

## POSTER 17

### AISHAPE RESOLVES ARCHITECTURE OF TERTIARY INTERACTIONS WITHIN THE RNA EXPORT ELEMENT OF MURINE LTR RETROTRANSPOSON

Michal Legiewicz<sup>1</sup>, Hiroaki Uranishi<sup>2</sup>, Andrei Zolotukhin<sup>2</sup>, Guy Pilkington<sup>2</sup>, George Pavlakis<sup>3</sup>, Barbara Felber<sup>2</sup>, and Stuart Le Grice<sup>1</sup>

<sup>1</sup>RT Biochemistry Section, DRP, NCI; <sup>2</sup>Human Retrovirus Pathogenesis Section, NCI; <sup>3</sup>Human Retrovirus Section, Vaccine Branch, NCI

Nuclear export of retroviral or endogenous retrotransposon RNAs is an essential step in the replication cycle of these retroelements, requiring direct interaction between protein factors and distinct structural domains within viral and retrotransposon mRNA(s). Studying the structures of these RNA domains and their interactions with cellular or viral factors not only provides insights into the evolution of such elements, but also offers the potential for development of novel therapeutic agents. We resolve the secondary structure of one such domain that is crucial for nuclear export of endogenous murine LTR retrotransposon, type D (MusD) RNA using a biochemical mapping technique, SHAPE (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension). This approach is based on selective chemical modification of RNA at the 2'-hydroxyl groups of constituent nucleotides, where the degree of reactivity is determined by local flexibility. Using this technology, a pair of kissing loops and a complex dual pseudoknot within the region of MusD implicated in nuclear export were identified. The existence of these motifs was verified by conventional mutational analysis and aiSHAPE (antisense interfered SHAPE), a novel extension of SHAPE in which long-range intramolecular interactions are probed with thermo-stable antisense oligonucleotide probes. The functional importance of the kissing loops and pseudo-knots *in vivo* was confirmed in cell culture experiments involving relevant mutants. Mutations disrupting kissing loop or pseudoknot interactions caused inactivation of nuclear export.