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Context-dependent splicing regulation

Exon definition, co-occurring motif pairs and tissue specificity

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Splicing is a crucial process in gene expression in higher organisms because: (1) most vertebrate genes contain introns; and (2) alternative splicing is primarily responsible for increasing proteomic complexity and functional diversity. Intron definition, the coordination across an intron, is a mandatory step in the splicing process. However, exon definition, the coordination across an exon, is also thought to be required for the splicing of most vertebrate exons. Recent investigations of exon definition complexes provide insights into splicing dynamics. That splicing regulators act in a context-dependent mode is supported by a large collection of evidence. Splicing contexts generally can be classified as cis-element and trans-element contexts. A widespread cis-element context is defined by co-occurring motif pairs to which splicing regulatory factors bind to direct specific molecular interactions. Splicing regulation is also coordinated by trans-element contexts as exemplified by tissue specific splicing, where alternative exons can be coordinately regulated by a few splicing factors, the expression and/or activity of which are concertedly higher or lower in the corresponding tissues.

Key words: splicing motif, exon definition, co-occuring pair, alternative splicing, tissue specificity

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Introduction

Pre-mRNA splicing is an essential step in gene expression in higher eukaryotic organisms as most human genes contain multiple copies of long non-coding introns interspersed between relatively small exons that embody the protein coding information.[1-4] Precise removal of introns and ligation of exons guarantee the accurate flow of genetic information from DNA to protein. Exons in pre-mRNA molecules can be spliced constitutively or alternatively: the former generates a single splicing outcome across all cell types and developmental stages and the latter produces a variety of mRNAs by splicing the exons of primary transcripts from the same genes in different arrangements. Alternative splicing regulation is one of the crucial mechanisms for gene regulation and is responsible for the greater proteomic and cellular diversity of higher eukaryotic organisms.[5] Around 95% of human multi-exon genes undergo alternative splicing,[6,7] sometimes producing two protein products with opposite functions.[8] The malfunction of alternative splicing regulation has been recorded in many human genetic diseases and cancer.[9]

Pre-mRNA splicing is performed by the spliceosome, a large ribonucleoprotein complex comprised of five small nuclear ribonucleoproteins (snRNPs U1, U2, U4, U5 and U6) and a large number of associated proteins cooperatively deployed to recognize the splice sites and carry out the two steps of the phosphodiester transfer reactions.[10] In vitro studies have revealed that pre-mRNA splicing is a highly concerted process, involving the sequential binding of the snRNPs to the target intron to form several distinct intermediate complexes termed H, E, A, B and C. The formation of an H complex can include the binding of the U1 snRNP to the 5' splice site of the intron and the association of many sequence-specific auxiliary proteins including hnRNPs.[11] The subsequent binding of the U2 snRNP auxiliary factor (U2AF) to a 3' splice site and the

recognition of the branch point by the splicing factor 1 (SF1) form the E complex. Then as the first ATP-dependent step in splicing assembly, the U2 snRNP interacts stably with the branch point sequence (BPS) to form the A complex. The B complex is generated by the subsequent association of the U4-U6-U5 tri-snRNP complex. The C complex finally forms by several structural rearrangements in the B complex and the disassociation of the U1 and U4 snRNPs to produce the active spliceosome that catalyzes the splicing reaction.[1]

The correct splicing of exons and removal of introns require, first and foremost, a mechanism to locate exons and introns. The core splice site sequences themselves do not have sufficient information.[12,13] Additional information is thought to lie in short degenerate cis-elements found both in exons and flanking introns.[13] These cis-elements are called exonic and intronic splicing enhancers and silencers (ESE, ESS, ISE and ISS) according to their location and the direction of their effect on splicing.[2,13] Many of these splicing regulatory elements have been found to be bound by specific RNA-binding proteins that either enhance or silence splicing in a context dependent manner.[2,3] Here we focus on recent progress on splicing mechanism derived from model gene constructs and genome-wide studies.

