

POSTER 19**DIRECT PROBING OF RNA NUCLEAR EXPORT MOTIFS BY SHAPE AND 3D MASS SPECTROMETRY**

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Export of mRNAs, retroviral RNAs, or retrotransposon RNAs from the nucleus requires the recognition of one or more RNA sequence and/or structural elements by nuclear export proteins. Unfortunately, while genetic experiments have helped determine which RNA motifs are essential for nuclear export, structural analysis of the RNA motifs involved in this process has proven difficult, primarily because functional motifs are typically too large for resolution by NMR and/or X-ray crystallography. To address this problem, our goal has been to develop efficient and reliable biochemical and biophysical methodologies to complement more established techniques for high-resolution RNA structure determination.

SHAPE, an acronym for Selective 2'-Hydroxyl Acylation analyzed by Primer Extension, is one such methodology. This technology allows for a rapid and quantitative distinction between constrained and flexible domains of RNA with single nucleotide resolution. We have used SHAPE to resolve the secondary structures of RNA Transport Elements (RTEs) - conserved motifs within intracisternal A-particle retroelement RNAs required for export of these RNAs from the nucleus. Two genetically distinct RTE subgroups, RTE-A and RTE-D, have been shown to interact with a common set of nuclear export proteins. Homologous core regions of RTE-A (241 nucleotides) and RTE-D (270 nucleotides) were analyzed. Despite only moderate sequence homology (70%), the secondary structures of the two elements contained several features in common, and are in good agreement with models obtained using computational RNA folding algorithms.

Having established the secondary structure(s) of the two motifs, these elements are currently being analyzed by mass spectrometric three-dimensional approaches (MS3D). By coupling chemical crosslinking of folded RNAs with electrospray ionization Fourier transform mass spectrometry (ESI-FTMS), we are able to obtain information about the spatial relationships among contiguous RNA secondary structural elements. Probed RNAs are treated with ribonucleases and analyzed by ESI-FTMS to obtain the correct position of chemically crosslinked nucleotides. This information, coupled with SHAPE data, is being used to generate 3D models via the constraint satisfaction algorithm provided by Mc-Sym and the energy minimization modules included in CNS. In recent work, the HIV-1 Rev-response element (RRE, 233 nucleotides) was been generated using a combination of SHAPE and MS3D approaches.