THE ROLE INTRON SPLICE ORDER IN THE COL1A2 GENE HAS ON THE EFFECTS OF SPLICE SITE MUTATIONS RESULTING IN OI.

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The effects of splice site mutations in collagen genes are not easy to predict on the basis of sequence changes alone. Recent studies suggest that the order of intron removal may influence splice outcome. To test this hypothesis we developed a strategy combining PCR amplification and fragment analysis on an ABI 310 (TM) using fluorescently labeled upstream and unlabeled downstream primer pairs which provided the opportunity to use a novel approach to observe the rate of intron removal from three regions of the COL1A2 pre-mRNA.

We established the order of splicing for three areas of the human COL1A2 gene; intron 19 - exon 23, intron 28 - exon 32, and intron 41 - exon 45 and examined the effects of mutations in those regions. Cells were treated with Actinomycin D to stop transcription, and nuclear RNA was harvested at intervals up to 40 minutes. After cDNA was made from each sample, using individual intron and adjacent exon pairs, we determined that introns 19, 28, and 41 were slow to be removed. Large fragment analysis which combined a labeled intron primer in a slowly removed intron with an exon downstream (spanning several introns) demonstrated a relative order of splicing.

The favored order of splicing for intron 19 - exon 23 was intron 22 first followed by introns 21, 20, and finally intron 19. For intron 41 - exon 45 there was one major and two minor splice pathways used. The major pathway was intron 42 first, intron 43, intron 44, and intron 41 last. The region from intron 28 - exon 33 spans very large introns (> 1000 nucleotides) and could not be analyzed using the large fragment approach.

Two mutations involving the 5' splice donor site of intron 21 demonstrated that adherence to the splice order dictated an intron 21 inclusion event. Splice paralysis surrounding exon 21 affecting introns 21 and 20 drove the reaction down a second pathway where intron 22 was still removed first but was followed by intron 19 and then splicing of exon 20 to exon 22 skipping exon 21. A mutation involving the 5' splice site of intron 43 also showed that adhering to splice order in the major pathway and splicing intron 42 out first necessitated the inclusion of intron 43. In the minor pathway of the intron 43 region intron 44 is spliced first. Splice paralysis surrounding exon 43 affecting both intron 43 and intron 42 occurs, and it is not until intron 41 is removed that an exon skipping reaction can take place, splicing exon 42 to exon 44.

These findings indicate that the order of intron removal governs, in part, the splicing reaction. Additionally, it provides insight into the mechanism of splice site mutation consequences and should lead to the ability to predict splicing outcomes from the mutation itself, and sequences around the intron.

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