multiple roles linked to the degradation of viral RNA, inhibition of transcription and translation of viral genes, and regulation of cellular genes involved in viral infection. Interestingly, Dicer-mediated restriction pathway is overcome by Vpr/CRL4 complex-mediated proteasomal degradation, implying the functional conservation of the Dicer pathway as an antiviral cellular response mechanism [23].

(Table 1) Part of reported interaction of viral-host cellular factors

Cellular protein	Viral protein	Cellular protein activity	Affected viral replication process	Effect on Viral replication
Restriction factors				
APOBEC3	Vif	Deaminase	Reverse transcription	Hypermutation
SAMHD1	Vpx (HIV-2)	Phosphohydorase	Reverse transcription	Nucleotide pool depletion
UNG2/ SMUG1	Vpr	Glycosylase	Reverse transcription	Regulation of mutation
Tetherin (BST-2)	Vpu	Trans-membrane protein	Viral release	Viral particle tethering
VprBP (DCAF1)*1	Vpr	E3 ligase adaptor	transcriptional regulation (?)	N/A*
Dicer	Vpr	Ribonucleaes	N/A*	RNA processing
Integrase interactor				
LEEGF (p75)	Integrase	Chromatin binding	Integration	Upregulation
Ku70	Integrase	DSB repair (NHEJ)	・Integration ・circularization	Upreguation
SNF5 (INI1)	Integrase	Chromatin remodeling	Integration	Suppression (increase fidelity)
Rad18	Integrase	・Translesion syntheis ・DSB repair	Integration	N/A*
Gemin2*2	・Integrase ・Reverse-transcriptase	DSB repair (HR)	Reverse transcription	Upregulation

*: N/A: not accessed.

*1: Romani and Cohen (2012)

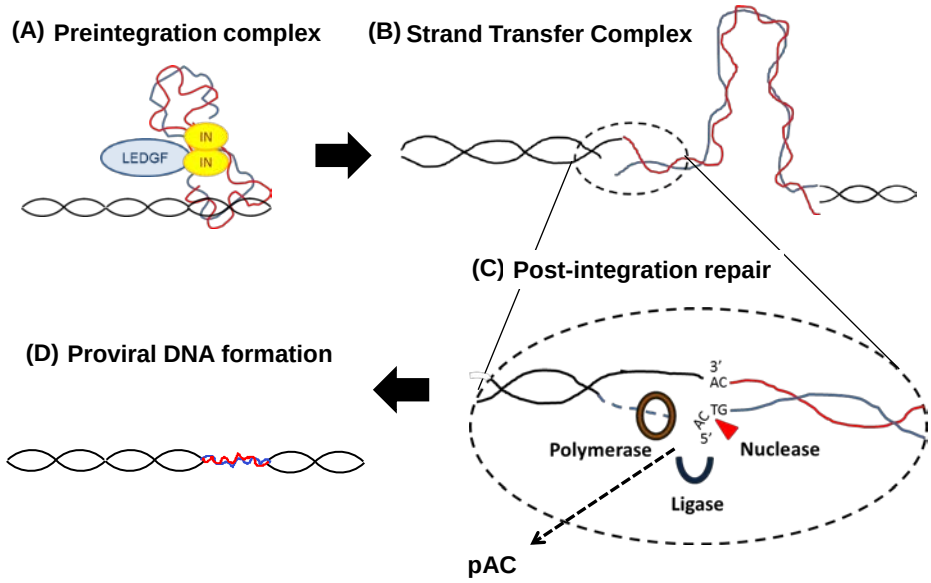
*2: Hamamoto et al. (2006), Takaku et al. (2011)

