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Alternative splicing regulation in plant

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Abstract

Alternative splicing of more genes is discovered to undergo AS in plants, AS is becoming more and more significant, but relatively little is known about the processes governing AS or the effects of AS on mRNA levels and protein function. To analyze AS in several genes, we have developed a precise and repeatable RT (reverse transcription)-PCR technique. New AS occurrences have been discovered in preliminary studies, indicating that the current estimates of the frequency of AS in plants are probably too low. Alternative splicing, or AS, is a process after transcription that controls the levels of mRNA transcripts and increases the complexity of proteins to control the expression of genes. In a process known as "combinatorial control," trans-acting protein factors and cis-acting signals in pre-mRNA (precursor mRNA) transcripts interact to regulate AS. Dynamic adjustments in AS patterns are a reflection of variations in splicing factor activity, composition, and abundance in various cell types as well as in response to cellular or environmental signals. We are using a high-resolution RT-PCR technique to analyze numerous AS events at once to answer fundamental concerns about AS in plants.

Keywords- Alternative Splicing, Splicing Factor, Development, Stress,

INTRODUCTION

By choosing different alternative splice sites, AS (alternative splicing) allows the same gene to produce multiple spliced mRNA isoforms. Such changes in transcript sequence have the potential to alter protein structure and function (activity, protein-protein or protein-substrate interactions, localization, protein modification, etc.), introduce premature termination codons that result in truncated proteins, or cause NMD (nonsense-mediated decay) to degrade AS isoforms. In contrast to plants, where it is currently estimated that only around 35% of genes (in Arabidopsis and rice) experience AS, 95% of multi-exon human genes undergo AS. Due to the relatively lower number of ESTs (expressed sequence tags) available in plants compared to, for example, humans or mice, as well as the fact that many

AS events are absent from or underrepresented in EST collections because they only occur in particular cells and tissues, growth conditions, or stages of development, this level of AS is likely to be underestimated. Several different biological processes, including splicing, signal transduction, development, and responses to biotic and abiotic stress, are influenced by the presence of AS in genes. These processes include seed development and quality, germination, and disease resistance. Even though there are increasing numbers of examples of AS in plant genes, little is known about (i) the dynamic changes in AS of the majority of annotated alternatively spliced genes, (ii) how such changes are regulated, or (iii) the functions of protein isoforms produced from the AS transcripts. To begin addressing the problems of dynamic modulation and control of AS, we developed a high-resolution RT (reverse transcription)-PCR system to track changes in AS isoform abundance of about 300 *Arabidopsis* AS events. The AS events, which occur primarily in transcription factors, RNA-binding proteins, or stress-related genes, reflect several forms of AS.

The AS RT-PCR system determines whether the factors affect AS and which events and types of AS events are impacted by using overexpression lines and knockout mutants of factors involved in splicing regulation and mRNA biogenesis [e.g., CBPs (cap-binding proteins)]. Similar to this, it is possible to discover AS isoforms that are vulnerable to NMD degradation by analyzing mutants defective in NMD. Even though the AS RT-PCR technique does not report on AS over the entire genome, the number of distinct events (about 300) is sufficient to show the degree to which specific proteins affect AS. Last but not least, the AS RT-PCR system is being used to evaluate splicing efficiency and AS in various plant organs, developmental stages, and under various growth and stress conditions, in addition to exploiting the genetic resources of *Arabidopsis* (in terms of mutant lines and transgenic overexpression lines) (e. g. temperature). For instance, a subset of intron-retention events that induce a core exon junction complex protein to relocalize to the nucleolus has been employed to study the efficiency of splicing in plants under anoxic conditions.

Alternative splicing in plant

The gene, which makes up more than 35% of the genes in both rice and *arabidopsis*, even though AS seems to occur less frequently in plant systems than in animal ones. Many plant processes, including growth and development, signal transduction, disease resistance, reactions to biotic and abiotic stress, blooming timing, and the circadian clock, are regulated by alternatively spliced genes. There is little to no knowledge of functional differences between the proteins generated by the great majority of the known alternatively spliced plant genes, and there is no mechanistic understanding of how alternative splice sites are chosen.

