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The Exon Junction Complex: A Multitasking Guardian of the Transcriptome

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Abstract

In a recent issue of *Molecular Cell*, Boehm et al. (2018), Blazquez et al. (2018), and Gonatopoulos-Pournatzis et al. (2018) uncover novel mechanisms by which the cell regulates splicing of cryptic splice sites and microexons.

RNA splicing, the process of removing introns from pre-mRNA, is carried out by the spliceosome. Significant attention has been focused on the remarkable ability of this enzyme to act on a vast array of substrates with relatively little sequence constraint due to the degenerate nature of splice sites. Nearly every mammalian gene undergoes splicing, and the spliceosome is able to recognize multiple introns (sometimes hundreds!) in a single RNA. However, this raises a different question: how does the spliceosome bypass the many splicing opportunities that this degenerate recognition might allow? In a recent issue of *Molecular Cell*, Boehm et al. (2018) and Blazquez et al. (2018) describe an unexpected player, the exon junction complex (EJC) and EJC-associated proteins, in suppressing the use of cryptic splice sites, including splice sites that are generated by newly formed exon junctions capable of recursive splicing. This quality-control mechanism limits loss of exonic information and prevents spurious formation of cryptic microexons (3–27 nucleotide exons). However, recursive and microexon splicing occur under physiological conditions. In fact, a specific class of microexons is critical for nervous system development and function. In the same issue, Gonatopoulos-Pournatzis et al. (2018) identify two SR-related proteins that regulate neuronal microexon splicing. Remarkably, one of these proteins, RNPS1, is the same factor that associates with the EJC to mitigate production of microexons from cryptic splice sites (Blazquez et al., 2018). Together these studies reveal novel mechanisms by which the interactions between RNA binding proteins (RBPs), including the EJC and EJC-interacting proteins, regulate the use of cryptic splice sites and activate authentic splice sites to shape the mammalian transcriptome.

The EJC consists of the core proteins EIF4A3, MAGOH, RBM8A, and CASC3/BTZ (Boehm and Gehring, 2016) and is deposited onto mRNAs ~24 nt upstream of exon-exon junctions in a splicing-dependent but sequence-independent manner. The EJC is involved in

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numerous posttranscriptional processes, including mRNA export, translation, and nonsense-mediated decay (Le Hir et al., 2016). Recent reports suggest a role for the EJC in RNA splicing, although the mechanism for this has been unclear (Le Hir et al., 2016).

The EJC interacts with a number of auxiliary proteins to direct its specific functions. One of these is the PSAP complex, comprised of RNPS1, SAP18, and the splicing coactivator Pinin (PNN). To further understand the role of the EJC in regulating splicing, Boehm et al. (2018) depleted EJC-associated factor RNPS1 and observed widespread changes in splicing, including use of cryptic 5′ splice sites (5′SSs) as well as reconstituted exonic 5′SSs generated by the new exon junctions. In the absence of the EJC, these reconstituted 5′SSs are re-spliced to the 3′SS of a downstream intron, resulting in exon skipping. Boehm et al. (2018) also identify an RNPS1-independent role for the EJC in masking splicing signals to prevent usage of reconstituted or cryptic 3′SSs (Figure 1).

Blazquez et al. (2018) also show a role for the EJC in suppressing cryptic splicing of reconstituted splice sites. Remarkably, the authors determined that 16.2% of human transcripts contain at least one putative exon that can reconstitute a 5’SS at the exon-exon junction. Depletion of core EJC proteins, RNPS1, or PNN all lead to use of these reconstituted sites, resulting in recursive splicing and exon skipping. The authors also show that EJC repression of cryptic 5′SSs prevents inclusion of cryptic microexons (Figure 1).

These papers highlight that a key function for the EJC is to mark newly spliced exons so that cryptic or newly formed sites in the vicinity are not used by spliceosomes. This ensures that information is not lost by a spliceosome efficiently performing its catalytic function: recognizing splice sites and removing the adjacent “intronic” sequence. The EJC is deposited co-transcriptionally and immediately upon splicing, thus enabling it to rapidly shut down aberrant splicing events. While this mechanism presumes that the RNAs are co-transcriptionally spliced in a 5′→3′ order, this is not always the case (Pandya-Jones and Black, 2009). The EJC also protects transcripts in which introns are removed out of order by masking splicing signals to prevent splicing to a reconstituted 3′SS, which would lead to exon skipping.

