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The conserved N-terminal basic residues and zinc-finger motifs of HIV-1 nucleocapsid restrict the viral cDNA synthesis during virus formation and maturation

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ABSTRACT

Reverse transcription of the genomic RNA by reverse transcriptase occurs soon after HIV-1 infection of target cells. The viral nucleocapsid (NC) protein chaperones this process via its nucleic acid annealing activities and its interactions with the reverse transcriptase enzyme. To function, NC needs its two conserved zinc fingers and flanking basic residues. We recently reported a new role for NC, whereby it negatively controls reverse transcription in the course of virus formation. Indeed, deleting its zinc fingers causes reverse transcription activation in virus producer cells. To investigate this new NC function, we used viruses with subtle mutations in the conserved zinc fingers and its flanking domains. We monitored by quantitative PCR the HIV-1 DNA content in producer cells and in produced virions. Results showed that the two intact zinc-finger structures are required for the temporal control of reverse transcription by NC throughout the virus replication cycle. The N-terminal basic residues also contributed to this new role of NC, while Pro-31 residue between the zinc fingers and Lys-59 in the C-terminal region did not. These findings further highlight the importance of NC as a major target for anti-HIV-1 drugs.

INTRODUCTION

The inner structure of mature infectious retroviral particles is formed of the genomic RNA coated by several hundred copies of the nucleocapsid (NC) protein (1).

NC is encoded by the C-terminal domain of Gag, and found as the mature protein upon Gag polyprotein processing by the viral protease during virion formation and budding. NC is a small basic protein with either one or two highly conserved 'CCHC' zinc fingers, and has potent nucleic acid binding and annealing activities (1,2). Retroviral NC is multifunctional in that it acts throughout the virus replication cycle via a number of molecular interactions. At the late stage, NC as part of Gag selects and dimerizes the genomic RNA, which is thought to start the Gag assembly process in infected cells. During the early stage, mature NC molecules do extensively interact with the viral genome and reverse transcriptase (RT) to chaperone proviral DNA synthesis by reverse transcriptase, thus ensuring the *bona fide* synthesis of the proviral DNA flanked by the LTRs (1,2).

HIV-1 NC is characterized by a central globular domain composed of two highly conserved CCHC zinc fingers (ZF) linked by a basic RAPRKKG sequence, and flanked by flexible N-terminal and C-terminal domains (Figure 1A) (3,4). Upon Zn²⁺ coordination by the CCHC residues the ZFs fold into a specific structure (5–7), which is required for virus infectivity (Figure 1A).

The present view is that NC as part of the Gag polyprotein specifically binds the genomic RNA via tight interactions between the ZFs and the Psi packaging signal located in the 5' leader sequence (8–14). These specific NC–RNA molecular interactions are thought to promote genomic RNA dimerization and Gag oligomerization, where the viral RNA acts as an assembly platform in the course of virion formation (1, 15–17). The basic residues located in the N-terminal flexible region of NC also contribute to genomic RNA selection and HIV-1 assembly (18–21). Indeed, subtle changes in the ZF structure caused by mutating the CCHC residues or the aromatic amino

