

**POSTER 16**

## NOVEL RNA/DNA SUBSTRATES FOR THE CHARACTERIZATION OF RIBONUCLEASE H ACTIVITY

Tatiana Ilna and Michael A. Parniak

Department of Microbiology &amp; Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Ribonuclease H (RNH) is a nuclease that selectively degrades the RNA strand of an RNA/DNA heteroduplex. RNH activity has been identified in all organisms although its role in replication is still not certain. However, the importance of RNH in retroviral replication has stimulated increasing interest in the study of reverse transcriptase-associated RNH (RT-RNH) structure and function, and as a potential target for antiretroviral drug discovery and development. While RNH carries out a seemingly simple hydrolytic reaction, RT-RNH catalyzed hydrolysis of RNA in fact can be quite complex, comprising several different modes including "polymerase-dependent" or 3'-directed RNH cleavage, "polymerase-independent" or 5'-directed RNH cleavage, and non-directed RNH cleavage. These modes also include primary and secondary cuts. We have designed a variety of RNA/DNA heteroduplexes that enable facile observation of the position and kinetics of the various modes of RT-RNH (and other RNH's) cleavage events, including primary and secondary cleavage events. These heteroduplexes utilize an RNA strand labeled at the 5'-end with Cy3 and at the 3'-end with Cy5. We used these various substrates to compare the cleavage patterns produced by RT-RNH, the catalytically active RNH domain fragment from HIV-1 RT, human RNH, and *E. coli* RNH. Each of these enzymes provided specific, yet different cleavage patterns on any given substrate, possibly related to the sequence specificity for RNH-catalyzed RNA hydrolysis. We also used the 3'-directed substrates to show that HIV-1 RT-RNH cannot cleave the RNA template during processive DNA synthesis catalyzed by HIV-1 RT DNA polymerase. Finally, we have used these substrates to characterize the mechanism of inhibition by a variety of RT-RNH inhibitors developed in our laboratory, and found that certain classes of inhibitors preferentially inhibit the "polymerase-independent" or 5'-directed cleavage events. We feel that our substrates may have particular value in which of the RT-RNH cleavage modes may represent the best target for antiretroviral drug discovery.

Acknowledgement: This research was supported by NIH grants AI073975 and AI077424 (to MAP).