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Human immunodeficiency virus type 1 nucleocapsid zinc-finger mutations cause defects in reverse transcription and integration

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Abstract

The nucleocapsid (NC) protein from HIV-1 contains two zinc-fingers, both of which are necessary for virus replication. This is the first in-depth study that presents the effects of nucleocapsid zinc-finger substitutions on the kinetics of reverse transcription and integration. Over a 72-h time-course of infection, the quantities of viral DNA (vDNA) observed with viruses containing either the nucleocapsid His23Cys or His44Cys mutations were significantly lower than those observed in infections with virus containing wild-type NC. In addition, the kinetics of vDNA formation and loss were significantly different from wild-type. The kinetic profiles observed indicated reduced vDNA stability, as well as defects in reverse transcription and integration. Overall, the defect in integration was much more pronounced than the reverse transcription defects. This suggests that the principal reason for the replication defectiveness of these mutant viruses is impairment of integration, and thus demonstrates the critical importance of NC in HIV-1 infection.

Keywords: HIV-1; Integration; Kinetics; Mutation; Nucleocapsid; Provirus; Reverse transcription; Strand transfer; Zinc-finger

Introduction

Nucleocapsid (NC) of HIV-1 functions during many steps of the virus life cycle, and is critically important for virus replication (Buckman et al., 2003; Darlix et al., 2002). NC is highly basic in amino acid composition and contains two zinc-finger motifs of the sequence –Cys–X_2–Cys–X_4–His–X_4–Cys–, which are necessary for many of its activities. Because NC functions throughout the viral life cycle, in-depth mutational analysis of NC in infection processes has mainly been limited to studies on virion assembly and genomic RNA (gRNA) packaging. Several in vivo studies have been performed using mutant viruses to examine NC's roles in reverse transcription and integration (Buckman et al., 2003; Gorelick et al., 1999a; Tanchou et al., 1998). However, the majority of studies have been performed using in vitro systems that assay specific NC functions that are thought to occur during early infection events, including reverse transcription (Drummond et al., 1997; Guo et al., 2000; Ji et al., 1996; Klasens et al., 1999; Wu et al., 1996), viral DNA (vDNA) protection (Lapadat-Tapolsky et al., 1993; Tanchou et al., 1995), and integration (Carteau et al., 1999; Gao et al., 2003).

Based on these in vitro experiments, it is clear that a major function of NC is as a nucleic acid chaperone; that is, NC assists nucleic acids in obtaining the most thermodynamically stable annealed structures (Levin et al., 2005; Rein et al., 1998). During HIV-1 infections, NC melts nucleic acid secondary structures that would otherwise result in pausing or premature termination of reverse transcription, which has been observed in numerous in vitro studies (Drummond et al., 1997; Ji et al., 1996; Klasens et al., 1999; Wu et al., 1996). The two zinc-fingers have been shown to be important for this chaperone activity, as mutations eliminating either finger abrogate this function (Levin et al.,

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2005). A major strength of in vitro systems is that through careful design, it is possible to demonstrate discrete functions of NC. However, during an infection, NC functions as part of a complex comprised of viral and cellular proteins, as well as membrane and nucleic acid elements.

Early in vivo studies demonstrated that mutations that disrupt the ability of NC to bind zinc cause replication-defective phenotypes, but in many instances the reason was partially attributed to significant gRNA packaging defects (Aldovini and Young, 1990; Dorfman et al., 1994; Gorelick et al., 1990, 1993). To examine the nature of NC's true role in early infection events, it was imperative that nucleocapsid mutant viruses package significant levels of gRNA. To this end, a series of mutants was generated in which key cysteine or histidine residues were replaced by histidine or cysteine, respectively (Gorelick et al., 1999b). Although these mutations still enabled NC to bind zinc, many of them produced virions that packaged their gRNA poorly. Generally, alterations to the carboxyl-terminal zinc-finger were better tolerated than the equivalent alterations in the amino-terminal finger. Two of the zinc-finger mutants, His23Cys (NC_{H23C}) and His44Cys (NC_{H44C}), do package significant levels of gRNA and produce wild-type levels of virus particles, but are, nevertheless, replication-defective (Gorelick et al., 1999b). These two His-to-Cys zinc-finger mutants have been tested in numerous specific in vitro assays to measure enhancement of tRNA annealing (Rong et al., 1998, 2001), initiation of reverse transcription (Rong et al., 1998, 2001), strand transfer activity (Guo et al., 2002; Hsu et al., 2000), and integration (Carteau et al., 1999). The NC_{H23C} mutation does appear to alter the nature of tRNA annealing, thereby reducing reverse transcription initiation (Rong et al., 2001). Both NC_{H23C} and NC_{H44C} mutants (i) decrease the efficiency of strand transfer and (ii) impair NC's ability to promote coupled integration. In all these in vitro assays, the NC_{H44C} mutant is less defective than NC_{H23C}, which correlated with observations that, in NC_{H44C} mutant virus infections, occasional reversions to a wild-type genotype were obtained, while none occurred with the NC_{H23C} infections (Gorelick et al., 1999b).