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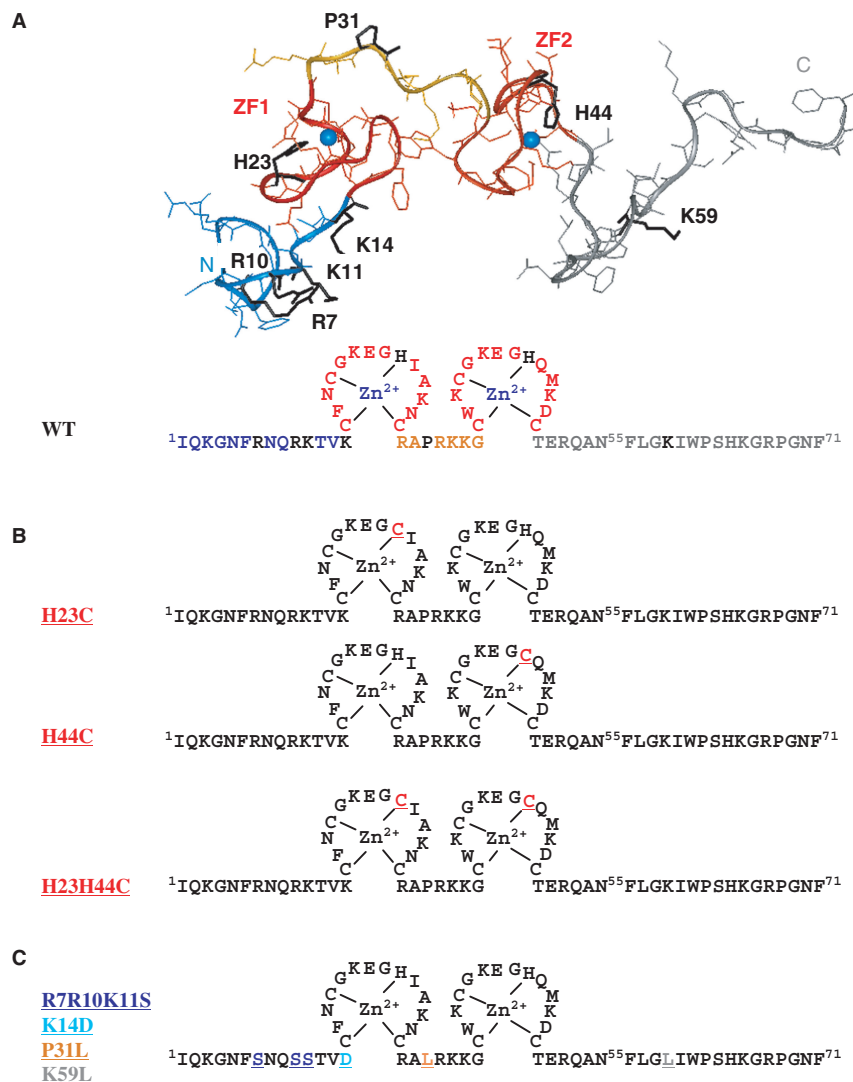


Figure 1. Sequence and structure of HIV-1 NC protein. (A) Sequence of the 1–71 form of NC and the 3D structure as determined by 1H-NMR are shown (4). Numbers indicate positions of amino acids. ZFs 1 and 2 are in red with the Zn ions shown as blue dots. The linker between the two ZF is in yellow and the N- and C-terminal domains (1–13 and 64–71, respectively) are in blue and gray, respectively. All residues important for this study are in black. (B) Substitution of the wt CCHC motifs by alternative zinc-coordinating residues, i.e. CCCC. Point mutations are indicated in red. (C) Point mutations targeting the N-terminus, the linker and the C-terminal domains of NC are shown.

acids result in the formation of replication defective viruses (1,2). Similarly, changing the basic residues to neutral ones in the N-terminus and the linker domains of NC results in the production of virions with large defects in the core structure and replication (12,22).

During the past years, detailed information has accumulated on the role of NC protein in viral DNA synthesis by RT both *in vitro* and in cell culture. For example, NC promotes annealing of the replication primer tRNA to the primer binding site (PBS) on the genomic RNA, and the two obligatory strand transfers that are required to generate the complete proviral DNA flanked by the two LTR (Figure 2) (1,2,23,24). NC also prevents nonspecific self-priming induced by the TAR stem-loop structure at the 3' end of minus strand ssDNA (25–27). Thus, it appears that NC has key roles as a nucleic acid chaperone during viral DNA synthesis by RT once the viral infection is completed.

Recently, we reported a new function for the NC in the virus life cycle. Indeed, we showed for the first time that NC regulates the timing of reverse transcription, by inhibiting premature cDNA synthesis during the process of HIV-1 assembly (28). By monitoring the effects of ZF deletions on the conversion of both the genomic and spliced RNA species into viral DNA, we showed that mutant virions unexpectedly contained a high level of viral DNA compared with wild-type (wt) virions (28). We showed that such intravirion DNA content of NC mutants did not result from endogenous reverse transcription in virus producer cells. These findings revealed that reverse transcription can occur before virion release, most likely during HIV-1 assembly. Then, we called this property “late reverse transcription” in accordance with the hepadnaviruses (e.g. hepatitis B virus) and foamy viruses, whose particles contain viral DNA due to reverse

