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Regulation of alternative splicing by RNA secondary structure

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Abstract

The alternative splicing process is controlled by trans-acting factors and cis-acting elements. Initially, it was thought that hnRNP proteins interact with the newly synthetized transcript and prevent the generation of a pre-mRNA secondary structure. However, there are many examples of the impact of secondary structure on the alternative splicing of genes. An alternative splicing regulation is presented based on: the recognition of 5′ and 3′ splicing sites and branch sites, regulatory cis-acting elements, long-range interaction.

Key words: alternative splicing, RNA secondary structure, splicing regulatory elements

Introduction

In many cellular processes, secondary structure plays an important role in the functional properties of RNA molecules (Wan et al., 2011). Regarding pre-mRNA, it is postulated that the structure of local motifs affect the process of alternative splicing. This is in contrast to tRNA molecules, as the global defined structure is responsible for their function.

The key step of alternative splicing is to identify the 5′ and 3′ splice sites. Select splice sites can be regulated by the secondary structure of regions involved in splicing (Maris et al., 2005, Hiller, Zhang et al., 2007). The secondary structure can inhibit or activate the spliceosome binding in pre-mRNA. In addition, the structural regulations may apply to elements acting in *cis*. Most of the splicing factors bind to between two- and ten-nucleotide-long pre-mRNA single-stranded regions which are most common in hairpin loops. Hiding and exposing regions such as exonic splicing silencers (ESS), exonic splicing enhancers (ESE), intronic splicing enhancers (ISE), and intronic splicing silencers (ISS) has a significant impact on the regulation of the alternative splicing process. Furthermore, the generation of specific structural motifs of pre-mRNA leads to an approximation of regulatory elements acting in *cis* which are positioned at a distance from each other in the sequence.

The effect of secondary structure on alternative splicing genes

The correlation between the occurrence of alternative splicing and the structure of the splice sites displays the impact of secondary structure on alternative splicing. The modelling of secondary structures of sixty-nucleotide-long fragments includes the following regions: 1) those subject to constitutive splicing (13 246 exons), 2) those containing 5′ and 3′ alternative splice sites (4179 exons), and 3) those controlling an exon skipping (8385 exons) which have revealed differences in thermodynamic stability (Shepard and Hertel, 2008). Splice sites, which are not part of the constitutive splicing form conserve structural motifs. Fragments within the alternative splice sites have a higher GC content; therefore, a secondary structure formed by these regions is more stable (Fig. 1A and 1B). The correlation between the stability of structural motifs and the alternative splicing events does not apply to exon skipping. It is thought that, in this case, exon size has a larger influence on splicing than the secondary structure (Fig. 1C).

A splicing pattern in four types of mice tissue has been created based on conserved structural motif data which regulate alternative splicing (Barash et al., 2010).

Impact of RNA secondary structure on the recognition of 5′ and 3′splice sites and branch sites

The RNA secondary structure influences the availability of splice sites in the pre-mRNA of the human GH1 growth hormone (Estes et al., 1992). Two isoforms of this protein are produced in the pituitary gland with shorter and longer variants depending on the choice of acceptor site B or B′. The pre-mRNA of this gene forms a hairpin structure, which includes a splice site and the branch point for the long isoform. The presence of such

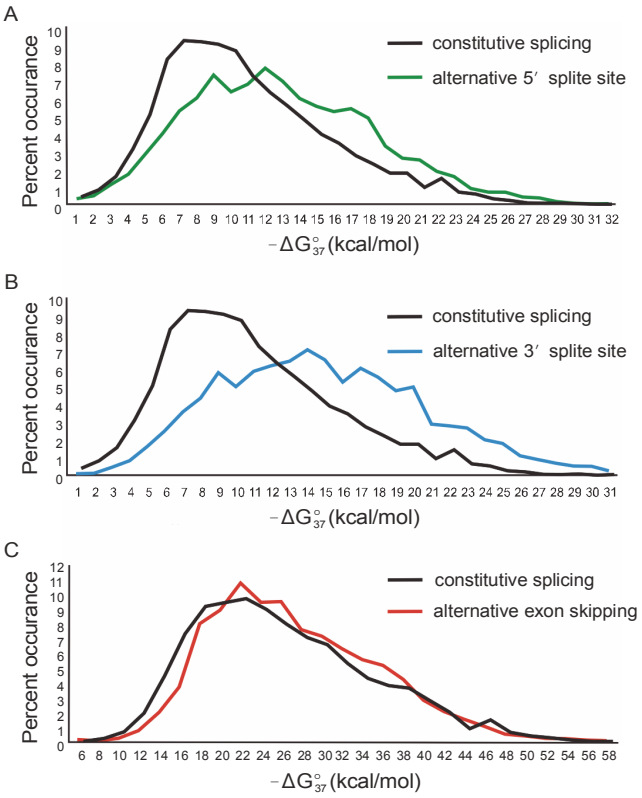

Fig. 1. Comparison of structure and thermodynamic stability of the pre-mRNA regions undergoing alternative splicing and constitutive splicing. A) Comparison of alternative 5′ splice site to constitutive splicing, B) Comparison of alternative 3′ splice site to constitutive splicing, C) Comparison of alternative exon skipping to constitutive splicing (Shepard and Hertel, 2008)

