

POSTER 8**A NEW FUNCTION FOR THE NUCLEIC ACID CHAPERONE ACTIVITY OF HIV-1 NUCLEOCAPSID PROTEIN IN REVERSE TRANSCRIPTION: INHIBITION OF NON-SPECIFIC PLUS-STRAND DNA SYNTHESIS**

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During minus-strand DNA synthesis, RNase H degrades viral RNA sequences, generating potential plus-strand DNA primers. However, selection of the 3' polypurine tract (PPT) as the exclusive primer is required for formation of viral DNA with the correct 5' long terminal repeat end and for subsequent integration. Here we describe a new function for the nucleic acid chaperone activity of HIV-1 nucleocapsid protein (NC) in reverse transcription, i.e., blocking mispriming by non-PPT RNAs. Three representative 20-nt RNAs from the PPT region were tested for primer extension. Each primer had activity in the absence of NC, but less than the PPT. NC reduced priming by these RNAs to essentially base-line level, whereas PPT priming was unaffected. RNase H cleavage and zinc coordination by NC were required for maximal inhibition of mispriming, although a modest effect of NC in the absence of RNase H was also observed. Importantly, a combination of different optical techniques (circular dichroism, fluorescence anisotropy, and temperature-dependent UV absorption) performed with short duplexes, demonstrated that the PPT is distinct from non-PPT complexes, in accord with the biochemical data. Specifically, while all non-PPT duplexes adopt an A-form helix, the PPT duplex resembles a B-form conformation. Moreover, RT binds with significantly higher affinity to the PPT duplex, relative to the non-PPT duplexes. The PPT duplex is also unusually stable and resistant to NC-induced destabilization. This result can explain the inability of NC to affect PPT priming. Taken together, our findings demonstrate that NC's nucleic acid chaperone activity has a major role in ensuring the fidelity of plus-strand priming and reveal a potential new target for antiviral therapy.

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