Furthermore, little is known regarding the coordination or higher-order combinatorial control of AS of gene sets. Numerous microarray studies have demonstrated how transcriptional patterns alter throughout development and in response to stressors and environmental variables. Similar to AS, patterns of AS vary across the genome depending on the environment, and numerous developmental and growth problems in plants overexpressing splicing regulators such as SR proteins serve as evidence of AS' critical function in plant development. Thus, transcriptional and AS regulation of these variables is influenced by environmental and developmental cues, which in turn modify AS of downstream targets to regulate metabolic and developmental processes. Particularly, AS of genes that encode RNA- or DNA-interacting proteins (such as SFs and TFs) can result in significant changes in expression profiles, amplifying signals, and responses.

ALTERNATIVE SPLICING REGULATION IN PLANT

The rise in interest in alternative splicing (AS) in plants over the past 10 years has mostly been driven by the introduction using high-throughput sequencing methods and groundbreaking studies showing an unexpectedly high frequency of AS in plants. (Filichkin *et al.*, 2010; Lu *et al.*, 2010; Marquez *et al.*, 2012; Syed *et al.*, 2012). the creation and control of alternative transcripts from certain transcribed genes by the choice of substitute splice sites affect all facets of eukaryote growth and response to adaptation to its changing environment. In particular, species of sessile plants need to be able to respond swiftly to Diurnal, seasonal, biotic, and abiotic changes. Since the separation from metazoans, splicing factors, which are components of the spliceosome in assembly, have been in charge of controlling the mechanism of splice site selection and the process of splicing of pre-mRNA. (Fedorov *et al.*, 2002). There are still many knowledge gaps that must be filled to define the prevalence of AS in various plant species and its effects on various biological processes, as well as to comprehend its mechanistic underpinnings to manipulate crops for critical traits needed for food security. Here, we present a Research Topic to the community of plant biologists that intends to highlight recent discoveries, new issues, and technical developments in the study of AS in plants.

The fundamental processes that control AS in various eukaryotes are remarkably conserved. The 5' splice site, 3' splice site, and branchpoint upstream of the 3' splice site are all locations of core sequence components (Wahl *et al.*, 2009; Meyer *et al.*, 2015). enhancing splicing and silencers that are present in introns and exons helps to better characterize certain splice locations. These sequence components are short and varied enough to allow for several alternative sequences, which, coupled with the diversity in splicing factors' expression, cause AS. But there are differences in animal and plant splicing that merit observation of oddities

in transcription, splicing, and gene and chromatin architecture. The distinctions between the regulation of AS in plants and people are discussed by (Chaudhary et al.) Splicing and transcription are known to be related in animals, and new research suggests that this relationship is retained in plants as well (Chaudhary et al.). The chromatin environment and the processivity of RNA Polymerase II have a significant impact on the results of splicing due to splicing's co-transcriptional behavior. AS, transcription and plant growth are coordinated in plants because light/dark transitions alter the RNAPII elongation rate, which in turn regulates AS. The part of a SKI-INTERACTING PROTEIN, which serves as both a transcription factor and a generic splicing factor, was summarised by (Cao and Ma.) In addition to being necessary for precise and effective genome-wide pre-mRNA splicing, it also slows down the blooming process by encouraging FLC transcription (Cao and Ma). A constituent of the RNAPII-related component 1 complex (Paf1c), ELF7, which controls transcription elongation, interacts with SKIP as well. SR45 (RNPS1 human ortholog) was described as a multifunctional splicing factor by (Cao and Ma, and Chen et al.) presented the results of a proteomic study of the mutant sr45-1, which showed that this factor is a part of the complex of proteins related to apoptosis and splicing (ASAP), which, by luring histone deacetylases to the FLC gene, is known to influence RNA metabolism at several levels. Additionally, the protein 18 linked to Sin3 in the ASAP component (SAP18), which instigates transcriptional silence in mammalian cells, was decreased in the sr45-1 mutant. Last but not least, the yeast equivalent in humans PRemRNA-PROCESSING PROTEIN 40 (PRP40), which was initially identified as a transcriptional regulator and later connected to pre-mRNA splicing, plays a function in the early stages of spliceosome complex assembly. (Hernando et al.) investigated a mutant of the Arabidopsis PRP40C, and they found that the element affected the development, growth, and stress responses of the plant in Arabidopsis by controlling gene expression and pre-mRNA splicing. These versatile splicing factors show how transcription and splicing interact in complex ways, which underlies how these two processes function together.

