

P020 Modulation of *NF1* exon 37 splicing: a disease-causing mutation changes an ESE into an ESS

Natasa Skoko, Emanuele Buratti, Anna Knezevich,
Francisco E. Baralle and Marco Baralle
*International Centre for Genetic Engineering
and Biotechnology, Trieste, Italy*

The mutation rate of the neurofibromatosis type 1 (*NF1*) gene is one of the highest reported for any human disorder with up to 50% of disease causing mutations that affect pre-mRNA splicing. Previously described nonsense mutations in exon 37 of patients suffering NF1, have raised the possibility of nonsense-associated altered splicing. We have established through the use of an eukaryotic minigene splicing assay that these mutations disrupt an exonic splicing enhancer (ESE). Secondary structure mapping experiments shows the region of interest in exon 37 to be in an open-loop structure which remains unchanged even when the pathogenic mutation is present. We show that this element is essential for the definition of exon 37 in that it aids the recognition of the 3' splice site. Unusually, this seems to occur through a mechanism that does not involve SR proteins.

UV cross linking and pulldown analysis have enabled us to identify two RNA binding proteins involved in the modulation of exon 37 splicing with opposite roles. In fact, one protein binds only to the wild type sequence while the other binds only to the mutated RNA. This observation indicates that the mutations change an ESE to an exon splicing silencer (ESS).