An initial analysis of vDNA synthesis upon infection of permissive cells with the NC_{H23C} or NC_{H44C} mutant viruses was performed to ascertain if these mutations affected nucleic acid chaperone functions in cell-based assays. At 24 h postinfection with either the NC_{H23C} or NC_{H44C} mutant virus, quantities of vDNA were considerably lower than those observed with wild-type virus. This suggested the presence of defects in reverse transcription and reduced vDNA stability, but it was unclear how the infection proceeded to this point. In addition, sequence analyses of 2-LTR circle junctions indicated a severe defect in integration for both the NC_{H23C} and NC_{H44C} mutant viruses (Buckman et al., 2003). A number of questions remained regarding (i) the rate of formation or degradation of vDNA intermediates in the mutant infections, (ii) whether strand transfer efficiency was reduced, and (iii) the magnitude of the integration defect.

The present study extends our previous report to provide an in-depth analysis of the impact of the NC_{H23C} and the NC_{H44C} mutations upon early infection. Quantities of vDNA were measured over a 72-h time-course to provide a kinetic picture of vDNA synthesis and loss. In addition, a real-time quantitative Alu-LTR PCR assay (Butler et al., 2001) was implemented to directly measure quantities of proviruses. This report shows that alterations in either the amino- or carboxyl-terminal zinc-finger motifs result in minor reverse transcription defects and somewhat reduced vDNA stability, but greatly decreased integration efficiency. The impairment to integration alone would account for the replication defects observed previously (Gorelick et al., 1999b).

Results

Expression of NC mutant viruses

Viruses containing either the NC_{H23C} or the NC_{H44C} mutation are replication-defective (Gorelick et al., 1999b), so it was necessary to limit wild-type infections to a single round. Otherwise, comparison of vDNA quantities between wild-type and the NC_{H23C} or NC_{H44C} mutant virus infections would be complicated at all time-points after 12 h when wild-type virus began re-infecting cells (Butler et al., 2002; Kim et al., 1989). Pseudotyped viruses were generated by co-transfecting $Env^{(-)}$ full-length proviral plasmids with a VSV-G expression plasmid (Burns et al., 1993) into 293T cells.

Because many NC mutations affect gRNA packaging (Aldovini and Young, 1990; Dorfman et al., 1994; Gorelick et al., 1990, 1993, 1999b), we examined the ability of the transfection-generated viruses to package gRNA (Table 1). Quantities were measured using real-time quantitative RT-PCR on viral pellets obtained from cell culture supernatants, were then normalized for equivalent exogenous template-RT activities, and reported as percent of wild-type gRNA levels (Table 1). In agreement with our previous report (Gorelick et al., 1999b), the NC_{H23C} mutant showed wild-type levels of gRNA

Table 1
Properties of mutant and wild-type virus particles

Virus	gRNA packaging (% of wild-type levels)[a]
(−)-Control[b]	0
NC_{H23C}	74 ± 20
$NC_{H23C}IN_{D116N}$	100 ± 7.6
NC_{H44C}	28 ± 4.3
$NC_{H44C}IN_{D116N}$	25 ± 3.3
IN_{D116N}	121 ± 4.9
Wild type	100

[a] "gRNA (genomic RNA) packaging" is expressed as a percentage of wild-type levels ± the standard error of the mean. The quantity of viral genomes per mL of transfection supernatant was divided by the exogenous RT activity (cpm [^{3}H]-TMP incorporated/mL) of transfection supernatant for each experiment. The resulting value, quantities of RNA per RT activity, was then divided by the value calculated for wild-type virus and this ratio was converted to a percentage (i.e. [*mG* / *m*RT] / [wt*G* / wtRT] × 100, where *m* = mutant, wt = wild-type, *G* = genomic RNA per mL, RT = RT activity per mL). The percentages shown are means from at least three separate transfection experiments. Genome quantities were determined as described in Materials and methods.

[b] "(−)-Control", transfection of 293T cells with sheared salmon sperm DNA.