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**Structural Determinants of HIV-1 5'-UTR in Virions and Infected Cells**

According to the most recent UNAIDS/WHO report, an estimated 33 million people worldwide were living with AIDS at the end of 2007, which caused 2 million deaths in 2008 alone. The effectiveness of the standard treatment for HIV infection, which consist of highly active-antiretroviral therapies (HAART), is undermined by the constant selection of drug-resistant strains. A possible unexplored target for the development of new therapeutic strategies is represented by the 5'-untranslated region (5'-UTR) of the viral RNA. This non-coding region governs key replication steps in which viral RNA acts either as genome intended for packaging, or as mRNA meant for protein expression. We plan on continuing our investigation of the structure-function relationships of 5'-UTR to understand the regulatory mechanisms that coordinate its multifaceted functions in the two distinct spheres of activity. Over the years, we have developed an approach based on structural probing and mass spectrometric detection (MS3D) to enable the structure elucidation of substrates that are not amenable to the established high-resolution techniques. Using this approach, we have determined the structure of different conformers assumed *in vitro* by 5'-UTR, which could constitute the basis for a regulatory riboswitch mechanism. We now plan on investigating the biological significance of these isomeric forms by performing structural probing directly in virions and infected cells, which will be supported by the ability of bifunctional probes to permanently crosslink any RNA or protein species that may come into mutual contact in the different viral/host environments. *In situ* crosslinking of juxtaposed functional groups will provide detailed snapshots of the actual RNA-RNA and protein-RNA interactions that define pertinent ribonucleoprotein complexes (RNPs) involved in 5'-UTR activities. Comparing the results obtained by probing the viral RNA in virions and selected subcellular compartments will allow us to investigate the architecture of 5'-UTR engaged in genomic or mRNA activities. The possibility of examining simultaneously the putative 5'-UTR structures and their protein binding preferences will provide valuable information about the recognition determinants of different viral/cellular factors *in vivo*. Further, studying how structural rearrangements affect the selection of such factors will offer unique insights into the structure-function relationships of 5'-UTR as a function of viral/cellular context. The survey of cellular factors interacting with specific signals will guide the design of small interfering RNAs (siRNAs) that will be used in multiplexed fashion to study the interactions involving a specific domain. The detailed maps of molecular contacts in the different contexts will provide the basis for mutagenesis studies involving multiple correlated sites to assess the role of specific interactions in controlling 5'-UTR activities. The proposed research will provide a comprehensive framework for rationalizing current structural and functional knowledge of 5'-UTR. On one hand, this framework will help validate the high-resolution structures obtained *in vitro* for isolated domains, will determine their contributions to the alternative folds assumed by full length 5'-UTR, and will confirm their direct interactions with cognate proteins *in vivo*. On the other hand, this information will provide the basis for understanding the extensive synergies and cross-talk between domains, which have been reported over the years. Correlating any variations of higher-order structure and intermolecular association with distinctive biological activities will provide the information necessary to evaluate the possible role of viral/host factors in coordinating the different functions of 5'-UTR. This information will enable the formulation of new therapeutic strategies aimed at interfering with 5'-UTR functions and regulation, which would constitute very desirable complements to current treatments endangered by the constant selection of resistant strains.