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HDV Ribozymes

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Abstract The self-cleaving RNA sequences, or ribozymes, in the genomic and antigenomic strands of hepatitis delta virus (HDV) RNA fold into structures that are similar to each other but distinct from those of small ribozymes associated with the RNA replicons that infect plants. HDV ribozymes have provided a tractable system for studying the mechanism of catalytic RNA, and results of biochemical and structural studies on the HDV ribozymes, from a number of labs, have enhanced our understanding and expanded our thinking about the potential for catalytic roles of RNA side chains. The results of these studies are consistent with models suggesting that both an active-site cytosine and a divalent metal ion have catalytic roles in facilitating the cleavage reaction in the HDV ribozymes. Despite recent advances, details about the catalytic mechanism of the HDV ribozyme continue to be debated, and these ribozymes should serve as a good system for further study.

1 Introduction

Self-cleavage sites in the RNA (ribozymes) of hepatitis delta virus (HDV) were hypothesized, identified, and initially characterized independently by Taylor and coworkers (Kuo et al. 1988; Sharmeen et al. 1988) and Wu et al. (1989). The ribozymes were proposed to process primary replication products, generated in rolling circle replication, to monomer size (Robertson 1992; MacNaughton et al. 1993; Lazinski and Taylor 1995a). Specific subfragments of each RNA strand were found to contain both the cleavage site and the sequences necessary and sufficient for self-cleavage (Kuo et al. 1988; Wu et al. 1989). Although the sequences associated with the cleavage sites in HDV RNA differed from the sequence that formed the familiar hammerhead and hairpin ribozyme motifs, the reaction was the same. Self-cleavage of the RNA backbone involves a rearrangement of the 3′,5′ phosphodiester bond to generate a 2′,3′-cyclic phosphate group and a 5′ hydroxyl group, suggesting nucleophilic attack of the adjacent 2′ hydroxyl on the scissile phosphate (Wu et al. 1989). The in vitro reaction required no protein or cellular factor but a divalent metal ion greatly stimulated cleavage rates (Wu et al. 1989). This chapter will review biochemical and structural data with emphasis on experiments and results that help us understand the catalytic mechanism used by the HDV ribozymes.

2 Sequence Requirements

2.1 Defining a Ribozyme Sequence

With examination of the HDV sequences required for self-cleavage, a native sequence of about 85 nucleotides was found sufficient for rapid cleavage and was defined as a minimal or core ribozyme domain (Perrotta and Been 1990, 1991). These core sequences, both genomic and antigenomic, could fold into similar secondary structures (Fig. 1) (Perrotta and Been 1991; Rosenstein and Been 1991). A single nucleotide 5′ to the cleavage site is sufficient for cleavage. The 3′ boundary is less precise in that the level of activity varied moderately—give or take a nucleotide or two at the 3′ end. Internal deletions that shorten the P4 duplex can reduce the minimum size to about 65 nucleotides before the rate of self-cleavage begins to be significantly reduced (Been et al. 1992; Thill et al. 1993).

The possible involvement or contribution of HDV RNA sequences that flank the core ribozyme remains of interest. In the process of elucidating