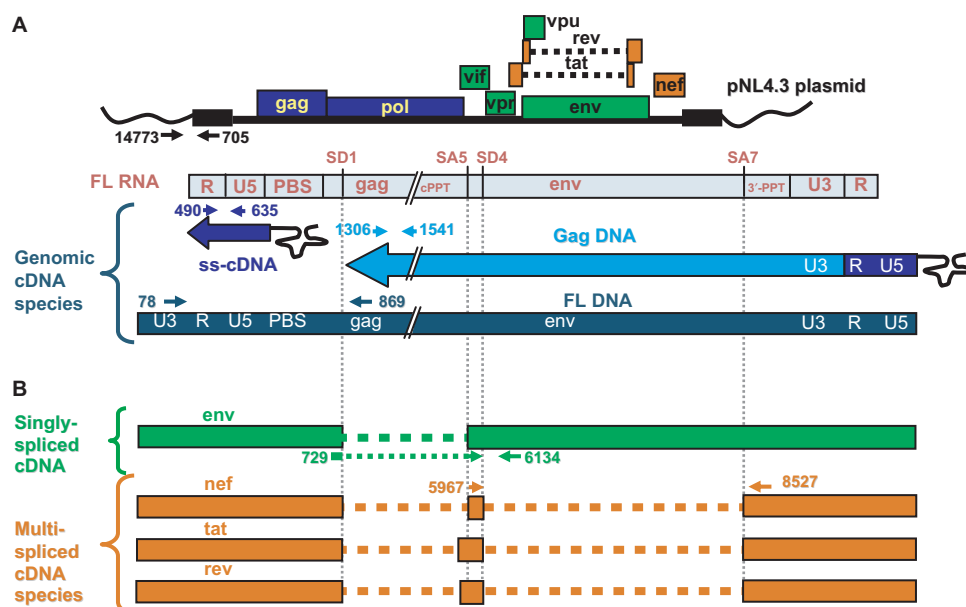


Figure 2. Strategy of qPCR to monitor the viral cDNA synthesis. (A) Conversion of the viral genomic RNA into FL DNA. Reverse transcription is initiated from the cellular tRNA^{Lys₃} (represented by a short black line attached to a 'clover-leaf') annealed to a complementary part of the genomic RNA called the primer binding site (PBS). Synthesis proceeds to the 5' end of the genome, creating a DNA fragment called minus strong-stop DNA (ss-cDNA). The direct repeat sequences located at the ends of the genome enable the ss-cDNA to transfer to the 3' end. This first jump is called the minus-strand transfer. Elongation of minus-strand DNA and RNase H degradation of reverse transcribed RNA continue (GagDNA), while plus-strand synthesis initiates by the polypurine tracts (PPT3' and cPPT). A second jump, called plus-strand transfer, is required to the plus-strand DNA synthesis and strand completion (FL DNA). The PCR-primer pairs used to specifically quantify the intermediate ss-cDNA, GagDNA, and the final product FL DNA or the pNL4-3 plasmid were schematically represented by blue and black arrows, respectively. Primer numbers refer to the position of the elongation start. (B) Spliced cDNA species monitored in the study. Only the splice sites (SD1/SA5 and SD4/SA7) important for this study are indicated. The *env* and the class of the multispliced (MS) cDNAs are represented in green and orange, respectively.

transcription taking place at the late stage of their replication.

In the present study, we investigated what might be the NC structural determinants involved in the temporal control of reverse transcription during the late stage of HIV-1 replication. To that end, we studied the consequences of subtle structural changes caused by single mutations in the first and/or the second ZF and of some conserved residues flanking the ZFs. Results reported here showed that the two intact ZF structures, as well as N-terminal basic residues are major determinants involved in the temporal control of reverse transcription by NC protein throughout HIV-1 life-cycle.

MATERIALS AND METHODS

Plasmids and cell culture

Different HIV-1 pNL4-3 plasmids with point mutations in NC were used: namely H23C, H44C, H23H44C, R7R10K11S, K14D, P31L and K59L. Construction of these mutants has been described elsewhere: H23C (29), H44C and H23H44C (30); R7R10K11S and K14D (18), P31L (31) and K59L (32).