Molecular mechanism of HIV-1 integration and post-integration repair

After reverse-transcription, a linear double-stranded DNA is formed with a copy of Long Terminal Repeats (LTR) flanked at each end [1,24] (Figure 1). IN engages the LTR ends (U3-end for upstream LTR, U5-end for downstream LTR) and forms a stable synaptic complex. IN site-specifically cleaves conserved GT dinucleotides from each LTR 3′-end, generating CA-hydroxyl 3′-DNA ends, stage at which the complex is called Pre-Integration Complex (PIC). After the nuclear translocation of PIC mediated by Lens Epithelium-Derived Growth Factor (LEDGF) (p75), the target chromosomal DNA is cleaved in a 5-bp staggered fashion [25]. Simultaneously, the 3′-end of viral DNA is covalently joined to the 5′-end of the target DNA at the site of integration, while the 5′ end of viral DNA is unjoined. This intermediate is termed as the “strand transfer complex”. The repair of the unjoined 5′-end of the viral DNA (i.e., Postintegration Repair, PIR) is mediated by cellular enzymes that remove two unpaired bases at the 5′-end and fill the single-strand gaps and ligate viral DNA and target DNA [26]. Finally, the integration of the viral DNA is completed by forming the proviral DNA that is flanked by duplicated sequences derived from the cut of the staggered DNA. The PIR process depends on several classes of cellular enzymes that include: (1) polymerases (beta, delta, and their cofactor, proliferating cell nuclear antigen - PCNA; (2) nuclease (flap endonuclease-1) and (3) DNA ligases (ligase I, III, or IV and its cofactor X-ray repair cross-complementing protein 4 - XRCC4 [26,27]. However, the actual contributions of these factors in each PIR step are not completely understood. Given that the proteins required for the DNA Double-Strands Break (DSB) repair are involved at multiple steps, including 3′-end processing and viral DNA-end protection, the host-viral DNA junctions are susceptible to frequent insertions or partial deletions if HIV-1 infects cell lines that are deficient of the DSB repair pathway [28]. During these steps, major two DSB repair pathways seem to play their function in a competitive manner: Non-Homologous End Joining (NHEJ) pathway and homologous recombination pathway, regulating positively or negatively the viral integration, respectively (Table 2)

Figure 1. Schematic view of viral DNA integration. (A) PIC is directed to chromatin by the function of LEDGF (p75). (B). Mode of strand transfer. Target chromosomal DNA is cleaved in a 5-bp staggered fashion. Then, the 3′-end of viral DNA (3′-HO-AC) is covalently joined to the 5′-end of the target DNA, while the 5′ end of viral DNA is left unjoined. This intermediate is termed as the strand transfer complex. (C). The repair of the unjoined 5′-end of the viral DNA (postintegration repair) is mediated by cellular enzymes that remove two unpaired bases (pAC) at the 5′-end (Nuclease), fill the single-strand gaps (Polymerase) and join viral DNA and target DNA (Ligase). (D). The integration of the viral DNA is completed and proviral DNA is formed.

Figure 1



Interestingly, retroviral infection elicits the DNA damage response via intermediates of viral DNA at multiple steps of the viral integration [29,30]. As a well-characterized intermediate that evokes the DNA damage response, unintegrated cDNA is recognized as broken-ends and activates the cellular pathway of NHEJ [31,32]. End-free circular fragments such as 1-LTR and 2-LTR are then formed in the nucleus, otherwise massive cell death is induced due to cytotoxicity [33]. Consistently, viral infection under NHEJ deficient conditions results in abortive infection. Furthermore, DNA strand breaks on host cell chromatin is inevitably induced during retroviral integration, leading to the activation of DNA damage response that include Ataxia Telangiectasia-Mutated protein (ATM), ATM Rad3-related kinase (ATR), DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and H2A histone family, member X (H2AX) phosphorylation. Of note, such DNA damage response depends on IN Catalytic Activity (IN-CA) [29,34]. It is plausible that these cellular responses could work as a cellular shield for viral transduction and prevent further propagation of infecting virus by inducing cytostatic and/or cytotoxic signaling. Thus, DNA damage response could represents a viral restriction mechanism and some studies showed that Vpr activates different DNA damage responses (ATM and ATR pathways, H2AX and replication protein A (RPA) phosphorylation, G2/M cell-cycle arrest and apoptotic cell death [35-37]. These observations imply that DNA damage responses enhance viral transduction. Therefore, the DNA damage response functions like dual sides of coin; besides what has been discussed above, it may also increase the efficiency of the viral infection in some circumstances, mentioned in the following section (Table 2).

