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Selection of fully processed HIV-1 nucleocapsid protein is required for optimal nucleic acid chaperone activity in reverse transcription

Tiyun Wu[a], Robert J. Gorelick[b], Judith G. Levin[a,*]

[a] Section on Viral Gene Regulation, Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2780, USA
[b] AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702-1201, USA

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ABSTRACT

The mature HIV-1 nucleocapsid protein (NCp7) is generated by sequential proteolytic cleavage of precursor proteins containing additional C-terminal peptides: NCp15 (NCp7-spacer peptide 2 (SP2)-p6); and NCp9 (NCp7-SP2). Here, we compare the nucleic acid chaperone activities of the three proteins, using reconstituted systems that model the annealing and elongation steps in $tRNA^{Lys3}$-primed (−) strong-stop DNA synthesis and subsequent minus-strand transfer. The maximum levels of annealing are similar for all of the proteins, but there are important differences in their ability to facilitate reverse transcriptase (RT)-catalyzed DNA extension. Thus, at low concentrations, NCp9 has the greatest activity, but with increasing concentrations, DNA synthesis is significantly reduced. This finding reflects NCp9's strong nucleic acid binding affinity (associated with the highly basic SP2 domain) as well as its slow dissociation kinetics, which together limit the ability of RT to traverse the nucleic acid template. NCp15 has the poorest activity of the three proteins due to its acidic p6 domain. Indeed, mutants with alanine substitutions for the acidic residues in p6 have improved chaperone function. Collectively, these data can be correlated with the known biological properties of NCp9 and NCp15 mutant virions and help to explain why mature NC has evolved as the critical cofactor for efficient virus replication and long-term viral fitness.

1. Introduction

Human immunodeficiency type 1 (HIV-1) Gag (Bell and Lever, 2013) is a multidomain protein, which contains (from the N- to C-terminus): matrix (MA), capsid (CA), spacer peptide 1 (SP1), nucleocapsid (NC, also referred to as NCp7), SP2, and p6 (Fig. 1A) (Henderson et al., 1992; Mervis et al., 1988). During or shortly after budding of virus particles from the infected cell, maturation occurs and the viral protease (PR) cleaves Gag at specific sites in an ordered and sequential manner to generate the mature virus structural proteins (Adamson and Freed, 2007; Ganser-Pornillos et al., 2008; Lee et al., 2012; Swanstrom and Wills, 1997). The initial Gag cleavage event yields two products: (N-terminal) MA-CA-SP1 and (C-terminal) NCp7-SP2-p6, referred to as NCp15. Further PR cleavage of NCp15 releases NCp9 (NCp7-SP2) and p6 and in the final cleavage step, processing of NCp9 produces the mature NCp7 protein and SP2 (Fig. 1A).

NCp7 is a small, basic nucleic acid binding protein containing two zinc-binding domains, i.e., zinc fingers (ZFs), each with the invariant CCHC motif, which are connected by a short basic linker peptide (Darlix et al., 1995, 2011; Levin et al., 2005, 2010; Rein et al., 1998; Thomas and Gorelick, 2008). NCp7 and the NC domain in Gag are essential for multiple events in the virus life cycle including viral RNA dimerization and packaging, virus assembly, reverse transcription, and integration (reviewed in Darlix et al., 2011; Isel et al., 2010; Levin et al., 2005, 2010; Lyonnais et al., 2013; Mirambeau et al., 2010; Piekna-Przybylska and Bambara, 2011; Rein et al., 1998; Sleiman et al., 2012; Thomas and Gorelick, 2008). Importantly, NCp7 is a nucleic acid chaperone, i.e., it remodels nucleic acid structures to form the most thermodynamically stable

Abbreviations: A3G, APOBEC3G; bp, base pair; CA, capsid; DTT, dithiothreitol; FA, fluorescence anisotropy; HIV-1, human immunodeficiency virus type 1; HTLV-1, human T-cell leukemia virus type 1; MA, matrix; NC, nucleocapsid; nt, nucleotides; PAGE, polyacrylamide gel electrophoresis; PBS, primer binding site; PR, protease; PI, protease inhibitor; R, repeat; RT, reverse transcriptase; SP, spacer peptide; SD, standard deviation; (−) SSDNA, (−) strong-stop DNA; TAR, transactivation response element; U3, unique 3′ sequence; U5, unique 5′ sequence; WT, wild type; ZFs, zinc fingers.