This raises the question: how exactly are the cryptic 5′SSs blocked? Given that the EJC must be close enough to the 5′SS to affect cryptic or reconstituted splice-site usage, it stands to reason that another protein (or proteins) might bridge the interaction between RNPS1 and the 5′SS to block or inactivate its use. Future studies will no doubt focus on identifying these factors.

Another important question raised by these studies is how the cell evades the EJC’s splicing control mechanisms. Under physiological conditions, some introns are indeed alternatively spliced via recursive splicing at reconstituted splice sites, and recursive splicing utilizing cryptic 5′SSs within the exon leads to inclusion of microexons (Blazquez et al., 2018). Sequence context, such as the presence of intronic/exonic splicing enhancers and silencers, will certainly determine the propensity for a particular constitutive or cryptic splice site to be used. It will also be interesting to determine if regulation of EJC protein levels contributes to differential EJC suppression. Since the entire complex is required for its activity (Boehm et

al., 2018), limiting levels of any of the EJC protein components or modifying them in ways that block their ability to form the EJC could impact its role in splicing. Consistent with this, there is evidence that EIF4A3 copy number contributes to splicing variation and disease (Bartkowska et al., 2018; Blazquez et al., 2018).

Recent studies have demonstrated that activation of a network of neuron-specific microexons is important for nervous system development and function (Quesnel-Vallières et al., 2018). Microexons in this network, in contrast to microexons arising from aberrant splicing, are highly conserved and maintain reading frame, thus expanding proteome complexity in the brain (Irimia et al., 2014). Gonatopoulos-Pournatzis et al. (2018) examine how these microexons are positively regulated and uncover an unexpected but familiar player: RNPS1. The authors generated microexon-splicing reporter neuronal cell lines that were sensitive to CRISPR-Cas9 targeting of endogenous splicing regulators. The screen identified over 200 proteins that affect microexon splicing, with SRRM4, a gene that has been previously implicated in microexon splicing, as a top hit. The authors also identified the SR-related proteins, RNPS1 and SRSF11. Knockdown of RNPS1 or SRSF11 resulted in skipping of the same neural microexons as knock-down of SRRM4. The authors show that RNPS1 and SRSF11 interact with SRRM4 and associate with pre-mRNA proximal to SRRM4-regulated microexons to promote spliceosome formation (Figure 1).

These three studies reveal that RNPS1 can both positively and negatively regulate microexon inclusion. The positive and negative roles are not mutually exclusive and depend on RNPS1's interaction partner: EJC interaction leads to association upstream of exon junctions and splicing inhibition, while interaction with SRRM4 and SRSF11 bound to an intronic splicing enhancer (ISE) leads to association with pre-mRNA and splicing. One can imagine a model whereby lower amounts of one RNPS1-interaction partner could contribute to it being free to engage with its other partner and vice versa. Interestingly, EIF4A3 levels are lower in the brain (Blazquez et al., 2018), which may contribute to the patterns of microexon splicing through modulation of RNPS1's positive and negative roles. Levels of the relevant RNA substrates could also determine RNPS1 activity. In neurons, for example, elevated expression of pre-mRNAs encoding microexons with SRRM4-SRSF11-RNPS1 binding sites may lead to increased association of RNPS1 with these pre-mRNAs. This may also explain the prevalence of recursive splicing in the brain if RNPS1 is sequestered away from EJC interactions.

The studies by Boehm et al. (2018), Blazquez et al. (2018), and Gonatopoulos-Pournatzis et al. (2018) reveal novel mechanisms by which the cell utilizes RBPs to direct spliceosome activity. These studies also continue an emerging theme that RBPs are repurposed to serve multiple, and in some cases opposing, functions. Continued exploration of the eukaryotic transcriptome under different conditions will certainly reveal new insights into the combinatorial capacity of RBPs like RNPS1 and the EJC.