(Table 2) Functional interaction of HIV-1 integration and DNA damage response

DNA damage response factor	Reference
Early damage sensor/transducer	
ATM	Ariumi 2005 (N), Lau 2005(+), Sakurai 2008 (+), Smith 2008(+)
ATR	Ariumi 2005 (N) Yang 2010 (+)
PARP-1	Baekelandt 2000 (N)*, Ha 2001 (+), Siva 2002 (N), Kameoka 2004 (+), Ariumi 2005 (N), Bueno 2013(N)
Nbs1	Smith 2008 (+), Sakurai 2009 (N)
Mre11	Sakurai 2009 (N)
NHEJ	
DNA-PKcs	Daniel 1999 (+), Daniel 2004 (+), Ariumi 2005 (N), Cooper 2013 (-)
Ku70	Baekelandt 2000 (N)*, Zheng 2011 (+)
Ku80	Jeanson 2002 (+)
Artemis	Sakurai 2009 (N/A)**
XRCC4	Daniel 2004 (+)
Lig4	Daniel 2004 (+)
Homologous recombination	
Rad51	Cosnefroy 2011 (-)
Rad52	Lau 2005 (-)
XRCC2	Lau 2005 (N)
XRCC3	Lau 2005 (N)
BRCA2	Lau 2005 (N)
Chromatin remodelling	
SNF5	Maroun 2006 (-)

*: lentivirus infection

**:N/A: not accessed.

(+): Positive effect on viral infection was observed. (promotional)

(-): Negative effect on viral infection was observed. (suppressive)

(N): No effect on viral infection was observed. (dispensable)

DNA damages are recognized by several sensor proteins and activate many types of gene products that function as downstream mediators or effectors. In a network of DNA damage responses, ATM and ATR are key players [38]. ATM activation is induced by DSB, replication stress, and structural changes of chromatin, whereas ATR can be activated by these ATM activators and single-strand break as well. Of note, ATR activation is achieved by loading RPA on the single strand region, which is a common process exerted during the repair of any kind of DNA damage. Additionally, in response to DSB, ATM is rapidly activated and phosphorylates target proteins, including the transcription factors, DNA repair, cell cycle checkpoint and apoptosis induction (e.g., p53, H2AX, Nbs1, Smc1 and Chk2).

Notably, [39] clearly demonstrated the requirement of ATM in HIV-1 infection by utilizing the ATM-deficient cell line and the novel ATM-specific inhibitor KU55933, which is a HIV-suppressive chemical targeting the cellular DNA repair pathway [39]. They showed that the decreased HIV infectivity in the ATM-deficient condition, without any negative effect on the HIV-1 catalyzing reactions (i.e., reverse-transcription, viral nuclear entry, and HIV cDNA integration into the host genomic DNA), being caused by the integrated HIV-1 DNA loss correlated with cell death. On the basis of these observations, it was proposed that ATM is required for efficient PIR, otherwise the host cells enter apoptosis. In addition to ATM, other cellular DNA damage response factors are evoked in HIV infection; ATR kinase could be also involved in HIV and retroviral infection [40]. However, controversial results were reported by different groups [41,42]. These conflicts may be derived from the different cell types, conditions, and methods for inhibiting ATR activity used in different studies. Principally, DSB-induced ATR activation depends on CTBP-interacting protein (CtIP), a human ortholog of yeast Sae2 that mediates the processing of DSB ends in the 5′ to 3′ direction [43]. Because CtIP mediated DSB ends processing that depend on the activity of Cyclin-Dependent Kinase (CDK) at the S/G2 phase, the distribution of the cell cycle is a critical determinant of the CtIP-mediated ATR activation. Yang et al. [40] reported that ATR, similarly to ATM, is required for efficient HIV transduction by supporting the cell survival after viral infection [40]. In such cellular response, a Phosphatidylinositol 3-Kinase (PI3K)-related kinase, a DNA-dependent protein kinase (DNA-PKcs) also plays an essential role in the early step of HIV infection [44,33]. DNA-PKcs associates with Ku70/Ku80 to form a DNA-PK complex that participates in the NHEJ pathway of the DSB repair [45]. NHEJ is a predominant cellular DSB repair pathway required for the efficient viral DNA integration and circularization of unjoined viral DNA. Thus, in the absence of DNA-PK activity, intermediates of both forms of viral DNA with free DNA ends should activate the cell death signaling, leading to the failure of the viral infection. Although these critical roles of DNA-PKcs in the viral infection have been reported, an apparently conflicting result was presented by Cooper et al. [34], who showed that DNA-PKcs recognizes the DNA damage induced by integration, not by unjoined cDNA, causing the killing of CD4(+) T-cells [34]. According to their observations, the activation of DNA-PKcs possibly leads to the depletion of CD4(+) T-cells during HIV infection. This conflicting dependency of viral infection on DNA-PKcs could be attributable to the features of the cell lines used in the aforementioned studies: lymphocytes are generally susceptible to DSB-induced cell death, whereas macrophages are