a structural motif is associated with an increased amount of the shorter protein isoform. It is believed that there is a balance between the two forms of the pre-mRNA GH1 genes in hiding or exposing the splice site. Because it is difficult to access the splice site responsible for the expression of the long isoform, the short protein version of the human growth hormone is produced about 10 times more. The usage of the splice site depends on the stability of the hairpin. The enhanced thermodynamic stability of this motif causes short human growth hormone isoform synthesis. There is a linear relationship between the use of splice site B′ and the regulating hairpin free energy.

The impact of RNA secondary structure on the alternative splicing of splicing factor hnRNP A1 has been observed (Blanchette and Chabot, 1997). As a result of exon 7B alternative splicing, two isoforms of this protein are produced which differ in their ability to bind to splice sites: hnRNP A1 and hnRNP A1B. A fragment called CE6IO, which is 84 nucleotides long, forms a very stable double-stranded region with exon 7B acceptor site and leads to skipping this exon. The formation of a stable duplex between CE6IO and the exon 7 acceptor site results in the reduced binding of U1 snRNP to this region.

The alternative splicing of the human β-tropomyosin gene (TPM2) is tissue-specific. As a result of this process, two isoforms containing exon 6A or exon 6B are expressed. TPM2 gene alternative splicing is regulated by an RNA secondary structure fragment comprising exon 6B and the intron between exon 6A and 6B (Sirand-Pugnet et al., 1995). The secondary structure that forms this region affects multiple steps in the alternative splicing of TPM2 pre-mRNA. Among other things, it reduces the recognition of the acceptor site, reduces the splicing factor binding to the branch site, and conducts regulatory elements. Nucleotide changes in this region reduce structure and thereby increase the efficiency of splicing. It remains unexplained why such regulation occurs only in selected tissues.

The alternative splicing of exon 10 of tau protein is regulated by the formation within the 5′ splice site of the RNA hairpin motif (Varani et al., 1999). In a healthy man, approximately the same amount of isoform with exon 10 (4R) and protein without exon 10 (3R) is created (Fig. 2A). Mutations within the regulatory motif mainly lead with the expression of isoform 4R, resulting in the aggregation of tau protein in cells and diseases such as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) (Hutton et al., 1998; Iijima et al., 1999; Varani et al., 1999; Stanford et al., 2000; Tolnay et al., 2000; Yasuda et al., 2000; Miyamoto et al., 2001; Stanford et al., 2003). These mutations do not alter the hairpin structure of this motif; they only change its thermodynamic stability, leading to dysfunction in alternative splicing (Fig. 2B). The ratio of 3R and 4R isoforms in mutant molecules were able to be changed with the use of small molecule ligands that stabilize regulatory hairpin and antisense oligonucleotides (Lisowiec et al., 2015).

Impact of the secondary structure on alternative splicing through regulatory *cis*-acting elements

Structural motifs occurring in pre-mRNA can also regulate the identification of *cis*-acting regulatory elements (Maris et al., 2005; Hiller et al., 2007). Most of the splicing factors interact with single-stranded regions, so in order to properly regulate alternative splicing, regula-

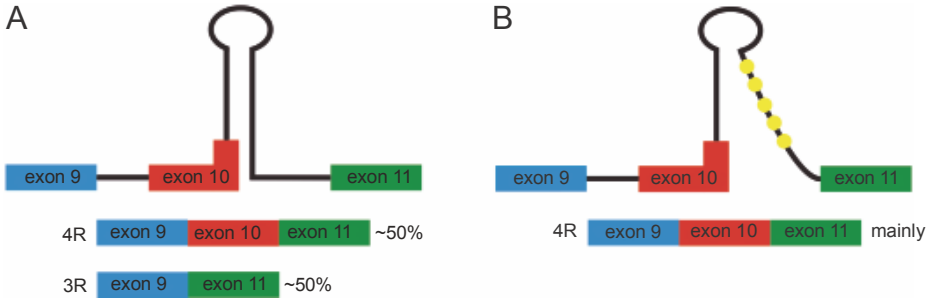

Fig. 2. A) Alternative splicing of exon 10 yields similar levels of the 3R (without exon 10) and 4R (with exon 10) protein tau isoforms. B) Mutations in alternative splicing regulatory hairpin disrupt alternative splicing of exon 10 cause 4R isoform to dominate

tory fragments should be in the single-stranded regions. By modulating the secondary structure, it is possible to hide or expose the sequence which enhances or silences splicing, leading to changes in alternative splicing.

