mRNA splicing

Many eukaryotic genes are mosaics, consisting of blocks of coding sequences separated from each other by blocks of non-coding sequences. The coding sequences are called Exons (or Expressed sequences) and the intervening sequences are called Introns. As a consequence of this alternating pattern of exons and introns, genes bearing non-coding interruptions are often said to be "in pieces" or "split".

The split genes of eukaryotes are transcribed into a single RNA copy of the entire gene. Thus, the primary transcript for a typical eukaryotic gene contains introns as well as exons. Introns are removed

from the pre-mRNA by a process called RNA splicing. This process converts the pre-mRNA into mature mRNA & must occur with great precision to avoid the loss, or addition, of even a single nucleotide at the sites at which the exons are joined.

Organization of the splicing Machinery :-

Nuclear pre-mRNA splicing is carried out by complexes of RNA-protein structures called spliceosomes. They contain a set of small RNA molecules called SnRNA (small nuclear RNA) and a set of proteins.

Five SnRNAs, called U1, U2, U4, U5 & U6 are involved in nuclear pre-mRNA splicing as components of the spliceosome. (SnRNA U3 is localized in the nucleolus and probably is involved in the formation of ribosomes). These SnRNAs do not exist as free RNA molecules. Instead they are present in small nuclear RNA-protein complexes called SnRNPs (Small nuclear Ribonucleoproteins). spliceosomes are assembled from four different SnRNPs and protein splicing factors during the splicing process.

Each of the SnRNAs U1, U2, and U5 is present by itself in a specific SnRNP particle. SnRNAs U4 and U6 are present together in a fourth SnRNP ; U4 and U6 SnRNAs contain two regions of the intermolecular complementarity that probably are base paired in the U4/U6 SnRNP.

Some RNA-free proteins are involved in splicing. one example, U2AF (U2 auxiliary factor), recognizes the polypyrimidine (Py) tract/3' splice site, and in the initial step of the splicing reaction,

helps another protein, named BBP (Branch point Binding protein), bind to the branch site.

Steps of Splicing Reaction:- Analysis of the reaction products and intermediates formed in vitro revealed that pre-mRNA splicing proceeds in two steps, which are as follows:-

i) First, the pre-mRNA is cleaved at the 5' splice site, and the 5' end of the intron is joined to an Adenine nucleotide within the intron (near its 3' end). In this step an unusual bond forms between the 5' end of the intron and the 2'-hydroxyl group of the Adenine nucleotide. The resulting intermediate is a lariat-like structure, in which the intron forms a loop.

ii) The second step in splicing then proceeds with simultaneous cleavage at the 3' splice site and ligation of the two exons. The intron is thus excised as a lariat-like structure, which is then linearized and degraded within the nucleus of intact cells.

These reactions define 3 critical sequence elements of pre-mRNAs; sequences at the 5' splice site, sequences at the 3' splice site, and sequences within the intron at the branch point (the point at which the 5' end of the intron becomes ligated to form a lariat-like structure). Pre-mRNAs contain similar consensus sequences at each of these positions, allowing the splicing apparatus to recognize pre-mRNAs and carry out the cleavage and ligation reactions involved in the splicing process.

Chemistry of splicing:- Splicing is achieved by two successive transesterification reactions in which phosphodiester linkages within the pre-mRNA are broken and new ones are formed.

i) The first reaction is triggered by the 2'-OH of the conserved 'A' at the branch site. This group acts as a nucleophile to attack the phosphoryl group ~~as a result~~ of the conserved 'G' in the 5' splice site.

As a consequence, the phosphodiester bond between the sugar and the phosphate at the junction between the intron and the exon is cleaved and the freed 5' end of the intron is joined to the A within the branch site. This is the first transesterification reaction.

ii) In the second, the newly ~~synthesized~~ liberated 3'-OH of the 5' exon reverses its role and becomes a nucleophile that attacks the phosphoryl group at the 3' splice site.

The second reaction has two consequences. First, and most importantly, it joins

the 5' and 3' exons; thus, this is the step in which the two coding sequences are actually "spliced" together. Second, this same reaction liberates the intron which serves as a leaving group. The newly liberated intron has the shape of a lariat.

Splicing pathways:- [Assembly of spliceosome & Rearrangements]

The steps of splicing pathways are as follows:-

A) Assembly:-

i) Initially, the 5' splice site is recognized by the U1 SnRNP (using base pairing between its SnRNA and the pre-mRNA). one subunit of U2AF binds to the Py tract and the other to the 3' splice site. The former subunit interacts with BBP and helps that protein bind to the branch site. This arrangement of proteins and RNA is called the Early (E) complex.

ii) U2 SnRNP then binds to the branch site, aided by U2AF and displacing BBP. This arrangement is called A complex. The base-pairing between the U2 SnRNA and the branch site is such that the branch site A residue is extruded from the resulting stretch of double helical RNA as a SS nucleotide bulge. This A residue is thus unpaired and available to react with the 5' splice site.

B) Rearrangement:-

i) The next step is the rearrangement of the A complex to bring together all three splice sites. This is achieved as follows: U4 and U6 SnRNPs, along with the U5 SnRNP, join the complex. Together these three SnRNPs are called tri-SnRNP particle (here U4 and U6 are held together by complementary base pairing between their RNA components, and the U5 is loosely associated through protein: protein interactions. With the entry of the tri-SnRNP, the A complex is converted into B complex.

ii) Then, U1 leaves the complex, and U6 displaces it at the

5' splice site. This rearranges that the base pairing between the U1 snRNA and the pre-mRNA be broken, allowing the U6 RNA to anneal with the same region. Thus the assembly pathway is complete.

c) Catalysis:-

i) It occurs as follows:- U4 is released from the complex, allowing U6 to interact with U2 (through the RNA:RNA base pairing). This rearrangement, called the C complex, produces the active site, i.e. the rearrangement brings together within the spliceosome those components (solely regions of U4 and U6 RNAs) that together form the active site. It also ensures the substrate RNA is properly positioned to be acted upon.

ii) Formation of the active site juxtaposes the 5' splice site of the pre-mRNA and the branch site, facilitating the first transesterification reaction. The second reaction between the 5' and 3' splice sites, is aided by the U5 snRNP, which helps to bring the two exons together. The final step involves the release of the mRNA product and the snRNPs. The snRNPs are initially still bound to the lariat, but get recycled after rapid degradation of that piece of RNA.