resistant to DSB-induced cell death. In future, it is important to re-evaluate the roles of ATM/ATR in the viral infection to primary CD4(+) T-cells and macrophages, helping us to understand whether inhibitors of ATM/ATR can give favorable clinical outcomes in HIV-1 patients.

Poly [ADP-Ribose] Polymerase 1 (PARP-1), an early response factor involved in DNA damage response, catalyzes the polymerization of ADP-ribose on target proteins and stimulates the recruitment of repair and signaling factors [46]. Although the targets of PARP-1 are proteins involved in DNA single-strand break and double-strands break repair, its involvement in HIV infection remains controversial [47-50,41]. One possible explanation of such an ambiguous requirement of PARP-1 is due to the presence of functionally redundant PARP enzymes that are differentially expressed in mammalian cells. However, in the experiment conducted by using DT40 cells, a chicken B lymphoblastoid cell line, the suppressive role of PARP-1 in viral infection was demonstrated. In this cell line, PARP-1 is the major PARP expressed. In wild-type cells, the viral gene expression was significantly suppressed compared to the PARP-1 knockout cells, whereas the reverse-transcription, viral nuclear entry, and viral DNA integration were not impaired. These observations suggest that PARP-1 is dispensable in the viral integration steps, but could also contribute to latent viral infection through epigenetic mechanism, such as CpG methylation.

Recently, novel ubiquitin-enzyme family members, ubiquitin-like molecules, and ubiquitin ligases/proteases with a different role compared to the classical function in proteasome-mediated target degradation, have been identified [51]. The non-classical ubiquitination pathway also modulates the activity of molecules involved in transcription, signal transduction and DSB repair, through regulating their localization, stability and interaction with other factors. In the field of HIV-1 research, most of the studies related to ubiquitination have been focused on the degradation of target proteins [4,5]. However, it is possible that both classical and non-classical ubiquitination pathways mediated by viral proteins enhance the viral transduction by tuning the activation of the DNA damage response.

There are several reports suggesting that DNA damage-induced signaling pathways exert positive effects on viral transduction. It was shown that DNA damaging agents upregulate the viral infectivity in cycling cells [52]. It was suggested that DNA damage-induced G2/M arrest is related to the upregulation of the infectivity. As a plausible explanation, the cell cycle arrest at G2/M would provide time for completing the integration of the viral DNA. Additionally, it was shown that retroviral promoters are upregulated at the G2 phase. In contrast, Smith and Daniel [53] showed the upregulation of the viral infectivity by DNA damaging agents using cell cycle-arrested or differentiated cells [53]. Their data clearly indicated that there would be another mechanism(s) for the upregulation of the viral infection by DNA damaging agents. On the basis, the finding that viral DNA levels were not changed after the induction of DNA damage, they proposed that a DNA damaging agent promoted the PIR step by activating the DNA repair pathway and/or upregulating the expression of host DNA repair genes [54]. Importantly, such enhancement of viral infectivity was not observed in ATM-deficient cells, suggesting that the ATM-mediated upregulation of the DNA repair pathway could contribute to PIR.

