

# Self Cleavage by the Hepatitis $\delta$ Virus Ribozyme

## Bimolecular Structure Project

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The hepatitis delta virus (HDV) is a RNA virus that has a single stranded and circular genome of about 1.6 kb. It is a defective virus and requires co-infection of the host with hepatitis B for replication. The genome encodes two interrelated proteins. The small delta protein is 195 aa in length and is required for viral replication. The larger protein product is 214 aa in length, inhibits viral replication and is required for capsid assembly. The virus replicates by direct transcription of the genomic RNA from an anti-genomic RNA template by the rolling circle process. The HDV ribozyme cleaves unit length RNA transcripts from the transcriptome during replication. There are two other curiosities in the replication of the viral genome. It is unclear how the ends of the rna are ligated to form a circular genome. A second problem is that upon ligation, a complete ribozyme sequence is created. It is thought that the structure of the ribozyme in the genome is different than during replication. A second ideas is that the 214 aa protein may inhibit ribozyme activity.

Crystallization of the ribozyme required engineering a RNA binding protein domain into a non-catalytic region of the ribozyme. The ribozyme was then co-crystallized with the U1A RNA binding protein (Fig 1). The crystals were solved at a resolution of 2.3 angstroms. Two interwoven pseudoknots dominate the structure of the ribozyme (Fig 2). Primarily Watson and Crick base pairings stabilize the pseudoknot structures, but there are non-canonical interactions as well. In Figure 3, there are two types of non-canonical interactions shown. In the left hand panel the cytosine is hydrogen bonded to oxygen on the backbone phosphate group. The right hand panel shows two different non-canonical interactions. There is a hydrogen bond between a base and a ribose sugar and a non-Watson and Crick hydrogen bond between two bases.

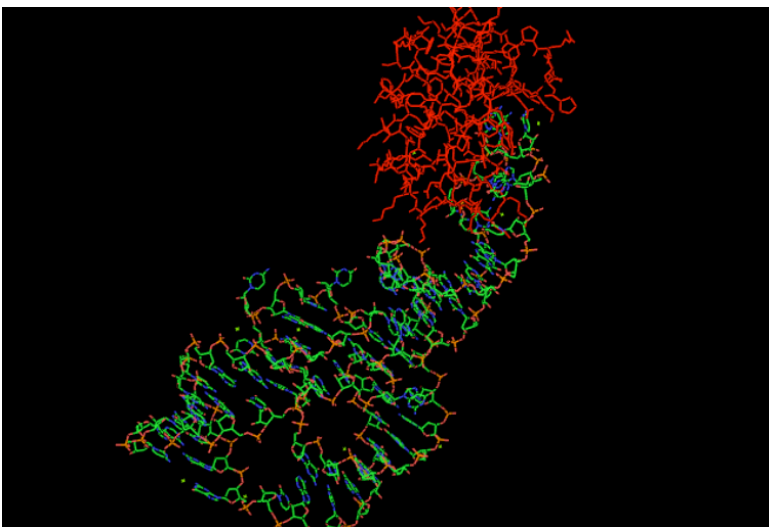


Figure 1: Co-crystallization of the HDV ribozyme and U1A rna binding protein ( U1A is red, HDV ribozyme is green)

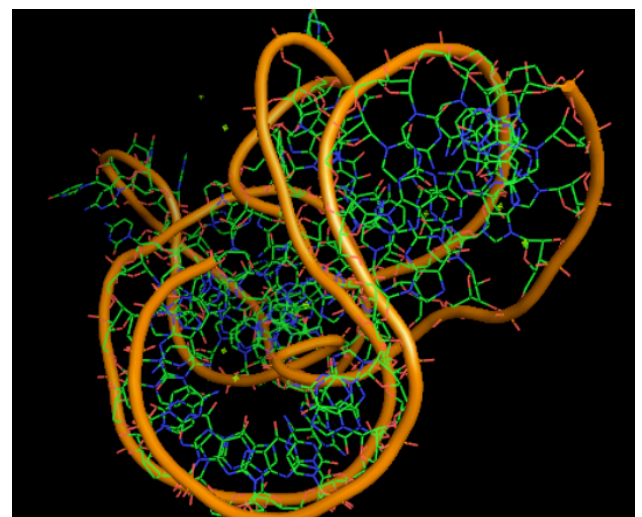
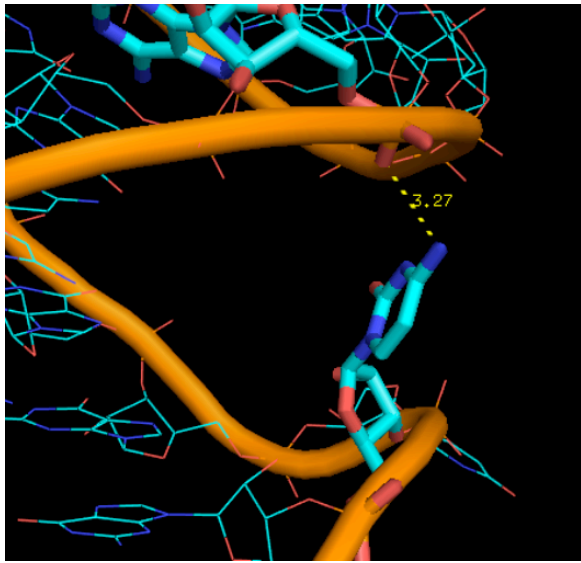


