Splicing from the Outside In

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A new study in this issue of *Molecular Cell* (Pleiss et al., 2007b) shows that changes in the environment rapidly alter the splicing efficiency of specific pre-mRNAs in yeast.

It is well known that extracellular ligands can modulate nuclear transcription and that these signals are often transmitted by signal transduction cascades. While the transcriptional consequences elicited by extracellular stimuli are clearly an important aspect of cell physiology, they encompass only a portion of what actually happens. In this issue of *Molecular Cell*, Pleiss et al. (2007b) demonstrate the extent to which pre-messenger RNA (pre-mRNA) splicing is impacted by changes in the extracellular environment in the yeast *Saccharomyces cerevisiae*.

Pre-mRNA splicing is the process by which introns are removed from a pre-mRNA and the remaining exons are ligated together to generate mature mRNAs. Intron removal is catalyzed by the spliceosome, an extremely large and dynamic macromolecular machine composed of five small RNAs and more than 100 proteins (Jurica and Moore, 2003). The assembly of the spliceosome on each intron is directed by elements within the intron that are recognized in a sequence-specific manner by spliceosomal components. The collective "strength" of these splice sites (i.e., the affinity of the spliceosomal components for these sequences) determines the efficiency of splicing—introns with strong splice sites are efficiently removed, while those with weak sites are spliced less efficiently.

In higher eukaryotes, the vast majority of genes contain multiple introns. This creates a situation in which the exons can be spliced together in different combinations to generate multiple mRNA isoforms from each gene. This process of alternative splicing plays important roles in increasing protein diversity and is a key aspect of gene regulation in higher eukaryotes (Graveley, 2001). In contrast, only ~250 of the ~6000 genes in *S. cerevisiae* contain an intron, and the vast majority of these contain only one intron (Spingola et al., 1999). Thus, although there are a few examples of alternative splicing in *S. cerevisiae*, it would appear that for the most part, pre-mRNA splicing in yeast is not highly regulated.

However, two recent studies by Pleiss et al. (Pleiss et al., 2007a, 2007b) suggest that splicing in yeast is much more malleable than previously thought. First, Pleiss and colleagues used a microarray platform to simultaneously analyze the impact of inactivating several components of the spliceosome on the splicing efficiency of all intron-containing transcripts in the *S. cerevisiae* genome (Pleiss et al., 2007a). Considering that the splicing factors tested are thought to be essential for splicing, it was surprising to discover that distinct but partially overlapping sets of introns were affected by each spliceosomal component examined. In the current study, Pleiss and colleagues extend these studies to examine whether environmental changes impact the efficiency of splicing (Pleiss et al., 2007b). They show that amino acid starvation has a profound impact of the splicing of introns in most of the ribosomal protein-encoding genes but little, if any, impact on the splicing of introns in other genes. They also demonstrate that the splicing of a distinct set of introns is affected when yeast are exposed to ethanol. Together, these results clearly show that environmental changes can regulate the efficiency of splicing in a transcript-specific manner in yeast.

Though microarrays are a powerful discovery tool, they frequently provide little insight into molecular mechanisms regulating the process being studied. In the current study, Pleiss and colleagues do not address this issue in much detail. However, there are a few clues in their data about possible mechanisms by which environmental changes can alter splicing. One of the most remarkable findings of this work is that the splicing changes occur extraordinarily rapidly, within 2 min of the onset of amino acid starvation or the addition of ethanol. Additionally, the transcripts that are impacted by amino acid starvation and the addition of ethanol are distinct. Thus, these changes in the efficiency of intron removal do not require new protein synthesis, and the splicing of each intron has a distinct response to various environmental cues.

So, what types of mechanisms could account for these rapid and specific changes in splicing? Perhaps the most obvious mechanism involves the posttranslational modification of a protein that modulates the efficiency of intron removal in a sequence-specific manner. There are, in fact, several known cases of signal-induced splicing regulation (Shin and Manley, 2004). For example, activation of the Ras signaling pathway results in phosphorylation of Sam68, which in turn binds to and modulates the alternative splicing of a variable exon within the *CD44* pre-mRNA (Matter et al., 2002). Interestingly, in *S. cerevisiae*, the accumulation of uncharged tRNAs that occurs during amino acid starvation is known to activate Gcn2, a kinase

that phosphorylates eIF-2α and represses translation. This prompted Pleiss et al. to test whether Gcn2 is involved in regulating the splicing of the ribosomal protein pre-mRNAs. However, these regulated splicing events are independent of Gcn2, as the same changes in ribosomal protein pre-mRNA splicing occurred in both wild-type and *Δgcn2* strains. This does not exclude the possibility that signal transduction is involved in the mechanism of amino acid response, as this pathway may require a kinase or other posttranslational modification enzyme other than Gcn2.

Even if posttranslational modifications are involved, it is not clear what proteins would be targeted as *S. cerevisiae* is relatively devoid of the types of proteins that regulate alternative splicing in higher eukaryotes (SR proteins and hnRNP proteins). How can the efficiency of splicing be so specifically modulated in the absence of known splicing regulators? One clue comes from the earlier study by Pleiss et al. (Pleiss et al., 2007a) and studies in *Drosophila* (Park et al., 2004) showing that perturbing the concentration or activity of several spliceosomal components that drive spliceosome assembly (i.e., Brr2, Prp5, etc.) can alter the pattern of splicing in surprisingly specific and distinct ways—the splicing events controlled by one splicing factor are distinct from those controlled by other splicing factors. These observations can be explained if introns have evolved to have different rate-limiting steps in spliceosome assembly. Thus, if signaling is involved in these rapid responses, perhaps the spliceosome components themselves are posttranslationally modified.

A second potential mechanism could involve the autoregulation of splicing. The splicing of several ribosomal protein genes is known to be autoregulated by the proteins they encode. For example, excess RPL30 can bind to the intron of the *RPL30* pre-mRNA and repress its splicing, thus reducing the level of free RPL30 in the cell (Eng and Warner, 1991). It is therefore conceivable that amino acid starvation results in either the disassembly of existing ribosomes or a decrease in the rate of new ribosome formation that leads to an increase in the levels of free ribosomal proteins. These free ribosomal proteins could then repress the splicing of their own introns.

Another mechanism, though significantly more speculative, could involve riboswitches—RNA sequences that adopt distinct structural conformations upon the binding of small molecules, especially metabolites such as amino acids. Recently, three introns in *Neurospora crassa* were shown to contain thiamine pyrophosphate-responsive riboswitches that control pre-mRNA splicing (Cheah et al., 2007). As the diversity and distribution of naturally occurring riboswitches is currently unknown, it is entirely possible that some of the ribosomal protein genes or ethanol responsive genes identified by Pleiss et al. contain amino acid- and ethanol-responsive riboswitches, respectively, that control the efficiency of intron removal.

These are but a few of the potential mechanisms by which these rapid and specific regulated splicing events can be triggered by changes in the extracellular environment. Identifying the pre-mRNA sequences required for these responses will assuredly provide insight into the actual mechanisms by which these changes occur. The surprising finding that changes in the environment can so rapidly and specifically modulate splicing in an organism that essentially uses intron retention as its only mode of alternative splicing certainly makes one wonder about the extent to which the environment can trigger transcript-specific alternative splicing in organisms such as *Drosophila* and humans and how this would be regulated. Finally, the fact that there is biological logic to these changes in yeast (i.e., the ribosomal protein genes are modulated in response to amino acid starvation) suggests that they are biologically relevant.

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