Mode of viral transduction independent of IN-CA

Recently, Ebina et al. [54] and Koyama et al. [55] showed that the DNA damage induced the increase of viral transduction of the IN-CA-defective virus [54,55]. Furthermore, Koyoma et al. [55] clarified the mode by using homing-endonucleases (I-SceI and I-PpoI) (see also [http://biomedfrontiers.org/infection-20139-33/]). The data indicated that the viral DNA was inserted into artificially induced DSB sites, implying that the DNA damage provides a platform where the viral DNA is inserted. The relative frequencies of these events were significantly increased when serum-starved cells were infected with the IN-CA–defective virus. Further analysis on the junction of host and viral DNA revealed the presence of pAC dinucleotides at the 5′-end of the viral DNA, suggesting that it integrated in a manner independent of IN-CA. Moreover, frequent deletions at the junction of host and viral DNA also support the hypothesis that the NHEJ pathway is involved in the IN-CA–independent viral transduction. The reason why the G0/G1 arrest by serum starvation increased the relative rate of the DSB site integration would be explained by the process in which viral DNA is captured in the DSB sites that are spontaneously generated during DNA replication. In addition to the cellular condition, the viral status also affects the frequency of the IN-CA–independent integration event. Under the IN-CA-defective condition, the presence of Vpr markedly increased the infectivity compared to the Vpr-defective virus. According to the reported function of Vpr associated with DSB, Vpr could play pivotal roles in the IN-CA-independent viral infection by provoking the DSB signaling and/or generating DSB itself.

Of note, the IN-CA-independent viral integration occurred even in the presence of IN inhibitors when viral infection was coupled with DNA damaging agents. This implies that the viral DNA integrates into spontaneously occurred DSB sites without the necessity of IN-CA. Additionally, it was shown that the inappropriate application of IN inhibitors could arouse aberrant integration and genomic rearrangement [56]. Finally, DSB site directed integration of exogenous DNA is observed in other kinds of viruses (hepatitis B virus and adeno-associated virus) and yeast Ty1 transposable element [57,58]. These observations suggest that the viral DNA integration into the DSB site depends on essential and common cellular activities, which would be involved in an uncharacterized mode of DNA damage repair. Notably, such cellular functions of capturing exogenous nucleic acids into the genome can possibly generate novel genes, but unfortunately result in the induction of genomic instability.

Candidate molecules involved in the IN-CA-independent viral transduction

Although the mode of the IN-CA-independent viral integration at the DSB sites is largely unknown, at least two modes of integration are expected. One is the active recruitment of PIC to the DSB flanking region by cellular factors. Another one is the accidental insertion of the viral DNA that is pre-loaded to chromatin in the region close to DSB. These modes of integration require common factors, but the former one needs additional recruitment factors. Here, we will introduce the candidate molecules, the function of which is associated with IN (Table 1, and Figure 2).