Examples shown by Hiller highlight the differences in alternative splicing depending on whether the regulatory element is in the loop or stem of the hairpin (Fig. 3) (Hiller et al., 2007). If the enhancing splicing element ESE of the CD44 gene comprises a region of the hairpin stem, it results in the exclusive formation of shorter isoforms, i.e., the regulatory element has not been recognized in the process. The model splicing enhancer element which was proposed also has a similar tendency, but the location of ESE in the hairpin stem does not completely eliminate splicing (Fig. 3A). In the case of splicing, the silencing element of the gene hnRNP A1 displays a distinct difference in the amount of isoforms while the regulatory element is in the hairpin stem or loop. The silencing element proposed by the authors perfectly displays the effect of secondary structure on the regulation of splicing by *cis*-acting elements. The location of the silencer in double-stranded regions results in an almost total retention of the alternative splicing process (Fig. 3B).

The EDA exon is part of fibronectin pre-mRNA, and its maturation is controlled by two *cis*-acting elements (i.e., ESE and ESS). Alternative splicing of this exon depends on the SR protein interactions with regulating elements. The secondary structure of the regulatory elements regulates the efficiency of the binding of these proteins (Muro et al., 1999; Buratti and Baralle, 2004).

There are two copies of the gene encoding the protein SMN (Survival motor neurons) in humans (Monani, 2005). Products of the expression of these genes differ in the presence or absence of exon 7, and there

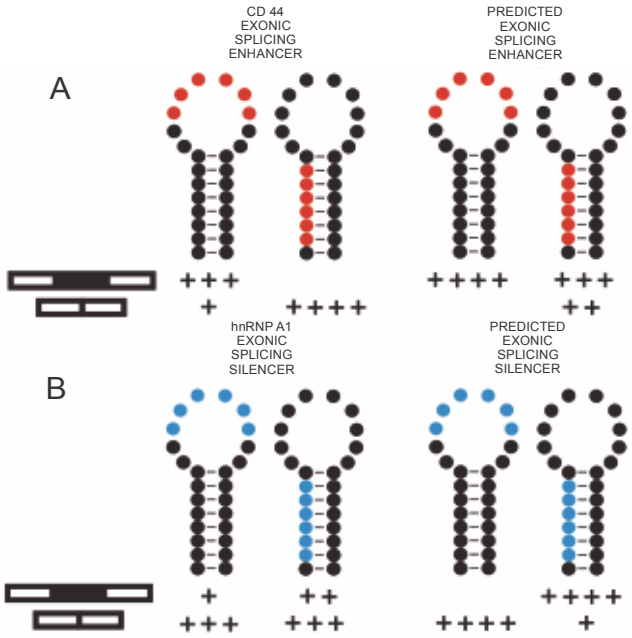

Fig. 3. Influence of splicing regulatory elements localization on splice site selection. A) Influence of exonic splicing enhancer localization (in hairpin loop or stem loop) on splice site selection, B) influence of exonic splicing silencer localization (in hairpin loop or stem loop) on splice site selection. The red circle represent exonic splicing enhancer, the blue circle represent exonic splicing silencer, the white rectangle represent constitutive spliced exons, the black rectangle represent alternative spliced exons (Hiller et al., 2007)

fore the SMN1 (with exon 7) and SMN2 proteins (without exon 7) can be distinguished. The difference in the sequence of both genes is a result of the point mutation C → T in exon 7. The change in the sequence does not change the amino acid in the protein, but affects the alternative splicing of exon 7. The mutation exposes the intronic splicing silencer element around the region 3′ splice site, resulting in the skipping of exon 7 and the expression of the SMN2 protein. This example shows

