Quadrant III – Notes

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Module Name: Splicing pathways And Alternative splicing

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Notes

Splicing pathways

1. Self-splicing

Self-splicing is a type of RNA splicing which occurs in some rare introns that are capable of promoting phosphodiester bond cleavage and formation without the help of other proteins or spliceosomes. These introns are unique as they can mediate their excision from precursor RNA and the subsequent ligation of the flanking exons in a simple salt buffer. This self-splicing reaction is facilitated by the tertiary structure of the intron, which provides the ability to recognize the splice sites of the precursor RNA and to perform the cutting and ligation reactions in a very precise manner.
The sequence present in such introns performs as a ribozyme that regulates the overall process. There are three types of self-splicing introns that are grouped as Group I, Group II, and Group III. Group I and Group II introns perform the splicing process in a mechanism similar to that by spliceosomes. These suggest that these introns might be evolutionarily related to the spliceosomes.

During self-splicing, the 5′ splice site is recognized by a short sequence element in the intron called the internal guide sequence. Besides, other strongly conserved sequences of the introns called P, Q, R, and S are needed to

‘catalyze’ the cutting and ligation reactions. Self-splicing follows a similar mechanism involving two transesterification reactions resulting in the removal of introns and ligation of exons.

2. tRNA splicing

Like in mRNA, the genes in tRNA are also interrupted by introns, but here the splicing mechanism is quite different. Splicing in tRNA is catalyzed by three enzymes with an intrinsic requirement for ATP hydrolysis. The process of tRNA splicing occurs in all three major lines of descent, the Bacteria, the Archaea, and the Eukarya, but the mechanism might differ in bacteria and higher organisms. In bacteria, the introns in the tRNA are self-splicing. In Archaea and Eukarya, however, the tRNA splicing reaction occurs in three steps where each step is catalyzed by a distinct enzyme, each of which can function interchangeably on all of the substrates. In the first step, the pre-tRNA is cleaved at the two splice sites by an endonuclease, resulting in two tRNA half molecules and a linear intron with 5’-OH and 3’-cyclic PO4 ends. The cleavage is then followed by the ligation of the two RNA half molecules in the presence of a tRNA ligase enzyme.
Finally, the PO4 ends produced from splicing are transferred to NAD in a process catalyzed by nicotinamide adenine dinucleotide (NAD)-dependent phosphotransferase.

Alternative splicing

Alternative splicing is a splicing process resulting in a varying composition of exons in the same RNA and creating a range of unique proteins. Alternative splicing of pre-mRNA is an essential mechanism to enhance the complexity of gene expression, and it also plays a vital role in cellular differentiation and organism development. Alternative splicing enables exons to be arranged in different combinations where different configuration results in different proteins.

The process of alternative splicing might occur either by skipping or extending some exons or by retaining particular introns, resulting in different varieties of mRNA formed.

Alternative splicing pathways:

1. **Exon inclusion/skipping/Cassette:** Some genes have exons that can be included or excluded, independent of others. If several cassette exons are present, high degree of diversity can be generated.

2. **Retained introns:** In some genes an intron may be spliced out or retained.

3. **Alternative 5' donor sites:** Introns of different lengths are spliced out to the availability of 2 of more different donor or acceptor sites (donor or acceptor of OH group during transesterification) at the 5' end of introns respectively.

4. **Alternative 3' acceptor sites:** Introns of different lengths are spliced out to the availability of 2 of more different donor or acceptor sites (donor or acceptor of OH group during transesterification) at the 3' end of introns respectively.

5. **Alternative promoter or poly A sites:** There are also genes having more than one alternative promoter sites (start points) at the 5' end (e.g. myosin light chain sites) poly A sites (cleavage sites) at the 3' end.

6. **Mutually exclusive internal exons:** In some genes, a pair of exons are present, which are neither spliced together, nor retained together, so that in one state one is spliced out and in the other state, the second exon is spliced out giving two isoforms.

Alternative splicing is also essential for other functions like the identification of novel diagnostic and prognostic biomarkers, as well as new strategies for therapy in cancer patients.Thus, alternative splicing has a role in almost every aspect of protein function, including binding between proteins and ligands, nucleic acids or membranes, localization, and enzymatic properties.