Figure 2

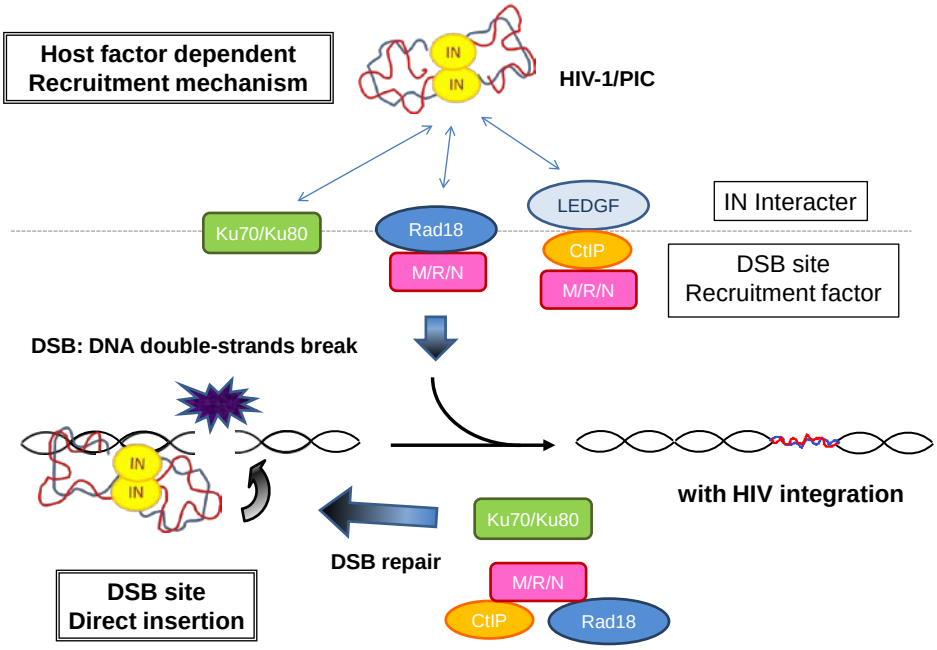


Figure 2 Hypothetical modes of IN-CA-independent integration. Upper panel, host factor dependent recruitment mechanism. PIC is actively recruited to the DSB flanking chromatin by the DSB site recruitment factors that are associated with IN. Then, viral DNA is inserted to DSB site using cellular DSB repair factors, some of which could be already associated with DSB site-recruited PIC. Lower panel, direct insertion the viral DNA into the DSB site. As an alternative, viral DNA that is tethered in the region close to DSB damage might be accidentally inserted to DSB site, which would be navigated also by the cellular DSB repair process.

LEDGF. LEDGF (p75) is a cellular factor that recruits IN onto euchromatin regions displaying active transcription [59]. LEDGF directly binds IN through an integrase-binding domain located in the C-terminus [60]. In addition, LEDGF also directly binds CtIP in a DNA damage-dependent manner [61]. As mentioned above, CtIP is involved in the processing of the DSB end, implying that LEDGF navigates IN and PIC onto the DSB site via CtIP. Additionally, CtIP might be involved in PIR, particularly in the removal of 5′-unpaired strand by DSB end processing.

Rad18. Rad18 is an E3 ubiquitin ligase that regulates translesion polymerases and is also involved in the DSB repair pathway through its ligase activity [62,63]. Furthermore, Rad18 interacts with Nbs1, a component of the meiotic recombination 11 (Mre11)/Rad50/Nijmegen breakage syndrome 1 (Nbs1) complex, which is involved in several DSB repair pathways through its versatile functions [64,45]. Interestingly, Rad18 associates with and stabilizes IN, further suggesting that Rad18 plays a positive role in the IN-CA–independent viral transduction, by recruiting IN and PIC to the DSB sites [65].

SNF5. Sucrose Nonfermentable 5 (SNF5) is a component of the SWItch (SWI)/SNF complex that is involved in chromatin remodeling [66]. SNF5 forms a ternary complex with IN and LEDGF, contributes to the stabilization of PIC, and enhances the fidelity of the integration reaction [67]. Furthermore, SNF5 in the SWI/SNF complex was thought to be required for viral DNA integration into chromatinized target DNA [66]. In contrast, however, SNF5 exerts a suppressive effect on the whole integration frequency [68]. Future studies are required for clarifying actual roles of SNF5 during viral transduction.

Ku70. Ku70 generates a heterodimer with Ku80 that rapidly accumulates at the DSB sites [45]. These Ku70/Ku80 complexes are essential for DSB repair protein since their association with DNA-PKcs is an early step of the NHEJ pathway. The Ku70 association with IN increases the IN stability, avoiding its degradation by ubiquitin [69]. The possible association of Ku80 with IN suggests the possibility that Ku70/Ku80 recruits PIC to the DSB sites and promotes the IN-CA-independent integration.

