An *in vivo* splicing assay in *C. elegans*

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Splicing is an essential mechanism removing introns and joining exons of the mRNA. Ribonucleoprotein complexes called spliceosomes recognize specific splice sites, thereby allowing to precisely excise introns. Splicing signals have been characterized in several species. Of particular interest in this study is the branch point located in the 3' portion of the introns. So far, in *C. elegans*, there is no evidence of splicing occurring through a branching nucleotide. By analysing more than 30 introns, our lab has shown the existence of a splicing branch point in *C. elegans*. This master thesis aims at setting up an *in vivo* assay for splicing in *C. elegans*, in order to identify nucleotides that are essential for efficient splicing. GFP reporter genes have been used to monitor splicing efficiency. By modifying splicing signals in the first intron and varying its length we observed a decrease of splicing efficiency by monitoring GFP intensity. Transgene copies have been determined in order not to bias the results. In addition we followed splicing efficiency by RT-PCR by comparing the spliced and the unspliced RNA for a given intron. We also investigated alternative splicing leading to two *tra-1* isoforms, *tra-1A* and *tra-1B*. We hypothesized that the two isoforms are in competition with each other for proper germline sex determination. Quantifications of *tra-1A* versus *tra-1B* have been done with extracts from staged worms having defects in splicing factors such as *rnp-3, mog-2* and *mog-1* and compared with wildtype animals. Our current working model is that decreased *tra-1A/tra-1B* ration leads to masculinized germlines in hermaphrodite.

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