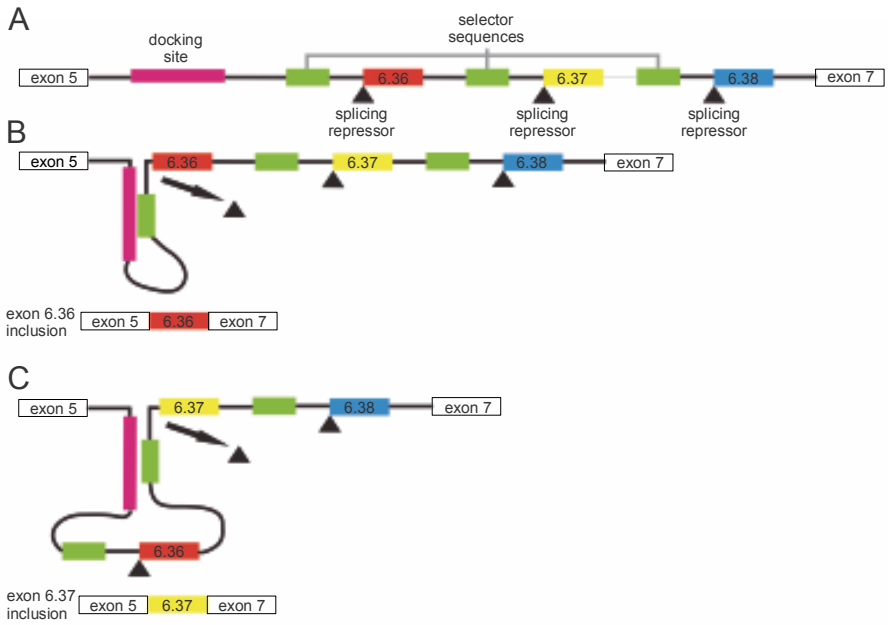

Fig. 4. Alternative splicing group of exons 6 of Dscam gene. A) Fragment of pre-mRNA Dscam gene with docking site after exon 5 and selector sequences after exon 6.36, exon 6.37, exon 6.38. B) Interaction docking site with selector sequence after exon 6.36 inactivates the repressor bound to exon 6.36 resulting in exon 6.36 inclusion. C) By analogy, interaction docking site with selector sequence after exon 6.37 inactivates the repressor bound to exon 6.37 resulting in exon 6.37 inclusion (Graveley, 2005)

that a mutation causing a change in the RNA secondary structure affects alternative splicing. In humans, SMN1 functions as a splicing factor. Spinal muscular atrophy (SMA) is a genetic disorder caused by a deletion in the gene encoding the protein SMN1. As a consequence, the protein does not have the regulatory function, leading to the degradation of motor neurons. SMN protein 2 cannot compensate for the absence of an active SMN1 protein because it has no exon 7. Changes in the regulation of the alternative splicing of exon 7 in the SMN2 gene is one of the main aims of SMA therapy.

Likewise, a point mutation does not alter the amino acid sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) in exon 12, but rather causes this exon to be reduced from 80% to 25% in the transcript. In this case, a single nucleotide change does not result in large structural changes. The mutation increases the length of single-stranded regions, including the exonic splicing silencer (ESS), and increases the stability of structures containing the exonic splicing enhancer (ESE) (Pagani et al., 2005).

The effect of RNA on the secondary structure can be enhanced by binding the splicing factor to structural motifs in order to increase their stability. The binding of a muscleblind like protein factor (MBNL1) causes alternative splicing of exon 5 in cardiac troponin T pre-mRNA (Warf and Berglund, 2010). The 3′ splice site of this exon forms an RNA hairpin which prevents further binding of U2AF65 to the splice site and protein MBNL1 additionally stabilizes the hairpin structure.

Impact of the secondary structure on alternative splicing through long-range interaction

Besides the local structural motifs, long-range interaction may also effect alternative splicing. The long introns can create 100-300 nucleotide hairpin structures with loops up to 15 000 nucleotides long, which makes it easy for them to be removed (Shepard and Hertel, 2008). The most known example of alternative splicing regulation by long-range interaction is the Dscam gene from *Drosophila melanogaster* (Graveley, 2005). The alternative splicing of this gene results in 38 016 different transcripts by skipping or retaining 95 different exons. The case of mutually exclusive exons in the exon 6 group of the Dscam gene is an example of how the regulation of the splicing of pre-mRNA involves so many exons. Behind exon 5, which is constitutively spliced, is located a docking site which interacts with the selector sequences that occur before the exons 6.36, 6.37, and 6.38. Depending on which region binds to the docking

sequence, different exons can be spliced (Fig. 4). The regulation of alternative splicing in the Dscam gene is an example of how stable double-stranded motif formation may loop-out a fragment of pre-mRNA and cause close regions to become sequentially spaced.

The pre-mRNA of fibroblast growth factor receptor-2 is also alternatively spliced, resulting in the formation of two isoforms with exon IIIb or IIIc (Baraniak et al., 2003). The isoform expression is tissue-specific. The process of pre-mRNA maturation is regulated by cis-acting elements which are sequentially located at a distance from each other. Within the region, a big RNA hairpin with a 735-nucleotide-long loop is formed. Such a rearrangement of this region causes the regulatory elements to be positioned close together and may affect the alternative splicing of the pre-mRNA.

In order to correctly splice the intron from the Saccharomyces cerevisiae rp51b gene, two short fragments that are sequentially separated by 200 nucleotides must interact with each other (Libri et al., 1995).

All of these examples show that secondary structure affects the alternative splicing of genes and is a part of the mRNA splicing code. This allows the process that a pre-mRNA molecule undergoes to be decrypted. It is also noteworthy that a single nucleotide polymorphism is sufficient to make changes in the folding and stability of mRNA (Shen et al., 1999), and thus it can lead to significant changes in the alternative splicing of pre-mRNA.

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