doi:10.1016/j.jmb.2008.08.046 *J. Mol. Biol.* (2008) **383**, 1112–1128

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How the HIV-1 Nucleocapsid Protein Binds and Destabilises the (–)Primer Binding Site During Reverse Transcription

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Received 22 July 2008; accepted 19 August 2008
Available online
26 August 2008

Edited by M. F. Summers

The human immunodeficiency virus type 1 nucleocapsid protein (NCp7) plays an important role in the second strand transfer during reverse transcription. It promotes annealing of the 18-nucleotide complementary DNA primer-binding site (PBS) sequences at the 3′ ends of (−)DNA and (+) DNA. NMR studies show that NCp7(12–55) and NCp7(1–55) interact at the 5′ end of the loop of ΔP(−)PBS, a (−)PBS derivative without the 3′ protruding sequence, in a slow-exchange equilibrium. This interaction is mediated through the binding of the hydrophobic plateau (Val13, Phe16, Thr24, Ala25, Trp37, and Met46) on the zinc finger domain of both peptides to the 5-CTG-7 sequence of ΔP(−)PBS. The stacking of the Trp37 aromatic ring with the G7 residue likely constitutes the determinant factor of the interaction. Although NCp7(12–55) does not melt the ΔP(−)PBS stem–loop structure, it opens the loop and weakens the C5·G11 base pair next to the loop. Moreover, NCp7(12–55) was also found to bind but with lower affinity to the 10-CGG-12 sequence in an intermediate-exchange equilibrium on the NMR time scale. The loop modifications may favour a kissing interaction with the complementary (+)PBS loop. Moreover, the weakening of the upper base pair of the stem likely promotes the melting of the stem that is required to convert the kissing complex into the final (±)PBS extended duplex.

Keywords: human immunodeficiency virus type 1 (HIV-1); nuclear magnetic resonance (NMR); nucleocapsid protein (NCp7); primer binding site (PBS); exchange

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Abbreviations used: HIV-1, human immunodeficiency virus type 1; PBS, primer-binding site; ITC, isothermal titration calorimetry; NOESY, nuclear Overhauser enhancement spectroscopy; TOCSY,total correlated spectroscopy; NOE, nuclear Overhauser enhancement; Rh6G, 6-carboxyrhodamine; DABCYL, 4-(4-dimethylaminophenylazo)benzoic acid; SL, stem–loop; ROESY, rotating frame Overhauser effect spectroscopy; EXSY, exchange spectroscopy; FCS, fluorescence correlation spectroscopy.

Introduction

The human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein NCp7 is a small, basic, nucleic-acid-binding protein generated by protease-mediated processing of the Gag polyprotein precursor.[1] NCp7 is characterised by two zinc fingers (Fig. 1a) with a common $CX_2CX_4HX_4C$ sequence that strongly binds zinc ions.[2,3] Solution structure of the protein showed that these two zinc fingers are strongly folded[4–7] and weakly interact.[7–9] They are separated by a short, flexible, basic region and surrounded by the N- and C-terminal domains.[6,10,11] NCp7 binds to nucleic acids through sequence-specific and non-specific interactions involving electrostatic and hydrophobic contributions.[12–14] Specific interaction of NCp7 with oligonucleotides is mainly supported by the finger domain,[14–17] which is also responsible for its DNA destabilising activity.[18–21] The NCp7 finger domain has a high affinity for guanine residues,[22] especially for those in TG (or UG) sequences[14,15,23] and GXG sequences (where X corresponds to either A, C, or U).[24,25]

NCp7 exhibits nucleic acid chaperone properties that direct the rearrangement of nucleic acid molecules into their most stable conformation.[26,27] Due to these properties, NCp7 plays key functions in both the early and late steps of the HIV-1 viral life cycle. NCp7 is notably thought to assist the reverse transcriptase to convert the HIV-1 RNA genome into linear double-stranded DNA (Fig. 2)[28,29] by promoting the annealing of the primer tRNA to the initiation site (primer-binding site, PBS) and by directing the two DNA obligatory strand transfer reactions.[30] During the second strand transfer, NCp7 chaperones the (+) strand transfer[31–33] by promoting the annealing of the (+)PBS to its (−)PBS

