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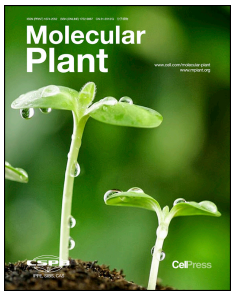

Environmental Stress and Pre-mRNA Splicing

Peng Cui, Liming Xiong

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Pre-mRNA splicing is an important step for gene expression regulation in plants in response to abiotic stress. In recent years, RNA-sequencing (RNA-seq) based on various next-generation sequencing technologies has been used to study dynamic changes of pre-mRNA splicing under conditions of diverse abiotic stress in several plant species. Accumulating results indicate that alternative splicing (AS) of pre-mRNA is closely linked with environmental stress response in plants.

FEATURES OF PRE-mRNA SPLICING IN PLANTS UNDER ABIOTIC STRESS

Recent genome-wide studies have revealed that a large number of plant genes extensively undergo AS under stress conditions. For example, Ding et al. analyzed global changes in pre-mRNA splicing under different concentrations of salt (NaCl) treatments (Ding et al., 2014). They detected that ~49% of all intron-containing genes were alternatively spliced under salt stress, 10% of which experienced significant differential alternative splicing (DAS). Interestingly, most genes that underwent DAS were not differentially regulated by salt stress, suggesting that AS represents an independent layer of gene expression regulation in response to stress. Likewise, Li et al. identified 313 genes with DAS in *Arabidopsis* roots under Fe deficiency (Li et al., 2013). Filichkin et al. identified a set of stress-associated AS events (Filichkin et al., 2010) and Gulledge et al. developed the Integrated Genome Browser to investigate stress-induced AS events in *Arabidopsis* (Gulledge et al., 2012). Collectively, these studies revealed several features of pre-mRNA splicing in plants under abiotic stress: 1) most stress-induced splicing

variants are associated with intron retention, 2) the expression level of these stress-induced variants is quite low relative to that of the major splicing variant(s), and 3) many stress-responsive genes are subject to AS.

Given the low expression level of stress-induced AS variants, it is likely that the majority of AS events could be ascribed to splicing errors or noise as a result of decreased efficiency or accuracy of pre-mRNA splicing under abiotic stress, rather than of active regulation. With the simultaneous production of a large amount of stress-inducible pre-mRNAs in response to abiotic stress, cells would need to immediately increase the recruitment of splicing and other factors for their co-transcriptional processing. This substantial increase in demand may strain the splicing machinery, and as a result, a significant portion of these transcripts may be inadequately processed, particularly when the splicing machinery is compromised.

What is the function of AS in stress response? There are reports that AS variants have been found to function in plant response and tolerance to stress (Reddy et al., 2013; Staiger and Brown, 2013). Nonetheless, many other variants of AS with retained introns or alternative splicing sites may represent ‘unproductive’ transcripts that cannot produce functional proteins. Furthermore, certain intron-retained transcripts are trapped in the nucleus and they are not subject to export or nonsense-mediated decay. It is tempting to speculate, however, that some of these intron-retained transcripts may constitute a reservoir that could later be post-transcriptionally spliced to generate functional proteins to boost long-term stress tolerance of the plants (Figure 1).

PRE-mRNA SPLICING AND STRESS TOLERANCE

In the last two decades, genetic studies have identified *Arabidopsis* mutants in RNA processing factors that exhibited altered sensitivity to various environmental stresses. Most of these mutants and their stress-related phenotypes have been summarized in a recent review (Staiger and Brown, 2013). Recently, RNA-seq has been used to study genome-wide pre-mRNA splicing in these RNA processing-related mutants. One study performed by Cui et al. demonstrated that depletion of the *Arabidopsis SAD1/LSm5* gene causes an inaccurate selection of splice sites, leading to a genome-wide increase in AS (or splicing errors) in the *sad1* mutant (Cui et al., 2014). These alterations were particularly evident under salt stress and the affected genes include those encoding known key determinants of salt tolerance such as SnRK2.1/2.2, SOS2, DREB2A, NHX1, WRKY33, WRKY25, STT3A, CAX1 and RCI2A.

