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Review

Regulated pre-mRNA splicing: The ghostwriter of the eukaryotic genome ☆

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ABSTRACT

Intron removal is at the heart of mRNA synthesis. It is mediated by one of the cell's largest complexes, the spliceosome. Yet, the fundamental chemistry involved is simple. In this review we will address how the spliceosome acts in diverse ways to optimize gene expression in order to meet the cell's needs. This is done largely by regulating the splicing of key transcripts encoding products that control gene expression pathways. This widespread role is evident even in the yeast *Saccharomyces cerevisiae*, where many introns appear to have been lost; yet how this control is being achieved is known only in a few cases. Here we explore the relevant examples and posit hypotheses whereby regulated splicing fine-tunes gene expression pathways to maintain cell homeostasis. This article is part of a Special Issue entitled: Nuclear Transport and RNA Processing.

1. Introduction

In general terms, prokaryotic genes are compact, can be grouped based on their functions, and are translated while still being transcribed. Gene expression in prokaryotes has evolved towards speed and efficiency. In contrast, most eukaryotic genes are isolated and, moreover, have their sequences interrupted, such that the coding pieces must be spliced together to produce a meaningful transcript or mRNA. Transcripts undergo further processing before being transported to the cytoplasm. Accordingly, mRNAs exist in the cell as RNPs (ribonucleoprotein) particles that act as both executors and bearers of their fates. In light of this complex mRNA processing, it is clear that eukaryotic gene expression is under a set of selection rules whereby speed and efficiency are only part of the story.

This review will focus on the diverse roles that pre-mRNA splicing plays in the control of the processes underlying gene expression. Elegant genetic, biochemical, and cell biological studies in the yeast *Saccharomyces cerevisiae* have made it possible to glean mechanistic insights into these processes and their interplay. As such, these will be the basis of this review. Nonetheless, many of the guiding principles learned from *S. cerevisiae* are likely to inform our general understanding of how regulation of splicing and the integrated gene expression networks that involve splicing contribute to cellular homeostasis.

2. Pre-mRNA splicing

Pre-mRNA splicing is the process by which the coding segments of a gene (exons) are spliced together, and the interrupting fragments (introns) are removed and discarded. Splicing is the task of the spliceosome, one of the most complex macromolecular machines in the cell [1]. This evolutionarily conserved assembly starts anew in each round of splicing (reviewed in [2]). First, once the intronic 5′ end (5′ splice site or 5′ss) is transcribed, it is recognized by the U1 snRNP (small nuclear RNP). This interaction is thought to be driven by the complementarity between the U1 snRNA and the 5′ss, although its protein components play important roles as well [3]. Subsequently, the 3′end of the intron, including the branch-site (BS) and the 3′ splice site (3′ss) are identified by the factors BBP and Mud2 in yeast or their homologs SF1 and U2AF in other systems (including human). These factors play a role in the initial recognition of the BS by the U2 snRNP. Early interactions between the U1 and U2 snRNPs on the same intron are thought to be essential to define the intron to be removed and, therefore, the exons to be joined. There is an alternative way to define the substrate for the spliceosome, based on "exon definition," which posits that interactions between an upstream U2 snRNP and a downstream U1 snRNP are critical for the further assembly of the nascent spliceosome, marking the pre-mRNA in between as an exon. This appears to be particularly important for genes that contain multiple, large introns, as is usually the case in metazoans [4] As we will further examine, recent studies in *S. cerevisiae* support the notion that cross-exon interactions are also important in yeast.

In a subsequent step, which remains somewhat obscure, molecular rearrangements lead to the interaction between U1 and U2 of the same intron (as in the previous "intron definition"), and spliceosome assembly

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proceeds with recruitment of the U4/U6.U5 tri snRNP. Subsequent remodeling steps, catalyzed by RNA-driven ATPases, displace U1 and U4 and facilitate the formation of the catalytically active spliceosome, which will form a lariat structure via a 2′–5′ phosphodiester linkage between the adenosine at the BS and the 5′ end of the intron, followed by exon joining and intron removal. Thus, a sophisticated molecular ballet culminates in two chemically simple transesterifications. That this rather straightforward chemistry involves such a complex cellular machinery is a testament to the relevance of spliceosome dynamics. By modulating spliceosomal function to produce a variety of splicing outcomes from a single pre-mRNA, evolution has developed a formidable tool to enlarge the coding potential of genomes.

The initial recognition of the pre-mRNA by U1 and U2 is an important target for regulation. Regulatory proteins can interfere with either the recognition of intronic sequences (leading to intron splicing or retention); or the interactions that occur during exon definition (promoting exon inclusion or skipping). Switching the 3′ss after spliceosome assembly has been shown in metazoans [5], indicating that regulation of the spliceosome goes beyond controlling initial splice site recognition. In some instances the pre-mRNA structure plays a role in intron recognition, and in many yeast introns it is critical for proper 3′ss selection [6,7]. Pre-mRNA intronic and exonic sequences, known as splicing enhancers or silencers, participate in the recruitment of splicing factors, such as SR proteins and hnRNPs. As is the case with many of the pre-mRNA sequences involved in splicing, the information content of silencers and enhancers is rather low, and the splicing outcome will also depend on the relative concentration of the splicing regulatory proteins. Thus, alternative splicing is a function of the pre-mRNA sequence (including exonic structure and RNA folding) and the concentration of splicing factors, which together make up a putative "splicing code". Although regulated alternative splicing has been best characterized in metazoans, studies of the yeast *S. cerevisiae*, in which regulated splicing primarily is seen in the form of intron retention, offers an opportunity to glean basic insights into the reaction.

