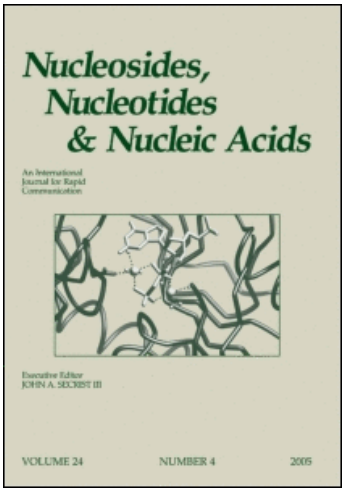

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^{1}H-NMR Assignment of Single Strand and Duplex Splice Domain of the Consensus Donor Exon:Intron Junction

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^{1}H-NMR ASSIGNMENT OF SINGLE STRAND AND DUPLEX SPLICE DOMAIN OF THE CONSENSUS DONOR EXON:INTRON JUNCTION

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Summary The high field ^{1}H-NMR assignments of a single strand consensus donor exon:intron junction and that of the duplex splice domain has been achieved using 2D-NMR and additional techniques.

Since the discovery that eucaryotic genes are fragmented and contain introns,[1-3] which are apparently not expressed,[1] between the functional exon regions there has been considerable interest in the nature, structure and function of the exon:intron junctions.[1-6] Examination of 139 sequences revealed that prototypical or consensus sequences exist for "donor" junctions (at the 5' end) and "acceptor" junctions (at the 3' end).[4] Since the splice site of a donor junction is G,C rich[5] it has been suggested as a potential target for alkylating anticancer drugs.[6] In order to examine its characteristics the following consensus junction was synthesized by the triester method[7] and

5'C - A - G - G - T - A - A - G - T3'

examined by ^{1}H-NMR at 400 and 500 MHz on Bruker WH and WM instruments on solutions containing ~20 mg oligomer/0.5 ml in 99.996% D_2O (40 mM potassium phosphate, 20 mM NaCl, pH 7.0) employing the following techniques. One dimensional NOE and 2D-NOESY methods, which revealed NOEs between the base and 1', 2'1, and 2'2 sugar protons, is most useful for assignment of sugar residues to their attached base as well as indicating connectivities between the sugar protons.[8] In addition 2D-COSY and ^{1}H-^{1}H INADEQUATE techniques show connectivity between the coupled pro-

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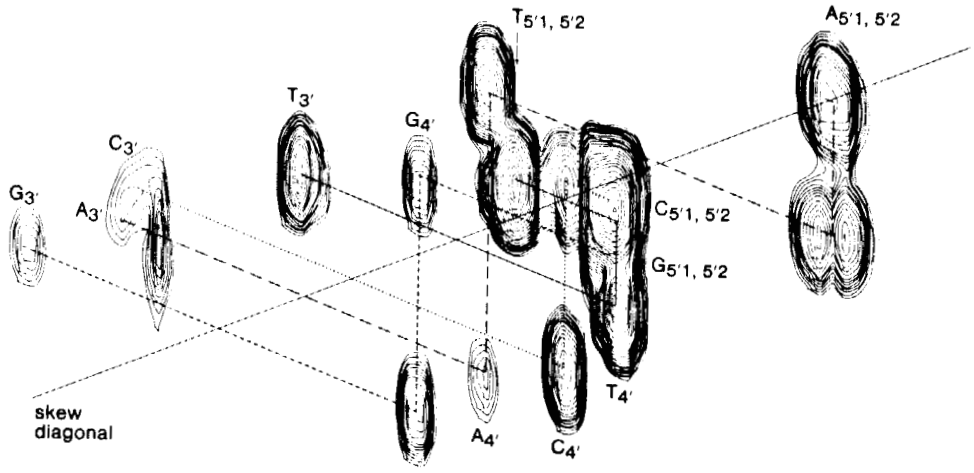


tons in each sugar unit as illustrated for d(ApCpGpT)[9] in FIG. 1. Owing to the anticipated complexity of the 18 bases of the full duplex nonamer consensus donor junction, it was decided to synthesize the complementary pentamer strand corresponding to the actual junction site 3'G - T - C - C - A5' (FIG. 2). Full ^{1}H-NMR analysis of this pentamer[7] was achieved using the techniques described above. The two

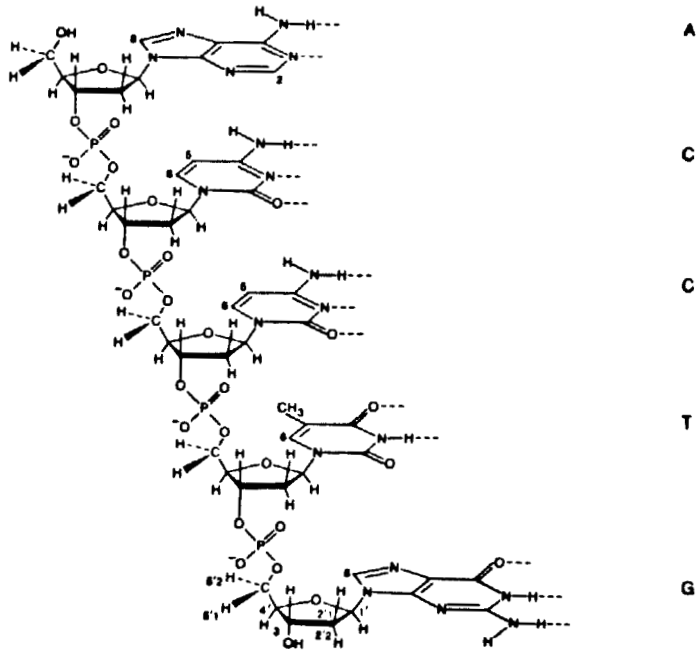
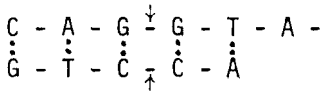


FIG. 2

strands were then annealed at 5°C. The duplex nature of the annealed splice junction domain under the conditions of the ^{1}H-NMR experiments was confirmed by: 5'-end labeling with ^{32}P-phosphate using T4 polynucleotide kinase at 20°C;[8] specific butt-end joining of the duplex region with T4 ligase at 20°C (although these conditions are suboptimal for the enzyme they correspond to the NMR experiment [8]), and gel electrophoresis with visualization by autoradiography. ^{1}H-NMR analysis then proceeded with the annealed oligomer. Attempts were made to deter-



mine the conformation of splice domain utilizing the data, particularly the inter and intra nucleotide NOEs. The susceptibility of the G,C rich splice junction (indicated with arrows above) to attack by anticancer agents will be reported in due course. The synthesis and characterization of this consensus donor exon:intron junction should be of value in projected isolation of splice enzymes using DNA affinity chromatography.

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