Pre-mRNA splicing is a process in which introns are removed from eukaryotic transcripts and exons are ligated together. Splicing is catalyzed by spliceosome, a large ribonucleoprotein complex composed of five small nuclear RNAs and more than 100 additional proteins, which recognizes 5′ splice site, branch point site and 3′ splice site and performs two transesterification reactions to produce mRNA molecules. 5′ splice site is recognized by U1 snRNP and U2 auxiliary factor (U2AF) is involved in branch point and 3′ splice site recognition in the early splicing complex. There is some evidence of splice sites cooperation during intron recognition in vitro but little is known about the situation in vivo. Using Fluorescence resonance energy transfer (FRET) and RNA immunoprecipitation (RIP) methods, we have investigated the early stages of spliceosome assembly. We have employed splicing reporters based on -globin gene and MS2 stem loops to detect interactions of proteins on RNA molecule directly in the cell nucleus. Results of FRET indicate that intact 5′ splice site is required for U2AF35 interaction with 3′ splice site and that U1C recruitment to 5′ splice site is partially limited upon 3′ splice site mutation. We have also confirmed by RIP that U2 snRNP association with pre-mRNA molecule requires presence of 5′ splice site. Our results thus bring the first evidence of in vivo cooperation of spliceosomal components in the process of intron recognition.