Exon Definition

All pre-mRNA splicing reactions require a mandatory 'intron definition' as the pairing of the splice sites at the two ends of an intron is involved in the removal of an intron from between two exons in all pre-mRNA splicing reactions.[1] However, most human genes contain multiple relatively short exons (50–250 bp in length) separated by large intervening introns (often thousands of bp or more and highly diverse in length).[14] It is likely that most human splice sites are recognized in pairs across an exon, a process termed 'exon definition'. This model was first proposed to explain the phenomenon that attaching a 5' splice site downstream of the second exon in a two-exon splicing substrate greatly enhances the splicing of the upstream intron in vitro.[15,16] The exon definition model was supported by the predominant exon-skipping phenotype of single splice site mutations in internal exons[17-19] and the transcript geometry in vertebrate genomes.[1,14] Furthermore, comparative genomics studies reveal that there has been compensatory evolution among the splice sites, ESEs and ESSs of the same exon, with the exon as an entirety being conserved as a unit of splicing.[20,21] Exon definition may serve as a quality control function, preventing the splicing at an isolated splice site unless a bona fide exon is defined.

At the molecular level, exon definition is thought to be engaged by U1 snRNP binding at the downstream 5' splice site to promote U2AF recognition of the upstream 3' splice site[22] and subsequent U2 snRNP binding to the branch site across the exon. Exonic splicing enhancers located in between the two splice sites recruit SR proteins to stabilize a protein-protein interaction network across the exon, forming a connection between U2 and U1 snRNPs.[22-24] Further confirmation of the existence of U1 and U2 snRNPs as well as many additional proteins in the exon definition complex have recently come from the purification of three such internal exon complexes formed on in vitro spliced exons.[25-27]

House et al. provided evidence that the splicing repression of exon 4 in the CD45 gene was due to the formation of a stalled A-like exon-definition complex (AEC) that failed to convert into cross-intron complexes to catalyze the splicing reaction. Instead, the AEC, produced by the binding of hnRNP L to an exonic splicing silencer and interacting with the flanking U1 and U2 snRNPs, sequestered the ESS-containing exon such that it is skipped in the final product.[25] Studying the PTB-repressed splicing of the src N1 exon, Sharma et al. found that exon definition complexes that assembled on the exon downstream of src N1 could form regardless of the presence of PTB.[26] However, the presence of PTB stalled the splicing complexes at an A-like complex that failed to transition to a functional spliceosomal complex. Proteomic analyses of these complexes revealed that exon definition complexes (EDCs) contained the anticipated components of the U1 and U2 snRNPs, U2AF and SR proteins, these last presumably binding to ESEs within the exon. In addition, multiple hnRNPs were found within EDCs, suggesting they may also be involved in the formation of this exon definition complex. Schneider et al. took an important further step by isolating functional exon definition complexes and showing them to contain U4, U5 and U6 (the tri-snRNP) in addition to U1 and U2. They also found an alternative spliceosome assembly pathway in which the transition from the cross-exon complex to the cross-intron B-like complex can proceed directly without the preexistence of a cross-intron A complex.[27]

These exon definition complexes studies, along with other findings,[11,28-31] provide clear evidence that alternative splicing regulation can occur at multiple transitions along the spliceosome assembly and catalytic pathway,[10] and is not limited to initial splice site recognition.

Cis-Context Effect Exemplified by Co-Occurring Motif Pairs

Splicing regulatory elements are often found to act in a context-dependent mode.[32,33] The combinatorial effects of two or more splicing regulatory elements have been widely documented.[1-4,34] Many of these elements are found to be splicing regulatory factor binding sites and can be either enhancing or silencing depending on the positions at which they are located, e.g., the binding sites of hnRNP F/H, ASF, hnRNP L, Nova1/2, FOX and PTB.[35-45] The strength of splice sites (i.e., splice site consensus score) can also modulate the function of a splicing regulatory element. A recent study demonstrated that the splicing enhancing effect of intronic poly-Gs is highest if adjacent to intermediate strength 5' splice sites.[46] Independently, the binding of hnRNP L to an exon was found to repress strong splice sites but unexpectedly to enhance weak sites.[47] These findings could be interpreted as a context effect generated by distinct interactions between splicing factors that bind these splicing regulatory elements and spliceosomal components binding to splice sites. A context effect can also be created by

interactions among splicing factors that bind individual splicing regulatory elements. Specific combinations of motifs have been found to act cooperatively to promote exon skipping.[48] The binding of the same hnRNPs at the two ends of a long intron can promote splicing by interacting with each other to bring the two splice sites closer together.[49,50] Motifs co-occurring at intron ends revealed by a computational search were shown to enhance intron removal.[51]