The 293T cell line was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with glutamine (2mM), penicillin, streptomycin and 10% (v/v) heat-inactivated fetal calf serum.

DNA transfection and virus preparation

Transfections of 293T cells were performed with 3×10^6 cells divided 1 day before in 100 mm dishes by the calcium phosphate precipitation method with 8 μ g of HIV-1 or pSP72 (mock) plasmid DNAs. Cells were trypsinized 6 h after transfection, extensively washed with fresh medium and divided into two new plates to eliminate plasmid DNA in excess. After 48 h posttransfection, virions were purified from filtered culture supernatants by centrifugation through a 20% sucrose cushion at 30 000 r.p.m. in an SW32 rotor for 1 h 30 min at 4°C. The amount of HIV-1 particles in the pellet was determined using a HIV-1 CAp24 core antigen enzyme-linked immunosorbent assay (ELISA) Kit (Beckman CoulterTM).

DNA extraction and qPCR analysis

Nucleic acid extraction from virions was performed as previously described (28). Purified virions were incubated with 8 U of DNase (RQ1, Promega France, Charbonnières-les-Bains) at 37°C for 45 min before phenol/chloroform extraction and ethanol precipitation. DNase treatment reduced contaminations by the transfecting-plasmid DNA (pNL4-3) under the level of the FL DNA.

Cellular DNA was extracted with DNAzol (MRC) according to the manufacturer's instructions. To avoid any contamination with viral cDNA associated with the particles, cells were trypsinized and extensively washed with PBS before DNA extraction.

Nucleic acids were quantitated by measuring optical absorption at 260 nm. The qPCR was achieved with 25 ng of intravirion and cellular nucleic acid samples extracted from cells transfected with either wt or mutant pNL4-3 plasmid, or with empty plasmid as controls (mock). Background DNA levels measured in mock were subtracted from all assays. Nucleic acid levels in virions and producer cells have been normalized with respect to capsid protein p24 (determined by ELISA) and GAPDH gene, respectively (33). qPCR assays were performed with SYBR Green Kit (Roche Diagnostics Meylan, France) with the RotorGene (Labgene, Archamps, France) system. A standard curve was generated based on a range of 50–500 000 copies of pNL4.3 plasmid DNA. Sequences of primers and detailed PCR conditions will be provided on request.

RESULTS

Influence of point mutations in the NC ZFs on reverse transcription in virus producer cells

We previously showed that deletion of either the first ($\Delta ZF1$), the second ($\Delta ZF2$) or the two ($\Delta ZF1ZF2$) NC ZFs resulted in the extensive synthesis of viral DNA already in virus producer cells (28). In an attempt to understand how NC controls the reverse transcription process during virion formation, we studied the effect of changing highly conserved residues in the NC ZFs, namely His23, His44 or His23/His44 to Cys, referred as HIV-1 H23C, H44C and H23H44C, respectively (Figure 1B). These mutations maintain the Zn²⁺-binding residues (CCCC motifs), but lead to a partial misfolding of the NC central globular domain, that is essential for virus infectivity (22,31,34). These NC mutants have been previously tested in reverse transcription assays *in vitro*, and the NC H23C and H44C mutants showed a reduced ability to chaperone the initiation of reverse transcription (35,36) and of viral DNA strand transfer (37). However, no effect was seen in RNA chaperoning assays *in vitro* (Darlix and Gabus, unpublished results). Upon infection by the corresponding HIV-1 H23C and H44C, mutations caused a marked reduction of viral DNA synthesis in infected cells, together with a complete defect in proviral DNA integration probably due to a degradation of its LTR ends (38). Besides the decrease in the overall efficiency of reverse transcription, infection by spinoculation revealed that the H23C and H44C mutants showed amount of DNA increased during earlier time-points of infection (1 h) compared to wt (38), suggesting that DNA synthesis could begin within HIV-1 particles. Moreover, we recently showed that the deletion of either one or the two NC ZF lead to reverse transcription taking place in virus producer cells, before virion release (28). Therefore, we examined whether subtle point mutations in the NC ZFs could induce such a change in the timing of the reverse transcription throughout HIV life-cycle.