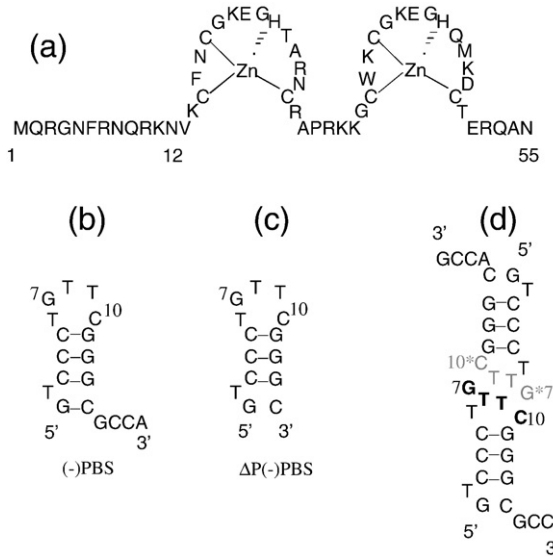

Fig. 1. Protein and oligonucleotide sequences used in this study: (a) NCp7(1–55), (b) (−)PBS, and (c) ΔP(−)PBS. (d) Model for the kissing complex homodimer.

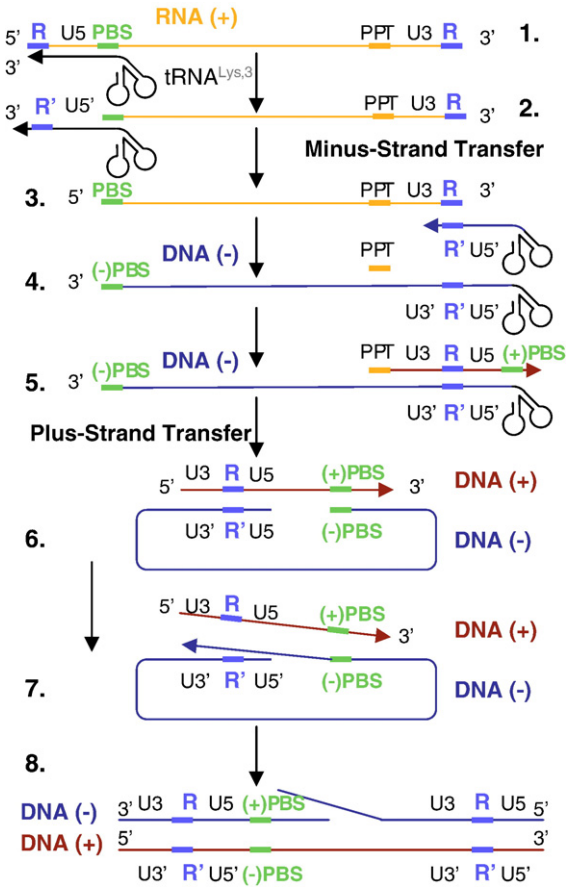

Fig. 2. Reverse transcription is initiated by the annealing of a specific cellular tRNA to the primer binding site (PBS) (1). Next, reverse transcriptase (RT) directs transcription to the 5′ end of the RNA genome, generating the minus-strand strong-stop DNA (ss-DNA), while the RNase H activity of RT concomitantly degrades the RNA template (2). Subsequently, a minus-strand DNA transfer to the 3′ end of the genome is required, favoured most likely by the interaction of the terminal repeat (R sequence) of the ss-DNA with the R sequence of the 3′ RNA (3), and reverse transcription is resumed to generate the cDNA(−) (4). Plus-strand DNA synthesis is primed by the polypurine track (PPT), an RNase H-resistant oligomer rich in purines located at the 5′ of U3, and continues to the end of the minus-strand template (5). The tRNA primer is specifically removed by RNase H and the second strand transfer occurs (6) to allow the completion of provirus synthesis (7 and 8). The HIV-1 nucleocapsid protein (NC) functions as a nucleic acid chaperone during the plus-strand transfer step in reverse transcription by facilitating annealing of the PBS sequence in the short plus-strand strong-stop DNA fragment [(+) SSDNA] (+PBS) (5) to a complementary site located near the 3′ end of the minus-strand DNA [(−) PBS DNA] (−PBS) (6).

complement present at the 3′ end of minus strand DNA (Fig. 1b and c).[31,33–35] This annealing reaction relies on the ability of NCp7 to destabilise the PBS stem–loops (SLs),[32,35] exposing nucleotides that are sequestered in the stem[35] and activating the fraying of the terminal G–C base pair.[32,36] NCp7 likely changes also the structure of the PBS loops, since