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Regulation of pre-mRNA splicing in *S. cerevisiae*: where RNA cooperates with proteins

Abstract

Removal of introns from protein coding transcripts occurs in two splicing reactions catalyzed by a large nuclear complex, spliceosome. The spliceosome is an extremely intricate and dynamic machine, wherein contributions of small RNA molecules and multiple proteins are coordinated to meet the requirements of absolute precision and high flexibility. For an intimate understanding of pre-mRNA splicing, it is necessary to unravel roles of individual components and to dissect the partial mechanisms.

In the first part of this work, we describe the role of the Prp45 splicing factor in *Saccharomyces cerevisiae*. Mapping of genetic interactions of a conditionally lethal allele *prp45*(1-169) suggests a relationship of Prp45 to the NTC complex and to the second transesterification. Two-hybrid assay and purification of spliceosomal complexes reveal a contribution of the Prp45 C-terminus in the Prp22 helicase recruitment and/or regulation. Numerous experiments with reporter substrates document the need of Prp45 for the efficient splicing of a specific subset of introns. Our observations suggest that the function of Prp45 in splicing is conserved in evolution.

The second part is devoted to the role of intron secondary structure in 3′ splice site (3′ss) recognition. We show that the stem-loop structures formed downstream of the branch point (BP) are required for the splicing of *COF1* and *UBC13* introns, which have extremely long distances between BP and 3′ss. Identified structures aid to efficient 3′ss recognition by bringing remote 3′ss to the BP proximity and by sequestering AG dinucleotides, which behave as potential cryptic 3′ss. Our analyses strongly suggest that the structure based mechanism of 3′ss recognition is employed in most introns with distant 3′ss in *Saccharomyces cerevisiae* and possibly in other *Saccharomycotina* yeasts.