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Critical Choice of RNA Molecules in Crystallization and Structure Solution of An RNA-Splicing Endonuclease Complex. Hong Li, Song Xue, Dept. of Chemistry and Biochemistry, Florida State Univ., Tallahassee, FL.

We will describe the efforts in co-crystallization and molecular replacement solution of an RNA-splicing endonuclease complex. The splicing endonuclease exhibits exquisite specificity in the recognition of RNA substrates, leading to the alteration of two RNA phosphodiester bonds. The majority of archaeal splice sites are located on a pseudosymmetric RNA motif that consists of two three nucleotide bulges separated by a four-basepair helix (bulge-helix-bulge, BHB). A number of challenges face the structural studies of the endonuclease-RNA complex. First, common protein-RNA interaction assays do not work for this system. Second, appropriate chemical modifications on RNAs are required to inhibit the splicing reaction during crystallization while maintaining sufficient binding energy of the RNAs for the enzyme. Thirdly, it is difficult, yet highly desirable, to capture structures of reaction intermediates by crystallography. We have obtained crystals of an endonuclease in complex with either an RNA inhibitor or cleavage products by combining enzyme activity assays and crystallization screening. Crystals of the current RNA-endonuclease complex diffracted to ~ 4.0 Å with severe anisotropy. However, a molecular replacement solution by using previously determined endonuclease structure could be obtained. Packed endonucleases in the C2 crystal leave significant packing gaps that are presumably filled by the bound RNA. Despite the significant progress, a model for the bound RNA could not be built at the moment because its electron density appears to be smeared. Close examination on RNA oligos used for co-crystallization suggests that this is likely due to the alternating arrangements of the pseudosymmetric BHB RNA with respect to the crystal lattice. Structure solution of a new crystal containing a 21mer symmetric BHB RNA and the endonuclease should provide the final proof of this hypothesis. We hope that our experiences in splicing endonuclease-RNA complex structural studies will help others who are engaged in similar studies of RNA-enzyme complexes.