Plants develop tolerances for daily, seasonal, and enduring stresses as well as for changing environmental conditions. Increased levels of glucosinolate biosynthetic enzymes, which are crucial for disease resistance, were found in the sr45-1 proteome (Chen et al.). By stating that, according to the data from the vast majority of the Arabidopsis exemplar transcript DNA repair genes are alternatively spliced (about 80%), (Nimeth et al.) untapped AS's potential in plants' DNA damage response (AtRTD2). The PRP40C mutants' differing susceptibility to salt pressure and their improved tolerance to Pseudomonas infection was discovered by (Hernando et al.) It was discovered that more than 600 transcripts were

enhanced for genes related to reactions because of Biological and abiotic stresses. Increased intron retention rates were seen in both instances, suggesting a potential method for controlling the expression of stress response genes and a function in fine-tuning transcriptome functionality (Hernando et al.). Numerous intron retention transcripts are restrained in the nucleus to prevent mRNA decay caused by nonsense. Some could be translated to create truncated proteins that could affect how the fully spliced protein functions (Chaudhary et al.). For plants to reproduce successfully and grow, developmental transitions from seed germination to flowering must be precisely controlled. In reaction to variations in temperature and light, many transitions are started. For instance, the change from seed dormancy to germination, which requires red and far-red photoreceptors, depends critically on light perception. In evidence of an important role for AS at germination, (Tognacca et al.) demonstrated that a pulse of red light alters the AS of numerous genes, largely involved in splicing regulation, light signaling, or dormancy/germination. A complex web of variables that incorporate environmental and developmental cues controls the timing of flowering. A gene's known functions when it is alternatively spliced that are necessary for the flowering time were reviewed by (Park et al.) For instance, alternate splicing of CONSTANS (CO) results in CO and CO protein isoforms, which may result in heterodimers that do not bind to DNA during photoperiodic blooming during protein synthesis and turnover (Park et al.). A temperature-dependent transactivator of the MADS-box and encoded by the alternatively spliced FLOWERING LOCUS M gene, which functions as a floral repressor (FLM). The useful floral suppressor isoform FLM-, whereas FLM- suppresses FLM- and has less DNA-binding capacity. According to research by (Nibau et al.), the cyclin-dependent kinase G2, together with cyclin L1 (CDKG2) which it is paired with (CYCL1), have an impact on FLM's AS and balance the quantities of FLM- and FLM- isoforms over a temperature range. Therefore, it's crucial to fine-tune the start of blooming through the induction of AS by thermosensory and developmental signaling. These genes translate alternatively spliced transcripts into protein isoforms that appear to be functional. However, it is still unclear to what scope of alternative transcripts are translated and contributes to protein diversity. While alternative transcript levels fluctuate during the day, CO and CO protein levels do not. The sr45-1 mutation displays a notable drop in SAP18 protein but not a notable reduction in SAP18 RNA (Chen et al.). The detection of proteins in proteomic investigations and alternatively spliced transcripts do not correlate well. It is not yet evident how this difference came about. The results of the proteomic analyses may eventually be influenced by transcript abundances, transcript stability, retention in the nucleus, and other mechanisms. Proteomic results may potentially be misinterpreted if

transcript translation is misinterpreted by ignoring real early termination codons and start codons. Alternatively, proteomic technologies may not be sensitive enough to identify the low-abundant proteins resulting from AS variations, in contrast to PCR or HTS procedures (Brown et al., 2015; Zhang et al., 2017).