Figure 2: The double pseudoknot structure of the HDV ribozyme. The phosphate backbone is shown in orange to highlight the structure



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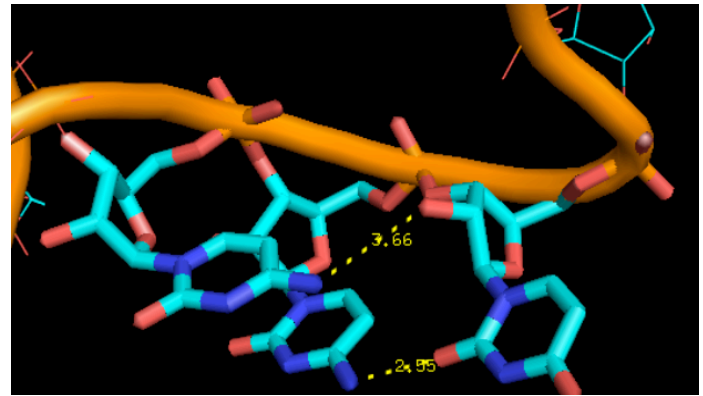


Figure 3: Non-canonical base pairing helps stabilize the pseudoknots. (A) A hydrogen bond between cytosine and phosphate backbone (B) Non-Watson and Crick hydrogen bonds between bases

A magnesium ion in the active site mediates the catalytic activity of the ribozyme. The magnesium atom is coordinated to four bases U20, C75, U23 and G1 (Fig. 4). Water molecules fill the remaining coordination sites and bridge the bond to U23. During catalysis the non-coordinated nitrogen on C75 forms a partial bond with the hydrogen on the 2' hydroxyl of U-1. The 2' hydroxyl oxygen also forms a partial bond with the phosphorous atom of the phosphate group (Fig. 5). Resolution of the transition state involves the concerted breaking and formation of several new bonds. The nitrogen on C75 abstracts the proton from the 2' hydroxyl on U-1 and the 2' oxygen forms a bond with the phosphorous atom of the phosphate group. This breaks the oxygen-phosphorous bond of the 3' oxygen on G1, cleaving the phosphodiester backbone and releasing the full-length genome from the rolling circle transcription complex. It is unclear if the 3' oxygen on G1 abstracts a proton from a water molecule coordinated to the magnesium ion or from the newly protonated nitrogen on C75.

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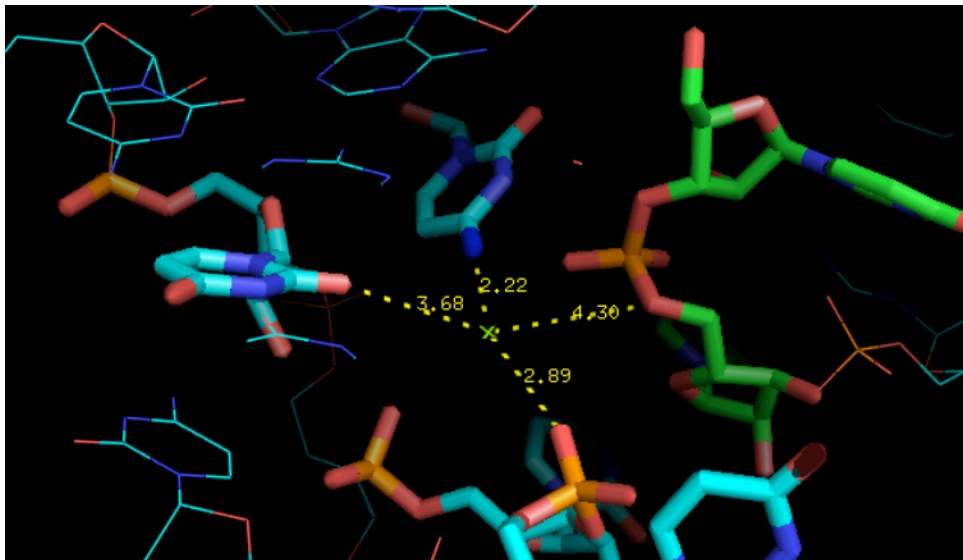


