**Abstract**

Eukaryotic gene expression requires the removal of non-coding introns and splicing of the coding exons, which is executed by the dynamic ribonucleoprotein (RNP) complex, spliceosome. In addition to cis-acting RNA elements, spliceosomes have evolved to require trans-acting factors. These factors help in assembly and activation of the spliceosome, leading to constitutive and regulated RNA splicing. In my thesis work I report function of the conserved tri-snRNP factor Snu66/SART1 in RNA splicing. A novel and highly conserved motif at the C-terminus of Snu66, termed Snu66-CM, is essential for its splicing function. Biochemical and splicing assays in the budding yeast Saccharomyces cerevisiae show that Snu66-CM is critical for splicing of precursor mRNAs with non- canonical 5’ splice sites (5’ss). We have extended our study to intron-richer fission yeast Schozosaccharomyces pombe and found that not only Snu66 but also Snu66-CM is essential for cell viability. We isolated a conditional mutant of Snu66-CM which was temperature sensitive and showed defects in constitutive RNA splicing. Importantly, splicing of pre-mRNAs with non-canonical splice signals was severely affected in this mutant, suggesting an additional role of Snu66 in regulated RNA splicing. We next identified that S. cerevisiae Snu66 interacts with Sap1, a AAA-ATPase which binds to covalent conjugates of the ubiquitin-related protein modifier SUMO (AAA-ATPase family proteins are reported to be involved in diverse functions like protein degradation, DNA replication & repair, microtubule motors, etc.). We studied function and regulation of Sap1. From the single gene, two protein isoforms of Sap1 are expressed from alternatively transcribed mRNAs in a carbon source- dependent manner. Interestingly, Sap1 bound Snu66 through its CM. While Sap1-Snu66 interaction indicated a role of this complex in RNA splicing, we could not find any splicing role for the complex. Instead, we discovered an unexpected and intriguing role of the complex in homologous recombination. The lack of Sap1 made yeast cells prone to homologous recombination involving DNA circles, and this activity appears to be mediated by Sap1 interaction with Snu66-CM. Thus, the splicing factor Snu66 is critical not only for constitutive and regulated RNA splicing; the protein also regulates homologous recombination by associating with the AAA-ATPase Sap1. S. cerevisiae SRC1 intron has two non-canonical 5’ splice sites (referred to as alternative and constitutive 5’ss). Spliceosomes uses both 5’ss to promote SRC1 alternative splicing. The usage of the alternative 5’ss requires non-covalent interactions of the ubiquitin-like protein Hub1 with splicing factors Snu66 and Prp5. However, additional proteins are likely to participate in SRC1 alternative splicing. Here we show that this process requires proteins of the spliceosomal core, including thePrp8-101 surface, and subunits of the retention and splicing (RES) complex. Hub1’s proximity to the spliceosomal core proteins is needed for SRC1 alternative splicing, whereas the RES complex appears to function by a different mechanism. The collective list of SRC1 alternative splicing factors indicates a general mechanism; spliceosomes slow down at SRC1 5’ss thereby promoting splicing using both sites. Thus, since SRC1 alternative splicing via the competing 5’ss needs a set of diverse spliceosomal proteins and regulators, other forms of alternative splicing might similarly require concerted action of distinct molecules.