In summary, the IN-CA-independent viral integration would require following sets of molecular events and related factors: (1) DSB in the absence of strand transfer activity of IN, the integration of viral DNA completely depends on the acceptor site that captures and incorporates the viral DNA after NHEJ; (2) Repair factors - once the viral DNA is captured by the broken DSB ends, the IN-CA-independent integration steps are completed depending on the general DSB repair pathway and, (3) recruitment factors – in general, DNA strand breaks are quickly repaired through the DNA repair pathway to maintain genomic integrity. To achieve the viral DNA insertion before the completion of chromosomal DSB repair, PIC should be actively recruited to the DNA damage site. There are several candidate factors that influence the DNA repair and bind IN. Among these, Ku70, Rad18, and LEDGF are rapidly recruited to the DSB site and promote the DSB repair process [64,61,45]. Moreover, there are multiple putative host factors that promote IN-CA-independent viral transduction and facilitate viral DNA integration into DSB sites. The activities of these repair factors are regulated by the cell cycle and are depend on the state of chromatin. For example, the activity of LEDGF/CtIP is restricted to the S/G2 phase, while LEDGF/CtIP shows strong preference for active chromatin loci [61,43].

Roles of the Vpr-induced DNA damage signal

Vpr possesses a variety of biological roles, such as a nuclear translocation of PIC inducer, a viral promoter activator and apoptosis promoter [70-73]. Although it has been proposed to trigger the DNA damage response through its chromatin binding activity, no nuclease activities of Vpr have been reported [74-76]. However, lines of evidence suggest that Vpr induces DNA damage. Markedly, the expression of Vpr induces high molecular genomic DNA fragmentation, which was detected by a pulse-field gel electrophoresis [77]. On the basis of the previous observation, there are candidate mechanisms for explaining the Vpr–induced DNA damage: (1) degradation of uracil DNA glycosylase 2 and single-strand selective mono-functional uracil DNA glycosylase 1 (UNG2/SMUG1) by the downregulation of the cellular DNA repair pathway that leads to the accumulation of spontaneous DNA damage breaks [78]; (2) induction of mitochondrial dysfunction by the accumulation of reactive oxygen species [79]; (3) degradation of Dicer that induces miRNA

pathway disruption and transcriptional and epigenetic regulation failure, leading to chromosomal instability [80,81,23] and, (4) modulation of cellular DNA metabolism through ubiquitin-mediated modification of unknown target(s). Interestingly, Belzile et al. [75] reported that Vpr interacts with different ubiquitinated proteins, which is required for ATR activation [82]. Moreover, the suppression of the ubiquitin-K48 chain formation, specifically abrogates Vpr-induced H2AX phosphorylation, suggesting that the response to DNA damage or related structure observed under Vpr expression is dependent on ubiquitination. The identification of primary target(s) by Vpr-induced ubiquitination is an open question for clarifying the mechanism of Vpr-induced DNA damage.

Perspectives

Here, we have highlighted the virus-host interactions. We first focused on the counteracting functions of viral proteins for overcoming the cellular antiviral pathway, and then we discussed how the virus hijacks the intrinsic DNA repair pathway to achieve the IN-CA-independent viral transduction. In the post-Highly Active Antiretroviral Therapy (HAART) era, it has been claimed that the therapeutic regimens of combined anti-HIV-1 drugs cause side effects and are not sufficient to completely block the viral infection because of the evasion of the virus from the anti-HIV-1 compounds. Namely, the case of IN inhibitors is the typical example: emergence of resistant mutation, IN-CA-independent integration, and aberrant IN activity at suboptimal inhibition [54,83,55,56]. Although the clinical relevance of the latter two events is currently unknown, it has been claimed that HIV-associated malignant formation might be caused by aberrant IN activity [83,84]. Furthermore, DNA damage derived from endogenous or exogenous origins stimulates both IN-CA-dependent and -independent viral transductions. In the case of viral infection, Vpr has been shown to positively regulate the viral infection. Moreover, it was shown that DNA damage provides a platform for viral transduction, particularly in the presence of IN inhibitors. It is plausible that the analysis of the mode of DNA damage-dependent upregulation of the viral transduction will provide a novel viral-host interaction, further contributing to development of novel anti-HIV-1 compounds.

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