As a very early step in splice site recognition, exon definition is thought to require communication between the two ends of an exon, during which the two ends could be bound with proteins that are capable of specifically interacting with each other. Thus, the binding sites of those proteins would exhibit the occurrence of specific combinations across exons. Evidence for such exon-defining motif pairs was found in the intronic regions that flank exons.[52] These pairs are found around constitutive and alternative exons but not pseudo exons. They are preferentially associated with weaker exons. They are conserved in evolution and they exhibit a lower SNP frequency when paired. Paired motifs are located close to exon boundaries and display specificity with respect to distance from the exon ends and in constitutive versus alternative splicing. These pairs were shown to enhance exon inclusion synergistically and the enhancement exhibits specificity with respect to the exon in between.[52] Many of these motifs resemble the binding site of hnRNPs. This finding is consistent with a recent proteomic study of exon definition complexes (EDC) in which hnRNPs were found within EDC complexes.[26]

A recent comprehensive study identified splicing regulatory element features that are capable of predicting tissue-specific alternative splicing.[53] In post-processing these sequence features, the authors identified many pairs that significantly co-occurred, suggesting specific molecular interactions of factors that may bind to these features. Furthermore, by comparative analysis of mammalian genomes, Suyama et al. identified eleven conserved sequence motifs that may regulate alternative splicing; these motifs are not only enriched in alternative exons but also co-occur with each other, implying specific interactions among the factors binding to these motifs.[54] Zhang et al. applied Bayesian networks to integrate diverse data sets to predict combinatorial regulation by Nova and Fox in alternative splicing.[55] In the same vein, PTB-repressed exons were found to be significantly associated with Fox2 binding.[45] All these results indicate that specific molecular interactions in splicing regulation are prevalent and likely constitute the basis for much context-dependent splicing regulation.

Trans-Context Effect Exemplified by Splicing Regulatory Factor Tissue Specificity

Recent genome-wide studies have provided evidence that the alternative splicing of functionally coherent genes is regulated in a concerted manner among different tissue types and upon signal-induced activations.[38,56] In many cases, the concerted regulation of alternative splicing was found to be independent of that of the transcriptional events, indicating the cell has multiple gene expression regulation networks to ensure appropriate actions in complex and diverse processes.[4,57-60]

Concerted alternative splicing regulation in response to cell signaling may be carried out by a few splicing factors. This idea is supported by findings that these alternative exons share common cis-elements to which the cognate splicing factors bind. Analysis of exons that are alternatively spliced upon neuronal depolarization identified two essential regulatory sequences accountable for the coordinated action.[61] Similarly, the concerted skipping of the three variable exons of the CD45 gene in response to T-cell activation is mediated by a signal-responsive ESS inside each of the three variable exons, and the cognate splicing factors identified include hnRNP L, PSF and hnRNP LL.[62-66] Furthermore, a recent global study of alternative splicing events involved in insulin and wingless signaling revealed pathway-specific sequence motifs enriched near the 5' splice site of the regulated exons in Drosophila cells.[67]

Pre-mRNAs subject to tissue-specific alternative splicing may also be concertedly regulated by common intronic sequences resembling the binding sites of splicing factors that are preferentially expressed in the corresponding tissues, e.g., Nova in brain,[58] Fox in muscle and brain,[42,43,68,69] and ESRP1/2 in epithelial cells.[70,71,72] Genes harboring certain individual intronic splicing elements identified through comparative genomics displayed tissue-specific bias.[73] Splicing factors involved in tissue-specific alternative splicing regulation may also include ubiquitously expressed proteins, the levels of which also exhibit tissue-specific differences, such as SR proteins and hnRNPs.[74] Consistent with this picture, many motifs of co-occurring pairs involved in exon definition resemble binding sites of known splicing factors,[52] including hnRNPs A1/A2, C, D, F/H, G, I (PTB), K, L, M and 9G8. Specific pairs are enriched in tissue-specific genes, the higher expression of which correlates with that of the pertinent RNA binding proteins.[52]

Tissue-specific alternative splicing is one of the major signatures of tissue identities and is crucial for our understanding of developmental mechanisms and human diseases. The examples of the concerted regulation of alternative splicing in different tissues and cell statuses presented here, is in no way an exhaustive list. We are probably just seeing the tip of the iceberg of systematic programs of alternative splicing regulation.[4,38,56] With high-throughput technologies, better algorithms and systematic empirical validations, we expect to gain deeper insight into splicing mechanisms.[75] In addition, just like other levels of gene expression, splicing regulation does not exist in isolation. To understand how these levels of regulation interact warrants investigation, and the latest progress on the effects of nucleosome positioning on splicing regulation serves as a good example.[76-80]

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