To test whether the DNA synthesis could be achieved, at least in part, before virion release, we performed a detailed quantitative analysis of viral DNA synthesized in 293T cells expressing or not (mock) either one of the HIV-1 H23C, H44C or H23H44C mutants. To circumvent

the difficulty to discriminate by qPCR between the transfected plasmid DNA and the viral cDNA molecules synthesized in cells, we analyzed the levels of spliced cDNAs corresponding to the reverse transcription of the spliced viral mRNAs (Figure 2). Indeed, we previously showed that the viral spliced RNA were reverse-transcribed as efficiently as the genomic RNA (39), which makes these spliced DNA forms an ideal marker for viral DNA quantification (28). As previously described, the viral spliced DNA levels were monitored by qPCR in cell lysates of assays and mock transfected controls (28,39) (Figure 3). In the present study, we used a primer pair for the analysis of *env* sequences, that represent 74% of the singly spliced RNA class, and a primer pair specific for the SD4/SA7 splice junction that allowed overall quantification of the multispliced RNA converted into cDNA (MS cDNA) (33) (Figure 2B). In parallel, the GAPDH copy numbers were systematically determined in all DNA samples extracted from cell lysates for normalization.

Mutating the first ZF (H23C) induced a slight increase of the spliced cDNA levels, notably for *env* cDNA ($P = 0.02$), compared to wt. A more pronounced effect was observed with the H44C mutation in the second ZF, with a 90-fold increase of the *env* cDNA level ($P = 0.02$). Such an increase was not found with the double mutant (H23H44C), where the effects of H23C and H44C mutations did not appear to be cumulative, as already observed with the two ZF deletion mutant ($\Delta ZF1ZF2$) (28) (Figure 3).

Because the accumulation of the viral cDNA in producer cells could be a direct function of the efficiency of viral assembly and virus production, next we monitored the viral cDNA products in nascent virions.

Impact of the H23C, H44C and H23H44C mutations on the cDNA content in virions

To examine whether viral cDNA products were incorporated into released virions, we performed an extensive analysis of the purified virions produced by 293T transfected cells (see Material and methods section). Overall, these ZFs H23C, H44C and H23H44C mutations have a

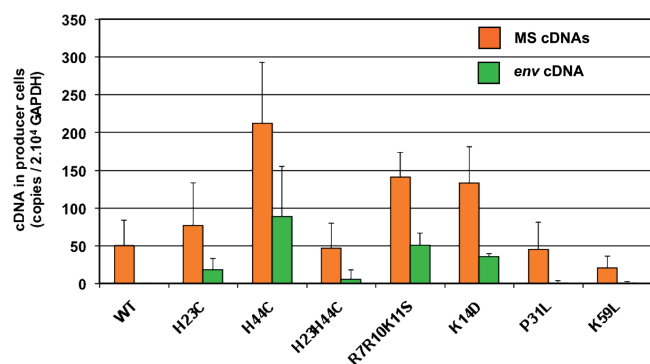


Figure 3. Viral cDNA in HIV-1 producer cells. Analysis of viral DNA synthesized in cells producing HIV-1 with either wt or mutated NC. Spliced viral DNAs were measured by qPCR in 293T cells transfected or not (control) with mutant or wt plasmid HIV-1 DNA. Mock controls were subtracted from assays ($n = 3 \pm SD$).

pleiotropic effect on virus structure and replication. Indeed, previous electron microscopy studies showed that these ZF mutations resulted in the production of replication defective virions with an immature core morphology, lacking the typical cone-shaped core structure (29,30). The mutations partially impair RNA packaging and modify the intracellular localization of Gag proteins (29,30,34,40).

In our experimental system, virus production, as monitored by a p24 ELISA assay on purified viral particles, was partially impaired by all the mutations (Figure 4). In contrast to previous observation (30), the double H23H44C mutant showed only 2% of wt virion release. The reason for this difference might be due in part to the different experimental conditions, since previously HeLaP4 cells were transfected and the virions collected 20 h post-transfection without previous split. The present results correlate with results of other mutations also targeting the NC ZF (41).

