

## Abstract

Eukaryotic genes contain non-coding sequences - introns that are removed during pre-mRNA splicing by the spliceosome. The spliceosome is composed of five snRNPs (U1, U2, U4/U6 and U5) which assemble on pre-mRNA in a step-wise manner and together with additional non-snRNP proteins catalyse splicing. Mutations in splicing factors can cause severe diseases, for example a point missense mutation (called AD29) in hPrp31 (U4/U6 snRNP specific protein) induces retinitis pigmentosa, disease often leading to complete blindness. In this PhD thesis we show that the hPrp31 AD29 mutant is unstable and is not properly incorporated into spliceosomal snRNPs. In addition, the expression of the mutant protein reduces cell proliferation, which indicates that it interferes with cellular metabolism (likely splicing) and could explain the induction of retinitis pigmentosa.

Next, we focus on a role of nuclear environment in pre-mRNA splicing. It was shown that new U4/U6·U5 snRNPs are preferentially assembled in non-membrane nuclear structure - Cajal body. Here we expand this finding and provide evidence that Cajal bodies are also important for U4/U6·U5 snRNP recycling after splicing. In addition, we analyzed a role of chromatin and particularly histone acetylation modulates in splicing regulation. Using inhibitor of histone deacetylases we change alternative splicing of more than 700 genes. Specifically HDAC1 deacetylase activity regulates alternative splicing of the fibronectin gene. We provide evidence that HDAC inhibition induces histone H4 acetylation and increases RNA polymerase II processivity along an alternatively spliced fibronectin elements. In addition, HDAC inhibition reduces co-transcriptional association of the splicing regulator SRp40 with the fibronectin alternative exon. We believe that there is a potential to use HDAC inhibitors in therapy of splicing related disorders in the future.