Early Spliceosomal Complex

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Crucial for the definition of the exon–intron junctions is the early spliceosomal complex (E complex), also called commitment complex (CC) in yeast. This minimal complex consists of the U1-snRNP, SF1, and U2AFand is sufficient to recognize all intron definingciselements. Base pairing between the 5′ ss and the 5′-end of U1 snRNA defines the start of the intron.

splicing spliceosome E-complex Prp2 5′ splicing site exon–intron junction fission yeast U2AF65

1. Introduction

Splicing of mRNA precursors is an essential part of regulated gene expression. The process consists in the excision of the introns (non-coding sequences) from the precursor mRNA (pre-mRNA), and results in the ligation of the coding sequences (exons), forming the mature mRNA. This is achieved by two consecutive trans-esterification reactions, which need to occur at nucleotide precision to avoid frame shifting with adverse consequences on the protein coding potential of the mRNA. To achieve this accuracy, sequence specific cis-acting elements on the pre-mRNA define the exon intron junctions. Evidence indicates that splicing has evolved during eukaryogenesis from self-splicing group II introns of prokaryotes together with the spliceosome acting in trans, to catalyze the splicing reaction [1][2]. Two types of spliceosomes are present across eukaryotes, namely the major and the minor spliceosome. Each spliceosome splices its own type of introns, the U2-type introns for the major spliceosome and the U12-type introns for the minor counterpart. The core mechanism of U2-type splicing is conserved from yeast to higher eukaryotes, as is the spliceosome [3]. However, U12 introns (and the associated snRNAs) are absent from the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe [4]. In this review, we will focus on the major spliceosome and U2-type introns and compare introns and the splicing machinery between S. cerevisae , S. pombe , and humans to highlight common ancestor mechanisms and how their increase in complexity over evolution might enable the transition from constitutive to regulated and alternative splicing.

2. The Spliceosome

The spliceosome is a multi-component machine composed of five small nuclear RNAs (snRNAs) pre-assembled with proteins into small ribonucleoproteins (snRNPs) and hundreds of additional proteins. The five different snRNPs are called U1, U2, U4, U5, and U6 [5][6][7]. The assembly of the spliceosome takes place through multiple

dynamic interactions that leads to the formation of different intermediate complexes: E (ATP independent), A, B, and C (ATP dependent) [8][9][10].

In the first step of spliceosome assembly, the exon intron junctions are defined by recognition and interaction with the cis-acting elements on the pre-mRNA termed the 5′ and 3′ splice sites (5′ ss and 3′ ss) [11]. The formation of the first spliceosomal complex (E-complex) is initiated by U1 snRNP interaction with the 5′ ss and the cooperative recognition of the 3′ ss by SF1 (Splicing Factor 1) and U2AF (U2 snRNP auxiliary factor). The A-complex is formed when U2 snRNP displaces SF1. Next, U4/U5-U6 tri-snRNP binding to 5′ ss results in the formation of the pre-catalytic B-complex and rearrangements of RNA-RNA and RNA-protein interactions lead to the catalytic active spliceosome. After completion of the first trans-esterification reaction, the C-complex is formed, which carries out the second reaction of splicing [11][12].

3. The Evolution of the Spliceosome

The core machinery of the U2-type spliceosome is highly conserved across eukaryotes [13][14]. Comparison of the spliceosomal components between S. pombe , S. cerevisiae , and humans confirmed this conservation [3]. There are, however, some factors that are present in fission yeast and humans that appear to be absent in budding yeast and several factors are present in humans that do not exist in either yeast. As the basic splicing mechanism is functional in all three organisms, the increase in the complexity of the spliceosome is thought to contribute to the evolution from mainly constitutive splicing in budding yeast to a highly regulated and alternative splicing in humans.

Due to the evolutionary conservation of the splicing machinery, studies in the fission and budding yeasts have been fundamental for the discovery of spliceosomal components and for the dissection of basic mechanisms of splicing [3][15][16][17][18][19][20][21][22]. There are, however, major differences in the splicing machinery between the two yeast species. While phylogenetic studies revealed that many proteins of the splicing machinery are well conserved, about 40% of the fission yeast splicing factors are more similar to the human proteins than to the budding yeast proteins [3][19]. Most of these factors are described to play a role in the recognition of the 3′ ss. [3][23]. In this line, members of the family of serine/arginine (SR)-rich proteins, which have been shown to interact with proteins that recognize the 3′ splice site, are found in both fission yeast and humans but are absent in budding yeast. The higher degree of conservation of transacting splicing factors parallels the high degree of degeneracy of splice site sequences in S. pombe , which closely reflects the observation in human transcripts [13]. For this and other reasons detailed below, it is considered that S. pombe represents an evolutionary intermediate between the constitutive mechanism of splicing in S. cerevisiae and the dynamically regulated process of splicing in humans, which allows alternative splicing of the same pre-mRNA into different mRNAs.

4. Co-Transcriptional Splicing of Long Introns and Intron Looping

The spliceosomal E-complex brings the two ends of the intron in close proximity to initiate the process of splicing and looping of the intronic sequence is a prerequisite. While these intron loops have already been described in early electron micrographs of Drosophila melanogaster embryonic transcription units [24][25], the underlying molecular mechanisms of intron looping have been just recently started to be explored in more detail. The cryoEM structure of a mammalian transcribing Pol II-U1 snRNP complex has been recently resolved [26]. The structure reveals that Pol II and U1 snRNP interact directly without involvement of the CTD of Pol II. The interaction instead is mediated by the protrusion domain in Pol II subunit RPB2 and the zinc finger domain in subunit RPB12, which contact with the RRM domain of the conserved and functionally essential subunit U1-70k from the U1 snRNP.

Using a CRISPR-based approach to halt RNA polymerase II transcription in the middle of introns, it has been demonstrated that the nascent 5′ splice site base pairs with a U1 snRNA that is tethered to RNA polymerase II during intron synthesis [27]. This mechanism relies on the strength of the 5′ ss–snU1 RNA and enables the co-transcriptional assembly of the E-complex by ensuring proximity of the U1 snRNP at the 5′ ss and the SF1-U2AF complex at the 3′ ss. In this way, not only short introns—as previously thought—but also long introns might be rapidly spliced co-transcriptionally if the 5′ ss-snU1 and the snU1-Pol II interaction is sufficiently strong to ensure looping of the intron, forcing the proximity of the intron borders.

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