These findings support the initial notion that some stress-responsive genes may sustain inaccurate splicing in these RNA-processing-related mutants. Most alternatively spliced transcripts were predicted to generate premature stop codon and truncated proteins if translated. Thus, the splicing defects would generally decrease the expression levels of the affected genes (Cui et al., 2014; Feng et al., 2015). This large-scale change in stress-responsive genes may collectively undermine plant readiness for the stress. Interestingly, in *SAD1/LSm5*-overexpressed plants, a genome-wide increase in splicing efficiency and accuracy of stress-responsive genes was observed, and plant resistance to salt stress also increased. This intriguing observation that overexpressing a single component of the multicomponent spliceosome machinery can enhance splicing efficiency prompted the proposal of a ‘dynamic model’ of splicing regulation. Complementary to the well-known ‘kinetic model,’ where transcription rate of RNA polymerase

II affects splicing, the dynamic model postulates that the availability of selected splicing factors may affect the dynamics or the assembly of the spliceosome complex, thus influencing splicing (Cui et al., 2014).

These findings suggest a new approach to improve plant stress tolerance, namely, by increasing the pre-mRNA splicing efficiency (Figure 1). Contrast to the approach of constitutively expressing stress-responsive genes that may cause unwanted side effects under normal growth conditions, enhancing pre-mRNA splicing efficiency does not affect gene expression under normal conditions, but would increase the ratio of more accurately processed transcripts for translation, thereby minimizing the negative impact on growth and development. It is thus of interest to further test whether enhancing the expression of other key splicing factors may improve splicing efficiency and overall plant stress tolerance.

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Peng Cui, Liming Xiong*

Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia

Peng Cui: Peng.Cui@kaust.edu.sa

Liming Xiong: Liming.Xiong@kaust.edu.sa

***Corresponding author: Liming Xiong liming.xiong@kaust.edu.sa**

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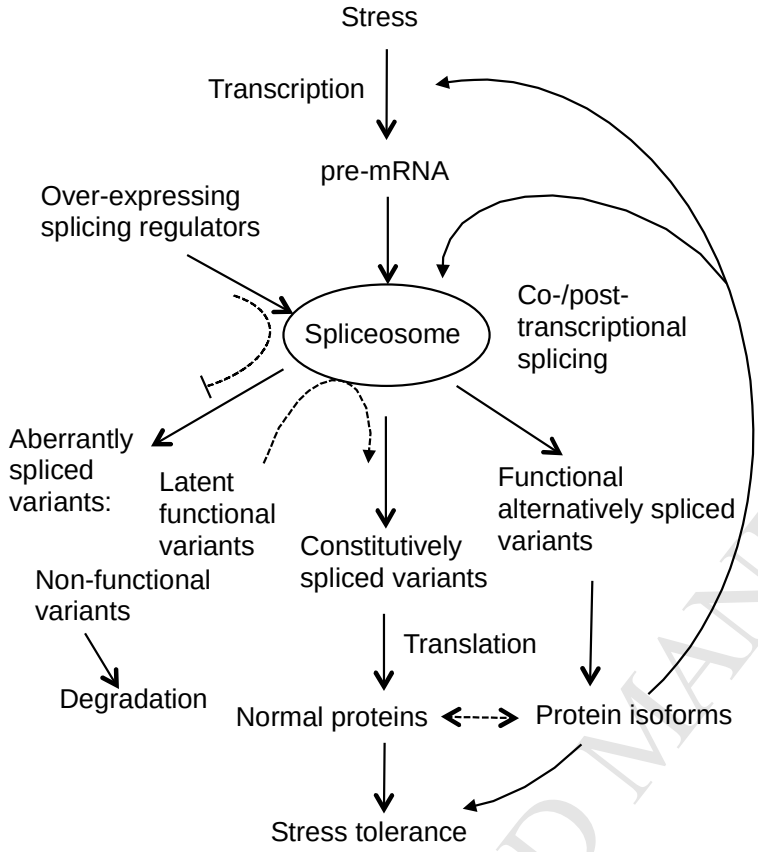

Figure 1. The interplay between abiotic stress and pre-mRNA splicing. Abiotic stress activates the transcription of many stress-responsive genes. The dramatically increased pre-mRNAs compete for the limited splicing machinery, leading to an increased amount of inadequately spliced variants along with constitutively spliced and stress-induced functional alternatively spliced variants. Some of the aberrantly spliced variants (e.g., intron-retained ones) are postulated to be posttranscriptionally spliced later and are potentially functional (dashed line). Normal proteins and the alternative isoforms may interact to regulate transcription or transcript processing or directly contribute to stress tolerance. Over-expressing positive splicing regulators

may enhance splicing efficiency and reduce splicing errors (dashed line) and increase plant stress tolerance.