3. Chromatin, transcription, and splicing converge to modulate gene expression

About 20 years ago, evidence emerged indicating that intron removal could occur on nascent transcripts. Elegant imaging studies by Beyer and co-workers revealed nascent pre-mRNA shortening caused by splicing [8,9]. Subsequently, a number of important studies using *S. cerevisiae* also showed that spliceosome assembly (beginning with intron recognition) occurred on the nascent transcript, although it was less clear whether the actual chemistry of splicing occurred co-transcriptionally [10–14]. Recently, RNA polymerase II has been reported to pause in a manner that facilitates both co-transcriptional assembly of the spliceosome as well as splicing [15,16], supporting the notion that most splicing occurs co-transcriptionally [16].

In light of this close spatial and temporal relationship between transcription and splicing, it is not surprising that polymerase activity can have important effects on splicing. For example, in mammals as in yeast, the speed of the polymerase influences recognition of splice sites, such that an exon that is normally excluded (exon skipping) is recognized and included when the polymerase rate is slowed [17,18]. Additionally, in mammals RNA polymerase pausing has been shown to affect exon recognition [19]. A more extensive survey of co-transcriptional splicing mechanisms can be found in a number of excellent recent reviews (see for example [20–22]). Nonetheless, these studies demonstrate a growing appreciation that in order to understand splicing and its regulation, it is important to view splicing within its co-transcriptional context.

More recently, it has become clear that one of the important mechanistic consequences of transcription-coupled splicing is that the state of the chromatin influences splicing outcomes. Analysis of genome-wide surveys from *Drosophila* and mammals have revealed that nucleosomes and, according to several of these studies, specific histone modifications are enriched in exon sequences, suggesting that there may be specific histone "marks" that are associated with splicing [23–29]. Additionally, proteins that bind to histones have been shown to facilitate the recruitment of snRNPs to the nascent transcript and influence efficiency of splicing and alternative splicing (Reviewed in [30,31]). The ability of chromatin to influence pre-mRNA splicing appears to be a conserved feature of co-transcriptional splicing. For example, Gcn5, the histone acetyltransferase that is part of the SAGA complex, has strong genetic interactions with components of the U2 snRNP, and its catalytic activity is required for co-transcriptional spliceosome assembly [32,33].

In light of the integral relationship between chromatin and splicing, one of the outstanding questions is whether the reverse relationship exists. Namely, can the splicing reaction or specific splicing factors influence transcriptional regulation through effects on chromatin—either by direct coupling of histone modification with intron recognition or via regulated splicing of components of the histone modifying machinery?

Several recent mammalian studies report that histone H3K36 methylation is mediated through splicing signals, although the mechanism for this is unclear [34,35], and it is not clear whether other histone marks may also be influenced by splicing. In yeast, splicing has been shown to affect histone H2B ubiquitination by regulating the expression of *SUS1*, a component of the histone H2B deubiquitinase machinery. However, thus far, no evidence of a direct relationship between histone H2B ubiquitination and splicing has been reported.

4. Yeast as a model to dissect regulated splicing

When facing complex scientific problems, a reductionist approach can often be a valuable tactic. The budding yeast *S. cerevisiae* has provided such a tool since the discovery of the *ACT1* intron [36]. With its streamlined genome and high functional conservation of the basic machinery [37] it has been particularly useful to uncover the intricacies of spliceosome assembly and catalysis. However, the scarcity of introns (~95% of yeast genes are intronless), and the lack of clear homologues of SR-proteins (ubiquitous splicing modulators in metazoan cells), has encouraged the view that the yeast cell does not use this important instrument to regulate gene output. While it is true that there are quantitatively fewer alternate mRNAs that arise from splicing changes in yeast compared to metazoans, regulation of splicing in yeast is essential to cellular fitness and meiotic differentiation (Fig. 1). Given that the fundamental aspects of the spliceosome are conserved in evolution, the study of these regulatory strategies is relevant to understanding splicing across eukaryotes, and likely it will reveal possibilities that may be harnessed by other organisms. In fact, its reduced intronic set simplifies the study of splicing at the genomic level. This, combined with the manageability of yeast as a model system, has helped to advance our understanding of some molecular mechanisms behind regulated splicing.

As a single-celled organism, yeast has been subjected to strong selection for rapid and adaptive growth. A growing yeast cell is under the Sisyphean task of constantly adjusting to an ever-changing environment and one might imagine that regulation of splicing is an important part of this business. Indeed, the cell uses splicing to fine-tune RNA biogenesis, as genes encoding proteins involved in a number of critical steps in RNA biogenesis can undergo regulated splicing—including translation, RNA export, RNA splicing, and transcription. This provides a powerful tool for regulating gene expression, as changing the capacity to translate mRNAs from the assembled and transported mRNPs can fundamentally alter gene expression. We will now focus on examples of how splicing affects these critical gene expression reactions.