

P042 Conformational Investigation of the pre-mRNA Branch Site of the Group II Intron

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We are investigating solution structural features of the branch site region of the autocatalytic Group II intron. Group II introns are noncoding regions of RNA, which catalyze their own removal from pre-mRNA and ligate the flanking coding regions (exons) in certain protozoa and eukaryotic organelles. All components necessary for recognition and catalysis are contained within the six contiguous secondary structural domains (D1-D6). The nucleophile for the first cleavage step is the 2'OH of a specific adenosine residue within D6, called the branch site because of its branched product. Our goal is to examine structural features of the branch site region in the context of D6 alone as well as conformational changes upon tertiary interaction with other domains that contribute to activation of the nucleophile for catalysis. Results of solution NMR studies of a D6 construct synthesized by *in vitro* transcription were consistent with the predicted stem loop secondary structure. Addition of Mg(II) resulted in marked conformational change involving residues attributed to the internal loop. Results of fluorescence experiments of D6 fragments in which 2-aminopurine (2AP), a fluorescent analogue of adenine, was substituted for the branch site adenosine, indicated that the D6 branch site adenosine remains extrahelical in solution.