To assess the DNA content in the HIV-1 ZF mutant particles, purified virions were treated with DNase to remove any contaminating plasmid DNA due to cell transfection. We used qPCR for the quantitative monitoring of the different intermediates of reverse transcription such as the minus strong-stop, Gag and the full-length viral cDNAs as well as the so-called MS and *env* cDNAs in assays and mock controls (Figure 2) (28). All NC mutant particles harbored an increase of 10- to 40-fold of the viral FL DNA levels compared to wt virions (Figure 5).

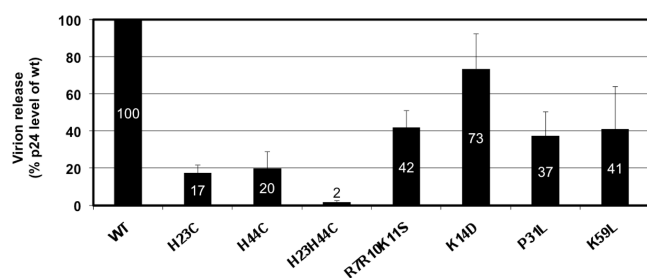


Figure 4. Analysis of the viral particles produced by cells expressing HIV-1 NC mutants. Amounts of viral p24 capsid present in pelleted virions were quantitated by ELISA and values were normalized to wt level ($n = 3 \pm SD$).

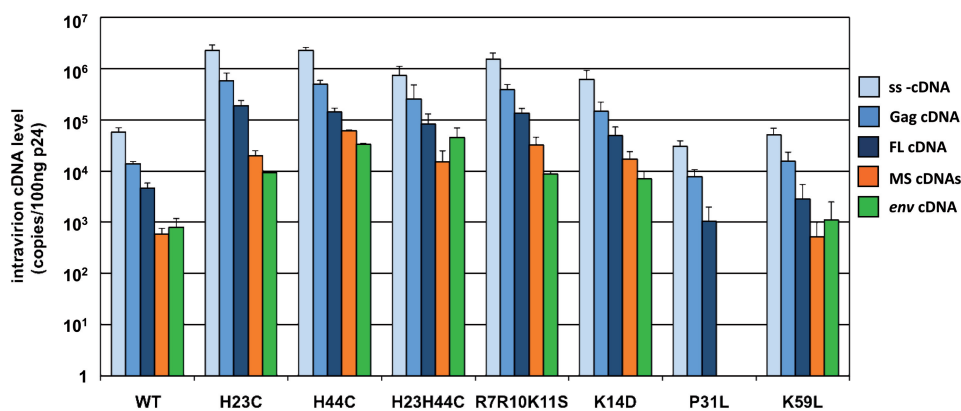


Figure 5. Quantitation of intravirion viral cDNAs in particles with NC mutations. Levels of viral strong-stop, Gag, FL, *env* and multispliced cDNAs were determined by qPCR in wt and NC mutant virions. Copy numbers of viral DNA were given for 100 ng p24 corresponding to $\sim 6.25 \times 10^8$ viruses (4000 CA/virus used for calculation) ($n = 3 \pm SD$).

There was also a large enhancement of the MS and *env* cDNA in these mutant virions, with about a 100-fold increase in the H44C viral particles. These results extend previous ones on the consequences of deleting the ZF motifs ($\Delta ZF1$, $\Delta ZF2$, $\Delta ZF1ZF2$) (28), and indicate that subtle structural alterations of the NC ZFs have an important impact on the timing of reverse transcription throughout the virus replication cycle.

Role of N-terminal basic residues of NC in the temporal control of reverse transcription

To seek for other NC determinants involved in the control of viral DNA synthesis during HIV-1 virion formation, we examined the consequences of mutating conserved basic residues in the N-terminal region that precedes the first ZF. To that end, we selected the NC R7R10K11S (basic residues Arg7, Arg10 and Lys11 changed to neutral Ser residues) and K14D (basic to acidic) mutants (Figure 1C) because such NC mutations cause a severe defect in virus replication. This might be related to a strong defect in viral DNA synthesis in infected cells and the fact the viral DNA appeared to be unstable during the early phase of cell infection (18,19,42–44). In addition, the HIV-1 R7R10K11S and K14D mutants were found to package the genomic RNA at level of 20–50% (12,18,42,44) and 90% (18) of the wt level, respectively. But genomic RNA dimerization remained unaffected for the R7R10K11S mutant (42). Presence of cDNA synthesized in cells transfected by the mutant HIV-1 R7R10K11S or K14D DNAs was monitored as before by qPCR. As shown in Figure 3, there was an enhancement of the spliced cDNA levels in cells expressing these HIV-1 mutants, notably a 40-fold increase in the *env* cDNA.

