

POSTER 18**IDENTIFICATION OF PSEUDOKNOT STRUCTURES IN THE RNA TRANSPORT ELEMENT OF MURINE LTR RETROTRANSPOSON TYPE D WITH HIGH THROUGHPUT CHEMICAL PROBING MODULATED WITH ANTISENSE LNAs**

Michal Legiewicz¹, Bruce A. Shapiro², Hugo Martinez³, Wojciech Kasprzak⁴, Hiroaki Uranishi⁵, Andrei Zolotukhin⁵, George Pavlakis⁶, Barbara Felber⁵ and Stuart Le Grice¹

¹RT Biochemistry Section, HIV Drug Resistance Program, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702; ²Nanobiology Program, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702; ³Nanobiology Program, Center for Cancer Research - Contract, Frederick, MD 21702; ⁴Basic Research Program, SAIC-Frederick, Inc., Frederick, MD 21702; ⁵Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702; ⁶Human Retrovirus Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702

Replication of retroviruses and transposition of endogenous retroelements depend on complex posttranscriptional regulation to export the full-length mRNA. The presence of a distinct RNA export element is essential for nuclear export. These RNA transport elements, utilizing either CRM-1 or NXF-1 nuclear receptors, have been attractive targets for disruption in antiviral therapies. Studying their structure and interactions with cellular or viral factors is bringing not only deeper insights into the evolution of such elements but also a promise in the fight against relevant diseases. Retrotransposons are genetic elements that amplify themselves via RNA intermediates and are frequent in eukaryotic genomes. In mammals, about a half of the genome comprises transposons or their leftovers. In this work, we focus on the structure of a RNA transport element that is crucial for nuclear export of endogenous murine long terminal repeat retrotransposon type D, named MusD. We applied innovative SHAPE technology to map the secondary structure of MusD. SHAPE assesses local flexibility in RNA by analyzing reactivity of the 2'-hydroxyl group with a chemical agent (NMIA), that allows distinguishing between single and double stranded regions with single nucleotide resolution. Using the SHAPE data set, we constrained an RNA folding algorithm to calculate a MusD secondary structure. Although a majority of the structure agreed well with the SHAPE reactivities, a few tracts were not predicted properly. In order to improve SHAPE's agreement with the secondary structure we manually rearranged a domain of the RNA to introduce pseudoknot structures. The presence of the pseudoknots significantly improved the agreement of the SHAPE data with the obtained structure. Proposed interactions have been validated with SHAPE-LNA Integrated Modulation (SLIM) targeted to pseudoknots. We have undertaken the task of computationally modeling the proposed MusD structure. Using the software 'RNA2D3D', we interactively modeled from the secondary structure representation a three-dimensional model. The 3D structure, although putative, revealed that the proposed pseudoknots are plausible in three-dimensions and allows us to use it as a working representation for further consideration.