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POLYPURINE TRACTS (PPTs) ARE DESIGNED FOR HIGH AFFINITY BINDING TO THEIR COGNATE REVERSE TRANSCRIPTASES, A PROPERTY THAT CAN BE EXPLOITED TO PRODUCE NOVEL PPT-BASED ANTIVIRALS

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Retroviruses carry purine-rich segments in their RNA genomes known as polypurine tracts (PPTs) that are used by reverse transcriptases (RT) for initiation of second strand synthesis. Because RT has to identify and selectively bind this short nucleotide sequence, it is not surprising that the PPT may also be optimized for the tightest possible binding to RT (DeStefano and Cristofaro, *Nucleic Acids Res.* 34:130). Systematic Evolution of Ligands by Exponential Selection (SELEX) has been used by several groups to identify high affinity RNA and DNA aptamers to RT and other proteins from large random nucleotide pools. Using a unique SELEX approach, we demonstrated that HIV-RT has a very strong sequence preference for specific primer-templates. The selected DNA primers closely resembled the HIV RNA PPT (5'-AAAAGAAAAGGGGGG-3'), raising the possibility that RTs and their cognate PPTs may have co-evolved for tight binding and proper orientation of RT for extension. Conceivably, because of the stringent PPT requirement by RT for priming second strand synthesis, resistance to inhibitors that closely resemble the PPT may be rare. To this end, we are currently testing closely related retroviral RTs from Moloney murine leukemia virus (Mo-MuLV, PPT: 5'-AGAAAAAGGGGGG-3') and avian myeloblastosis virus (AMV, PPT: 5'-AGGGAGGGGGA-3'), as well as the LTR-retrotransposon Ty3 that has a significantly different PPT (5'-GAGAGAGAGGAA-3') which does not contain homopolymeric nucleotide runs. These RTs are being used in SELEX experiments to see if they, like HIV-RT, also select for cognate PPT sequences. Binding affinity assays with synthetically designed retroviral DNA-PPT substrates are also being conducted. Data indicates that MuLV- and HIV-RTs have similar affinities for one another's DNA-PPTs which they bind 25-100 fold more tightly than random sequence primer-template. They have lower affinity for the slightly divergent AMV DNA-PPT, and even lower for the highly dissimilar Ty3 DNA-PPT. In contrast, AMV-RT binds its own DNA-PPT more tightly than those from MuLV or HIV. Finally, single stranded loop-back aptamer inhibitors of HIV-RT based on our SELEX experiments are strong RT inhibitors in vitro, and ongoing cell culture experiments indicate they also inhibit virus replication, thus opening up an exciting new vista of possibilities.