Then, we examined the cDNA content of the mutant particles produced by the transfected cells. In contrast to the ZF point mutations, changing the basic residues had only a moderate effect on virus production (Figure 4) but, mutant particles harbored an altered core morphology with an enlarged particle size (18). Quantification of the different cDNA species present in DNase treated virions, i.e. ss-cDNA, Gag, FL DNA and spliced cDNAs, revealed a much higher level of viral DNA (Figure 5).

In conclusion, the basic residues located in the N-terminal domain of NC are important determinants for the control of reverse transcription mediated by NC, during virion formation.

Effect of mutating residues in the ZF linker and the C-terminal domain of NC

The Proline residue at position 31 (Pro31) is unique in the HIV-1 NC, and is located in the highly conserved ²⁹RAPRKKG³⁵ region linking the two ZF motifs (Figure 1C). This structural kink caused by Pro31 is thought to be involved in the respective spatial orientation of the ZF motifs (3,4,11). Such a role in NC conformation prompted us to study its importance in the control of late reverse transcription. To that end, we analyzed the effect of changing Pro31 to Leu31 (P31L), which is expected to strongly modify the structure of the linker and of the spatial orientation of the two ZFs. The P31L mutant has been reported to produce noninfectious viruses containing only minimal levels of mature RT (31,32). In our experimental conditions, we found that the P31L mutant produced 37% of the wt virion level, as measured by the p24 ELISA assay (Figure 4). The viral cDNA was monitored as above in both the producer cells (Figure 3) and the virions (Figure 5). Results indicate that the cDNA levels in the producer cells were similar to those found with the wt HIV-1. In the P31L virions, the strong-stop, Gag and FL cDNA levels were found lower than viral cDNA levels in wt particles. In addition, the spliced cDNA species remained undetectable in P31L particles. Thus, changing Pro31 to Leucine in the ZF linker appears to result in a possible increase in the control of reverse transcription in virus producing cells. In agreement with this notion, the P31L form of NCp7 was found to possess potent RNA chaperoning activities and to extensively interact with the RT enzyme *in vitro* (Darlix and Gabus, unpublished data).

Last, we wanted to study the influence of the C-terminal domain of the NC precursor (1–71) because NC (1–55), thus missing residues 56–71, was shown to have a reduction of its activity in reverse transcription *in vitro* (45). The large and short forms of NCp7 are found in HIV-1 virions recovered every 2–4 h [Berthoux, L., thesis; (46)]. To study the influence of the C-terminal region of NC on the control of viral cDNA synthesis, we used the HIV-1 NC mutant where Lys59 was changed to Leucine (K59L). The HIV-1 K59L mutant was about ~1000-fold less infectious than the wt virus (Darlix, J.L. and Berthoux, L., unpublished data) and contained 50% of wt genomic RNA level (32) with most of it in a dimeric form (42). While the main defect that has been reported for this mutant was in the efficiency of the pr55Gag processing (32,43,47), this has only weak effect on virus production (Figure 4). Quantification of the viral cDNA levels in producer cells (Figure 3) and in virions (Figure 5) showed that cDNA levels were similar to those found with the wt HIV-1, indicating that the K59L mutation has little or no effect on the control of reverse transcription in virus producer cells.

DISCUSSION

The results reported here indicate that HIV-1 reverse transcription can occur in HIV-1 producer cells upon mutating conserved residues in the NC (Figure 3) such as H23C and H44C that convert the first and the second CCHC motif to CCCC found in variety of cellular proteins (48). This has been named 'late reverse transcription'. These results confirm our first study showing the importance of the NC ZF motifs in the regulation of the RT timing throughout the HIV-1 life-cycle (28). Note that the double mutant (H23H44C) did not exhibit efficient late reverse transcription in cell as did the H23C and H44C mutants. Late RT could be achieved upon virion formation at the assembly sites, as supported by our previous study (28). Then, the lower late-RT activity of the double mutant was likely related to its severe defaults in RNA packaging and virion release.

