POSTER 9

INVASION-MEDIATED STRAND TRANSFER DURING HIV-1 REVERSE TRANSCRIPTION MUST BE COMPLETED OVER SHORT DISTANCES AND REQUIRES VARIOUS MODES OF RNASE H CLEAVAGE BY RT

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Influences on the mechanism of HIV-1 reverse transcriptase (RT)-catalyzed strand transfer were analyzed. Strand transfer begins as RT degrades the RNA template (donor) concomitantly with primed DNA synthesis. Donor cleavage leads to gaps of ssDNA where a second RNA (acceptor) invades and hybridizes with the DNA primer. The acceptor-primer hybrid expands and drives transfer of the DNA 3′ terminus to the acceptor. DNA synthesis and RNA hydrolysis by RT were assayed using a unique RNA-DNA hybrid substrate containing a 20 nt gap of ssDNA that functioned as a pre-created invasion site (PCIS). Previous work with the PCIS substrate showed that acceptor-primer hybridization initiating at the PCIS advances only through short lengths of donor-primer hybrid. Donor RNA hydrolysis by RT and chaperoning by nucleocapsid protein (NC) increased the effective distance of the PCIS. Current work examines the contributions of various modes of RNase H cleavage by RT on the creation of invasion sites and subsequent steps. RT can cleave the RNA in polymerization-dependent (pol-dep), polymerization-independent (pol-ind), primary, and secondary modes of cleavage. Results indicate that with or without NC all modes of RNase H cleavage support invasion site formation. In the presence of NC, only nicks generated by pol-dep, primary cleavages enhanced acceptor-primer hybrid propagation initiated at the PCIS. Results suggest that after invasion an acceptor-primer intermediate efficiently propagates with assistance from NC and only nicks in the donor RNA. Slowing RT synthesis on the PCIS substrate while excluding RNase H activity yielded no change in strand exchange efficiency. Allowing RNase H cleavage led to increased transfer efficiency. Moreover, predictions by random walk modeling indicate an accelerated increase in time required for forward movement of branch migration. Strand exchange results are consistent with random walk predictions suggesting a precipitous decrease in branch migration rate with increasing distance.