* Corresponding author at: Section on Viral Gene Regulation, Program in Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Building 6B, Room 216, 6 Center Drive, Bethesda, MD 20892-2780, USA. Tel.: +1 301 496 1970; fax: +1 301 496 0243.
E-mail address: levinju@mail.nih.gov (J.G. Levin).

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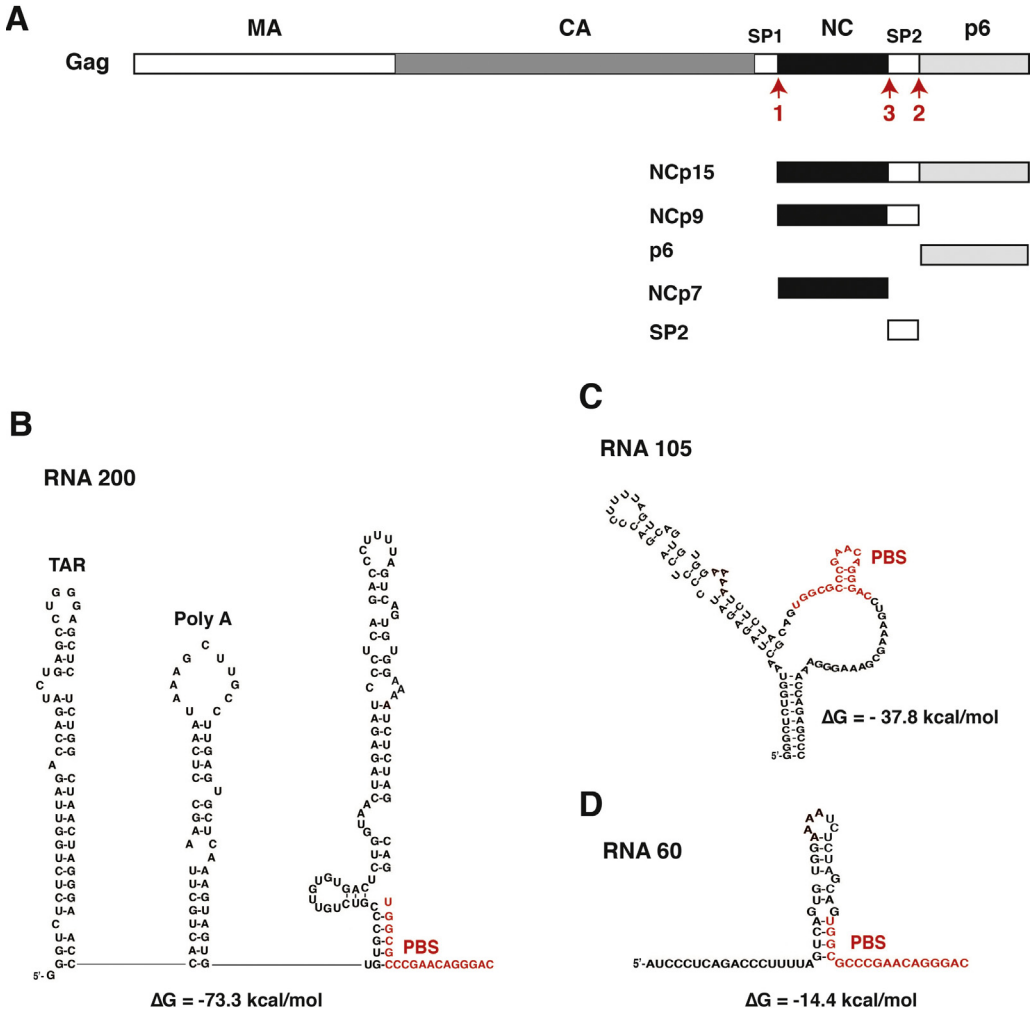

