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Target site selection for RNA-cleaving DNAzymes from combinatorial libraries

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The 10-23 DNAzyme is capable of cleaving RNA with high sequence specificity at sites that contain purine-pyrimidine (R-Y) junctions. Although they are abundant in mRNA, many of these potentially cleavable junctions are protected from DNAzyme activity by secondary structure. To optimize the process of target-site selection in long RNA substrates, we have designed a strategy of identifying accessible cleavage sites in hepatitis C virus (HCV) nonstructural gene 3 (NS3) RNA from a pool of random DNAzymes. An active DNAzyme library, a pool of random sequences 58 nucleotides long that contained the previously identified 10-23 catalytic motif, were tested for their ability to cleave the target RNA. The library was incubated with target RNA in the absence of magnesium, and bound library pools were isolated and PCR-amplified. The active preselected library pool was incubated at 37 °C with target RNA in the presence of magnesium, and cleavage products were identified on sequencing gels by the reverse transcription primer extension method. The protocol identified 5 sequence-specific cleavage sites in the HCV target mRNA of 1.54 kb length. DNAzymes that were selected and targeted against these cleavage sites showed target-specific cleavage activities in the presence of magnesium only. This method could be applicable for locating accessible sites for other nucleic acid-based gene suppression strategies.