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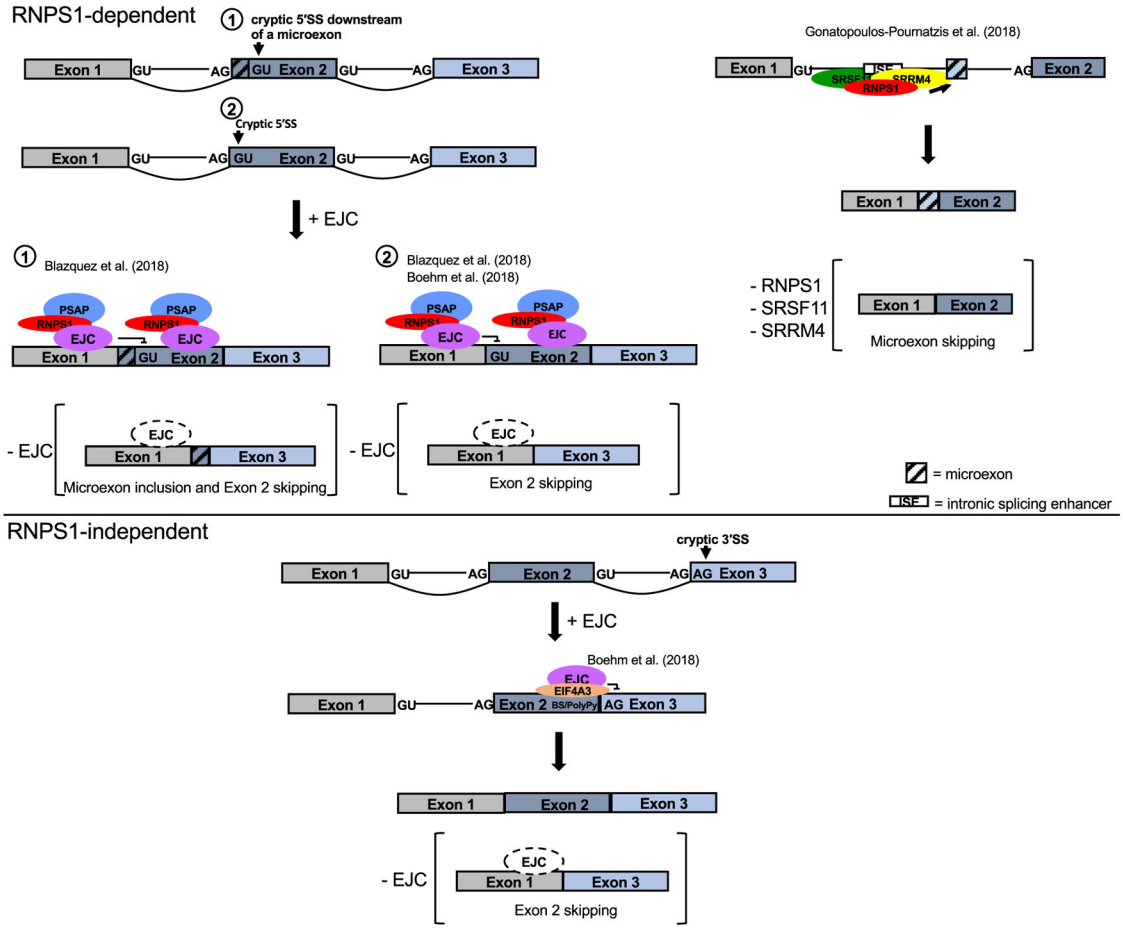

Figure 1. The EJC and Auxiliary Factors Regulate Splicing of Cryptic Splice Sites and Microexons

The EJC suppresses usage of cryptic and reconstituted 5′ splice sites (SSs) in a RNPS1-dependent manner. In scenario 1, a cryptic 5′SS downstream of a microexon within Exon 2 is used, resulting in inclusion of the microexon and skipping of Exon 2 in the absence of the EJC. In scenario 2, a reconstituted 5′SS is generated upon ligation of Exons 1 and 2, resulting in re-splicing and skipping of Exon 2 in the absence of the EJC. Additionally, the EJC suppresses cryptic and reconstituted 3′SSs in a RNPS1-independent and EIF4A3-dependent manner. The EJC masks the branchpoint or the polypyrimidine tract of Exon 2, which suppresses usage of the cryptic 3′SS of Exon 3. In the absence of the EJC, the cryptic 3′SS is used and results in Exon 2 skipping. In neurons, SR-related proteins RNPS1 and SRSF11 regulate a program of inclusion of conserved and frame-preserving microexons through interaction with SRRM4, the neuronal-specific SR-repeat protein of 100 kDa (also known as nSR100), at splicing regulatory elements. These interactions promote spliceosome formation. Knockdown of RNPS1, SRSF11, or SRRM4 results in skipping of a neuronal microexon between Exons 1 and 2.