Using “old methods” to solve new structures: crystallization of the human CPEB3 ribozyme

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Catalytically active RNA called ribozymes play substantial roles in a variety of biological functions including protein synthesis (ribosome), tRNA maturation (RNase P) and RNA splicing (group I and II introns). Ribozymes are much more widely distributed than previously thought and understanding their structure and function is the focus of many studies. One of the best studied ribozymes is the hepatitis delta virus (HDV) ribozyme originally identified in the human pathogen. So far, the HDV ribozyme is the fastest naturally occurring ribozyme known with a cleavage rate of more than 1 per second at 65°C (**1**). Its crystal structure was reveled in 1998 revealing the complex fold in a nested double pseudoknot with 5 paired regions that form two coaxial stacks, which are linked by single-stranded joining strands (**1**). In 2006, the genome-wide search identified the human cytoplasmic polyadenylation element-binding protein 3 (hCPEB3) ribozyme as the first HDV-like ribozyme (**2**). This small ribozyme (68 nucleotides) is located within the second intron of a single-copy gene and is highly conserved in all mammalians. Its exact function is yet unknown but it may be involved in the regulation of CPEB3 mRNA stability. However, CPEB3 proteins are known to be crucial for synaptic plasticity and memory (**3**). Most of our knowledge on the CPEB3 ribozyme folding and activity is based on comparative studies with the HDV ribozyme (**4**). Like all HDV-like ribozymes, CPEB3 ribozymes folds into a double-pseudoknot structure (**5**) and possesses an active-site cytosine (C57) located in the J4/2. However, the hCPEB3 ribozyme cleavage rate is much slower compared to HDV ribozyme. Even if, the primary nucleotide sequence of both ribozymes are very different (with exception of severals nucleotides including catalytic cytosine), their secondary structures and their role in separation of multimeric precursor claimed the new hypothesis that HDV ribozyme may have arise from ancestral hCPEB3 (**6**).

In order to better understand the complex folding that leads to cleavage activity of hCPEB3 ribozyme, we aim o solve the three-dimension structure by X-ray crystallography. However, many factors affect crystallizability of RNA sample including purity, homogeneity, ligand binding and structural dynamics. In order to stabilize the structure of hCPBE3 and to improve its crystallization, we follow different strategies which will be presented.

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