Fig. 1. Schematic representation of NC proteins and RNA templates used in this study. (A) Proteins produced by C-terminal cleavage of Gag. HIV-1 Gag is shown with each domain indicated by rectangles depicted as follows: MA, open; CA, dark gray; spacer peptide 1 (SP1), open; NCp7, closed; SP2, open; p6, light gray. The proteins derived from the Gag C-terminus, i.e., NCp15, NCp9, p6, NCp7, and SP2, are also shown. The red arrows labeled 1, 2, and 3 refer to the primary, secondary, and tertiary PR cleavages at the C-terminus of Gag (Swanstrom and Wills, 1997). (B–D) Sequence of RNA templates and secondary structure, based on mFold analysis (Zuker, 2003): (B) RNA 200; (C) RNA 105; (D) RNA 60. Two major structural elements, i.e., the TAR and Poly A stem-loops, are present only in RNA 200. The other two templates contain varying amounts of sequence upstream of the PBS and in each case, the PBS is largely unpaired. RNA 105 also has unpaired bases downstream of the PBS in addition to a short 10-bp stem formed with bases upstream and downstream of the PBS. The PBS sequence in each template is highlighted in red. The predicted ΔG values are shown beneath the structures. The diagrams are not drawn to scale.

conformations (Tsuchihashi and Brown, 1994) (reviewed in Darlix et al., 2011; Godet and Mély, 2010; Levin et al., 2005, 2010; Rein et al., 1998) (also see more recent Refs. Hergott et al., 2013; Mitra et al., 2013; Wu et al., 2013, 2014). Effective chaperone activity depends on three properties: (i) aggregation of nucleic acids, which is important for annealing (associated with the basic residues); (ii) moderate duplex destabilizing activity (associated with the ZFs); and (iii) rapid on-off nucleic acid binding kinetics (Cruceanu et al., 2006a) (reviewed in Levin et al., 2005, 2010; Mirambeau et al., 2010; Wu et al., 2010a). This activity plays a critical role in ensuring specific and efficient reverse transcription and mediates primer placement, i.e., annealing of the $tRNA^{Lys3}$ primer to the viral RNA genome, synthesis of (−) strong-stop DNA [(−) SSDNA], and minus- and plus-strand transfer (Levin et al., 2005, 2010).

In addition to studies on the mature NC protein, the biological activity of the immediate NCp7 precursors, NCp15 and NCp9, has also been investigated. Under normal conditions, mature, infectious HIV-1 virions do not contain NCp15 and NCp9, which are transient intermediates in the virus assembly pathway (Henderson et al., 1992). In fact, blocking both C-terminal cleavage sites in Gag required for NCp15 processing, abolishes viral infectivity (Coren et al., 2007; de Marco et al., 2012) and results in assembly of virions with abnormal morphology (de Marco et al., 2012). If the cleavage site between NCp7 and SP2 is blocked giving rise to NCp9, there is some reduction (<2-fold) in the number of particles that exhibit WT morphology (de Marco et al., 2012; Ohishi et al., 2011), but there is no clear consensus as to whether mutant virions are infectious, possibly due to differences in the constructs used or the different mutations used to maintain NCp9. For example, in one study it was reported that NCp9 mutants produced very little early viral DNA (<10%), implying that the virions were replication-negative (Ohishi et al., 2011), and in another, it was shown that blocking release of SP2 completely abolished replication (Kafaie et al., 2009). In contrast, other investigators have observed that NCp9 mutant virions were infectious in a single-cycle assay (Briggs and Kräusslich, 2011; Coren et al., 2007; Müller et al., 2009). However, in one study it was found that after four weeks in cell culture, normal processing was restored and the particles contained NCp7 instead of NCp9 (Coren et al., 2007). Studies on the behavior of NCp15 and NCp9 in several different experimental contexts, e.g., single-molecule DNA stretching (Cruceanu et al., 2006b), electron microscopic imaging of NC-DNA complexes (Mirambeau et al., 2007), biophysical and