

ABSTRACT

In the process of pre-mRNA splicing introns are removed from pre-mRNA and exons are joined together. Current studies show, that about 95 % of genes, which contain more than two exons, can undergo alternative splicing. In this process some exons are included in or excluded from the final mRNA. Majority of pre-mRNA splicing take place co-transcriptionally at this time RNA polymerase II is still attached to pre-mRNA. Alternative splicing is complex process that takes place in a close proximity of DNA and histones that might modulate alternative splicing decisions.

Futher studies have validated fibronectin gene (*FNI*) and his alternative exons EDA and EDB (extra domain A and B) as suitably model for studying alternative splicing. Study using *FNI* minigene reporter system, which is composed from EDA exon and two surrounding introns and exons, has proved that insertion of transcription enhancer SV40 infront of promotor, the level of EDA inclusion is decreased. So far, has not been proved if this mechanism can function in real genome context and if distal transcription elements can influence alternative splicing.

In this study, we have predicted transcription enhancer for *FNI* gene by using The Ensemble Regulatory Build and FANTOM 5. The predicted transcription enhancer, is located 23,5 kbp upstream of TSS for *FNI* gene and was 2 401 bp long. We have prepared by using CRISPR/Cas9 system three HeLa cell lines, from which was this transcription element removed.

Results from quantitative and semiquantitative PCR showed, that after removal of predicted transcription enhancer, increase level of transcription of *FNI* gene in all prepared clones. More importantly the deleted sequence wasn't transcription enhancer, but transcription silencer. From our results we concluded, that alternative splicing can be influenced even by distal transcription regulatory elements, which can promote splicing of one alternative splicing variant.

Key words: alternative splicing, transcription silencer, *FNI* gene, EDB exon, CRISPR/Cas9