Figure 4: The active site of the precursor ribozyme. The magnesium atom represented by the green "x" in the middle. Cytosine 75 is in the middle towards the back. Uracil 20 is on the left and Uracil 23 is on the bottom. The bond to Guanine 1 is on the right and bridged by a water molecule. (The water is not shown) Water molecules satisfy the remaining co-ordination sites. (not shown)

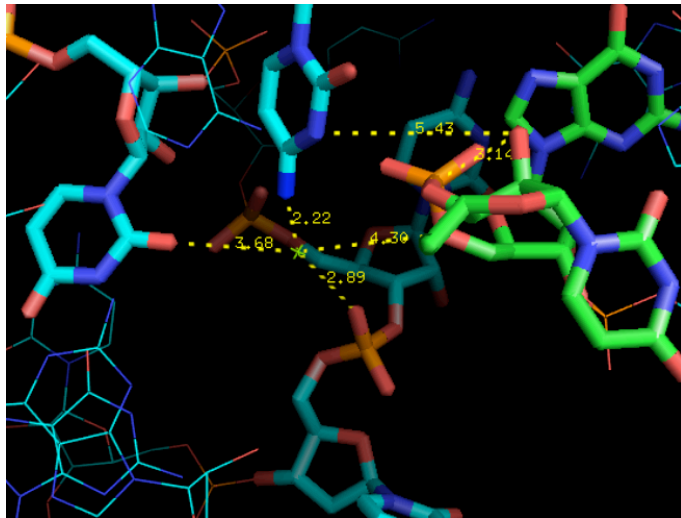


Figure 5: The transition state for cleavage of the phosphodiester backbone. The hydrogen on the 2' hydroxyl of U-1 forms a partial bond with the nitrogen of C75 and the oxygen forms a partial bond with the phosphorous of the phosphate group. In resolution of the structure, the hydrogen is abstracted by C75 and the 2' oxygen forms a complete bond with the phosphorous atom. This breaks the bond between the phosphorous and the 3' oxygen on G1 and releases the full length genome from the transcription complex.

After cleavage of the phosphodiester backbone, the magnesium atom is kicked out of the active site and C75 is shifted slightly down and to the right. A more dramatic change occurs with U23 (Fig. 6). This base flips nearly  $180^\circ$  from its position in the transition state. The phosphate group for this base is coordinated to the magnesium atom in the active site. When the magnesium atom is kicked out of the active site the negatively charged oxygen is no longer coordinated. The phosphate group presumably rotates or inverts so this negative charge can be stabilized through hydrogen bonds with water. This process is probably responsible for the large conformational change observed for U23.

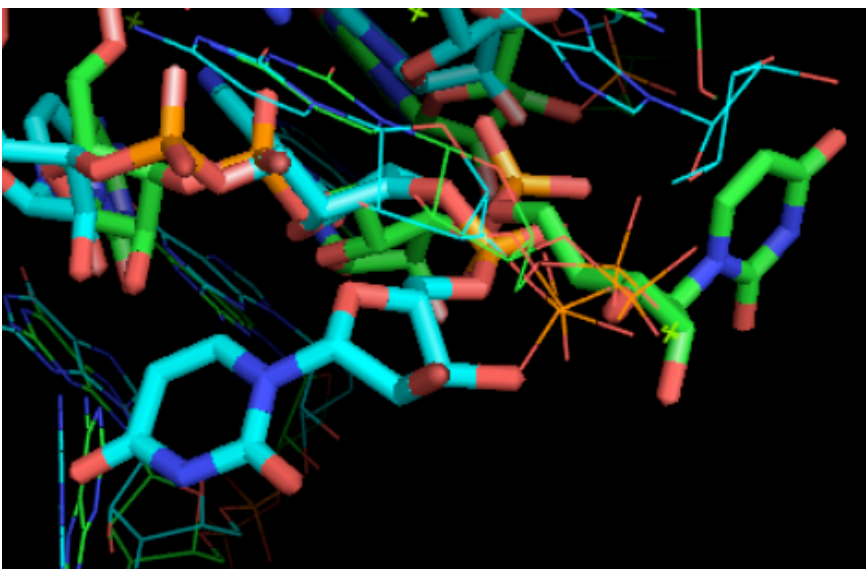


Figure 6: Uracil 23 flips  $180^\circ$  from the precursor (green) to the cleaved state (blue). This probably mediated by inversion of the phosphate group that is no longer coordinated to the magnesium atom in the active site.

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