Abstract

Splicing is a process, during which non-coding sequences (introns) are cleaved out of pre-mRNA, and exons are ligated. This whole process is catalysed by a multi-megadalton splicing complex, composed of five small nuclear ribonucleoprotein particles (shortly snRNPs), which each contains its own small nuclear RNA molecule and specific set of proteins. During the biogenesis of snRNPs, U4 and U6 snRNPs are assembled to form the di-snRNP, which further associates with U5 snRNP and gives rise to tri-snRNP. With the help of mass spectrometry, we have found previously uncharacterized protein interacting with U5 snRNP, called TSSC4. By immunoprecipitation, I confirmed TSSC4 as a U5 snRNP specific protein and identified the region of TSSC4 responsible for interaction with U5 snRNP. I also showed that TSSC4 interacts with PRPF19, a component of complex driving the catalytic activation of the spliceosome and that this interaction is U5 snRNP-independent. Knockdown of TSSC4 in HeLa cells results in accumulation of di-snRNAs and U5 snRNP in Cajal bodies, nuclear compartments involved in snRNP biogenesis. Similar phenotype was previously observed upon inhibition of tri-snRNP assembly. To analyse the importance of TSSC4 for tri-snRNP assembly, I separated individual snRNPs by glycerol gradient ultracentrifugation according to their sedimentation coefficient. Upon TSSC4 depletion in HeLa cells, I observed lower levels of tri-snRNP formation. Ineffective tri-snRNP assembly is further supported by the accumulation of 52K in Cajal bodies, which is U5 snRNP specific protein absent in tri-snRNP. I also show loosened interaction between two core components of U5 snRNP – PRPF8 and SNRNP200 upon TSSC4 depletion in HeLa cells. I thus propose TSSC4 as a novel U5 snRNP specific factor important for tri-snRNP assembly.

Keywords

Spliceosome, small nuclear ribonucleoprotein particles, TSSC4