The number of transcript isoforms can be significantly influenced by genetic variation in core splicing regions, splicing regulatory sequences, or genes encoding trans-acting splicing factors. Phenotypic diversity and terrestrial adaption are impacted by these changes. The 666 different natural inbred *Arabidopsis* ecotypes used by (Khokhar et al.) were derived from populations along the east-west axis of Eurasia. To determine how genetic alterations affect the function of AS patterns of genes involved in stress response, flowering, and the circadian clock, they carried out quantitative trait loci splicing (QTL) analyses. Under various field circumstances, (Dantas et al.) analyzed the C4 sugarcane's AS events containing clock genes. The authors discovered pronounced variations in the seasonal AS patterns in these genes, which might be caused by temperature variations from winter to summer (Dantas et al.). The close connection between AS and environmental cues like temperature, nocturnal, and seasonal fluctuations demonstrate how crucial a role AS plays in many different adaptation processes. A class of AS regulators known as SR proteins is extremely conserved. (Melo et al.) identified 16 genes for *Physcomitrella patens* SR. with the use of an in-silico investigation that corresponded to the six traditional plant SR protein subfamilies. SR subfamilies range significantly in size and quantity from vascular plants to aquatic green algae. The authors propose that SR proteins play a part in speciation as well as adaptation to new terrestrial environments (Melo et al.). talk about how AS evolved in plants versus vertebrates. In their comparative analysis of the transcriptomes of both closely related and distantly related plants, they found that, except for AS events that result in premature termination codons, there is little AS conservation between species (Ling et al.). (Clark et al.) integrated mRNA, EST, and RNA-seq data from 27 published projects to examine tomatoes' genome-wide AS. They discovered an AS frequency of about 65%, which is comparable to the frequency identified for *Arabidopsis* (Clark et al.). Last but not least, talk about the advantages, drawbacks, and potential of HTS technologies to overcome constraints brought about by low coverage of certain genomes, high ploidy levels, and sequencing error rates (Bedre et al.). To describe the *Physcomitrella* SR gene, differences in big datasets that were brought on by insufficient gene annotation curation, occasionally missing support from expression data, were found. (Melo et al.). However, new HTS and bioinformatics approaches will probably encourage further in-depth identification of AS patterns on a genome-wide scale in a variety of plant species. When combined with the

fundamental mechanistic studies, these efforts will probably provide answers to important questions about the role of AS in the adaptation and evolution of plants on this planet.

Future challenges in alternative splicing research in plants

In the last four to five years, research on AS in plants has advanced significantly. Understanding the mechanisms and regulation of AS as well as its activities is crucial, as evidenced by the rising number of plant genes containing AS and the processes in which they participate. The functional significance of AS is among the most crucial issues, in large part because there are so few instances of AS where multiple protein isoforms have been shown to have distinct activities. To find out how widespread AS is in plants, we draw a comparison. Only 1.2 percent of plant genes were thought to be AS-displaying ten years ago. This number has increased to over 61 because of dramatically enhanced technologies. Similar to this, the growing number of plant genome sequences and the production of enormous amounts of transcriptome data will enable computational analyses to pinpoint the conservation of AS events across species as well as the regulation of AS that is tissue-, developmental-stage-, and environment-specific, providing evidence of functionality. A greater interest in AS is being sparked in the plant community by the increasing number of functional instances of AS, whether at the level of protein function or mRNA transcript stability. High-throughput sequencing will also explore the dynamic changes in AS that occur during development and under various environmental conditions and stresses, as well as the role that variation in AS patterns in different ecotypes and polyploids plays in the plasticity and adaptation of plant species. We also need to comprehend the interactions between transcriptional and AS networks and how signaling pathways affect splicing factor activity directly or indirectly through chromatin alteration. Plants control and fine-tune the expression of their genes mostly through AS. The functional significance of AS will become increasingly well-known over the next five years, and understanding how it contributes to the complexity of gene expression will open up new possibilities for techniques to change plant function for better phenotypes

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