In addition, the present results clearly show that an intact structure of ZF1 and ZF2 is required for the negative control of the late reverse transcription process. As observed for complete ZF deletion, the substitution of single conserved residues in the ZF also resulted in late reverse transcription in virus producing cells, notably for H44C, while causing only subtle changes in the conformation of the central globular domain of NC (1,4,31).

The 3D structure of HIV-1 NC has revealed the importance of the kink induced by Pro31 in bringing the two ZF motifs into close proximity, while Pro31 was hydrogen-bonded to ZF1 (3,4,11). Though overall 3D structure of the ZFs appeared to be critical, the P31L point mutation had no influence on the late reverse transcription process. The NMR structure of NCp7 bound to the SL3 RNA stem-loop of the HIV-1 packaging signal showed that the SL3 stem-loop interacts with N-terminal basic residues such as Arg7, Arg10 and Lys11 via electrostatic interactions (11). Interestingly, these conserved basic residues, Arg7, Arg10, Lys11, Lys 14, of the N-terminal domain are important determinants for the control of late reverse transcription, since their substitutions activated late reverse transcription (Figure 3). Besides their role in viral RNA binding, these basic residues are also involved in Gag–Gag and Gag–ABCE1 interactions important for virion assembly (20,21,49,50). This led us to hypothesize that late reverse transcription is probably taking place in intracellular core structures at the time of assembly, resulting in the production of viral DNA containing particles. Therefore, mutations in the ZFs and N-terminal basic residues may slow down the kinetics of the coordinated assembly and budding processes, allowing more time for viral DNA synthesis before virion release. In that respect, the current view is that Gag assembly relies on specific Gag–RNA interactions and Gag oligomerization, and also depends on the implications of cellular factors such as, Alix, APOBEC3G, ABCE1 (HP68) (1,51), Staufen (41) and IMP1 (insulin-like growth factor II mRNA binding protein 1) (52). These proteins are all important for optimal HIV-1 assembly, and they have been reported to interact with the Gag–NC domain through either the ZF or the conserved basic residues. Therefore, mutating these specific NC residues may well

interfere with the cellular environment where assembly takes place and indirectly promote late reverse transcription during assembly. Another parameter that could be implicated in the late reverse transcription reaction is the processing of Gag and Gag-Pol in virus producer cells (53). Interestingly, NC is also implicated in the protease mediated processing of Gag through RNA binding (53–56). Soon after infection, reverse transcription takes place in nucleocapsid structures in the form of reverse transcription complexes (RTC) containing the mature enzymes reverse transcriptase and integrase (IN), the genomic RNA and tRNA and NC protein molecules (1,57). Such mature viral proteins and enzymes are also present, to some extent, in producer cells and therefore would be responsible for the late reverse transcription. Consequently, the kinetic of maturation of the Gag and Gag-Pol precursors during the late phase of HIV-1 replication could greatly influence the late reverse transcription reaction. In conclusion, the NC ZF structures, as well as conserved N-terminal basic residues are important determinants involved in the control of HIV-1 reverse transcription during virus replication. In contrast, the linker and C-terminus domains of NC had no effect on viral DNA synthesis, suggesting that they might not be involved in the control of the late transcription reaction during late steps of HIV replication.

Late reverse transcription is also a property of the hepadnavirus [e.g. hepatitis B virus (58)] and foamy virus, which then release viral DNA-containing particles (59,60). Interestingly, HIV-1 carrying mutations in the ZFs and the N-terminal domain appear to share some of the foamy virus specific properties. Indeed, the characteristics that led the foamy viruses to be closely related to DNA viruses could rely in part to their lack of Gag-NC ZFs and to an original Gag maturation without classical processing into matrix, capsid and NC mature products (60).

Note that presence of viral DNA-containing particles has been observed *in vivo* in HIV-1 isolated from AIDS patients (61,62) and could result to late reverse transcription event. Such an intracellular DNA synthesis during virus assembly producing HIV-1 DNA-containing particles could be the source of HIV-1 re-emergence when antiviral therapy is halted. Our findings may complement ongoing efforts to block HIV replication with anti-NC drugs (1,63,64) and may also identify new strategies for the design of antiviral therapies.

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