Probing the CPEB3 structure by NMR

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This work is aimed at the investigation of the nuclear magnetic resonance (NMR) solution structure and folding mechanism of the cytoplasmic polyadenylation element binding protein 3 (CPEB3) ribozyme to better understand its catalytic activity. Ribozymes are RNA molecules that act as chemical catalysts in the cells. The discovery of ribozymes was a milestone in RNA research and disclosed the unique role of RNA in a multitude of cellular reactions. The CPEB3 ribozyme is until now the only confirmed ribozyme in mammals and its role remains still elusive.[1] As RNA function is directly linked to structure, structural studies are the basis to understand RNA function.

The CPEB3 secondary structure belongs to the Human Delta Virus (HDV)-like family of self-cleaving ribozymes.[2] Therefore it is suggested that the mechanism of self-cleavage reaction of the CPEB3 ribozyme is very similar to the one of the HDV ribozyme. The cleavage reaction of the HDV follows a Mg^{2+} facilitated acid-base mechanism that is based on a perturbed pK_a of the conserved cytosine C75 in the catalytic core. In analogy to C75 of the HDV, the CPEB3 ribozyme contains a conserved cytosine C57 that might also have an elevated p*K*a value, and could be therefore directly participating in the cleavage reaction. Therefore we started to determine the pK_a value of C57 in the absence and in the presence of Mg^{2+}. The first pH-titrations results of chimp CPEB3 construct with and without Mg^{2+} did not show a shift in pK_a. Another goal of this work is the elucidation of the NMR solution structure of the CPEB3 ribozyme. Using various truncated constructs and labeling schemes in multinuclear and multidimensional NMR spectroscopy, the vast majority of resonances could be unambiguously assigned. We are now introducing 5-fluoro-uridine-5'-triphosphate into the construct using in vitro transcription and apply ^{19}F NMR spectroscopy to support and facilitate the assignment, on the one hand, and on the other hand, to allow to directly follow the folding of the CPEB3 into its active state. These results will be the basis to understand the individual steps of the ribozyme work on a structural level and maybe help to enlighten the biological role of the CPEB3 ribozyme.

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