

**P019** Exonic and intronic regulatory elements affect the splicing of human apolipoprotein A-II exon 3 carrying an atypical 3' splice site

**Pablo Arrisi-Mercado, Maurizio Romano, Andres Muro and Francisco E. Baralle.**

*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy*

Human apolipoprotein A-II (apoA-II) intron 2/exon 3 junction shows a peculiar tract of alternating pyrimidines and purines (GU tract) that makes the acceptor site deviate significantly from the consensus. However, exon 3 is constitutively included in the mRNA. We have studied this unusual exon definition by creating a construct with the genomic fragment encompassing the whole gene from apoA-II and its regulatory regions. Transient transfections in Hep3B cells have shown that deletion or replacement of the GU repeats at the 3' splice site resulted in a decrease of apoA-II exon 3 inclusion, indicating a possible role of the GU tract in splicing. However, a 3' splice site composed of the GU tract in heterologous context, such as the extra domain A of human fibronectin or cystic fibrosis transmembrane conductance regulator exon 9, resulted in total skipping of the exons. This observation prompted us to look for regulatory sequences either within the exon 3 and its flanking introns. Deletions and site-directed sequence mutations of exon 3, have lead to the identification of an exonic splicing enhancer (ESE) spanning from nucleotide 87 to 113. Moreover, immunoprecipitation experiments using monoclonal antibodies against ASF/SF2 and SC35 showed that both SR proteins bind specifically to the ESE. Experiments also demonstrated that exon 3 splicing regulatory elements are placed within flanking introns. In fact, two regulatory sequences with opposite functions were found in IVS2 and IVS3 and actually the factors able to interact with these elements are currently under investigation.