Alternative splicing in plants

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Regulation of gene expression by AS (alternative splicing)

Pre-mRNA (precursor mRNA) splicing is the excision of intron sequences from pre-mRNAs mediated by the spliceosome. AS produces more than one mRNA from a single gene through the selection and utilization of alternative splice sites in the pre-mRNA [1]. AS is widespread in higher eukaryotes, with up to 74% of human genes containing one or more AS events [2]. The main consequences of AS are changes in protein structure/function, mRNA stability and regulation of gene expression. The inclusion or exclusion of whole or parts of exons or introns can alter protein domains, activity, localization, interactions with other proteins or substrates, and post-translational modification [3]. As such, AS increases the protein complexity of an organism (the ~30000 human genes produce 150000–200000 proteins). Gene expression can be affected directly by AS of TFs (transcription factors) and SFs (splicing factors), which modulate DNA recognition and binding, transcriptional complex assembly and splicing. In addition, AS can affect mRNA stability and turnover because many alternatively spliced transcripts contain premature termination codons and are therefore substrates for NMD (nonsense-mediated decay) [4,5].

The selection of alternative splice sites involves recognition of classical intron splicing signals or exonic/intronic enhancers or suppressors by RNA-binding factors that enhance or suppress use of particular splice sites [1]. Splice site choice is determined by virtue of their position relative to competing splice sites and/or through interactions or interference with other proteins or complexes. Major factors involved in alternative splice site regulation are families of SR (serine/arginine-rich) and hnRNP (heterogeneous ribonucleoprotein particle) proteins [1]. In addition, AS of specific gene transcripts or sets of transcripts is regulated by specific regulatory proteins. SR and hnRNP proteins have multiple roles associated with splicing, mRNA transport and translation, and often act antagonistically [6]. The relative amounts of alternatively spliced transcripts in different cells and tissues under different conditions reflect the relative levels and activities of the interacting factors, leading to the hypothesis of a ‘cellular code’ of trans-acting factors [6] (Figure 1). Further fine-tuning of expression is achieved by the co-ordinated AS of genes involved in the same biological pathway or process and the superimposition of such AS networks on transcriptional regulatory networks [7].

AS in plants

Although AS appears to occur less frequently in plants than in animal systems, it is clearly significant, with over 35% of genes in Arabidopsis and rice showing AS [8–10]. Alternatively spliced genes are involved in a range of plant functions, including growth and development, signal transduction, disease resistance, biotic and abiotic stress responses, flowering time and the circadian clock. For the vast majority of the reported alternatively spliced plant genes, there is little or no information on functional differences among the proteins produced or any mechanistic understanding of how alternative splice sites are selected. In addition, nothing is known about higher-order combinatorial control or co-ordination of AS of sets of genes.

Extensive microarray studies have shown how transcriptional profiles change during development and in response to environmental factors and stresses. Similarly, patterns of AS across the genome change under different conditions [11] and the essential role of AS is demonstrated by the many developmental and growth defects in plants

Key words: alternative splicing, Arabidopsis, precursor mRNA, splicing factor, transcription factor.

Abbreviations used: AS, alternative splicing, hnRNP, heterogeneous ribonucleoprotein particle, pre-mRNA, precursor mRNA, RT, reverse transcriptase, SF, splicing factor, SR, serine/arginine-rich, TF, transcription factor.

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External stimuli and developmental cues induce changes in transcription, splicing, protein modification and localization. The resulting changes in the levels and activities of different SFs (histograms) induce changes in expression and AS of TFs and SFs (which feedback to further regulate expression) and other downstream genes to produce and modulate the cell’s response.

Overexpressing splicing regulators such as SR proteins [12,13]. Thus environmental and developmental cues affect the transcriptional and AS regulation of these factors which in turn modulate AS of downstream targets to control metabolic and developmental processes. In particular, AS of genes encoding RNA- or DNA-interacting proteins (e.g. SFs and TFs) can generate major changes in expression profiles, thereby amplifying signals and responses (Figure 1).

Monitoring AS events in plants detects novel AS transcripts
To begin to address the functions of RNA-binding proteins in AS and the co-ordination of AS in genes involved in the same biological process, we have established an AS RT (reverse transcription)-PCR panel to monitor multiple AS events from multiple plant genes simultaneously. By measuring the relative amounts of alternatively spliced transcripts, we are able to reproducibly detect statistically significant changes in the ratios of AS products [14]. To date, we have detected changes in AS of numerous genes in seedlings grown in the light and dark, in different organs (flower, leaf and root) of seedlings grown under short and long days, in transgenic lines overexpressing SFs [14] and in mutants of proteins involved in mRNA biogenesis. Significantly, many new AS events were detected in over 25% of the genes studied. Characterization of some of these events confirmed that they were previously unidentified AS transcripts (Figure 2), demonstrating the much wider occurrence of AS than currently expected in plants.

Given the current level of knowledge of AS in plants and the number of potentially undescribed AS events, RT-PCR-based approaches are the most accurate method of directly quantifying and monitoring the relative amounts of different alternatively spliced transcripts. We are currently increasing the number of AS events analysed on the RT-PCR panels and are developing distinct sets of AS events to measure changes in AS in genes involved in developmental and biological processes. Nevertheless, to obtain a true picture of AS in plants, a concerted effort to discover and
characterize all AS events will be needed. High-throughput sequencing systems will undoubtedly help in the discovery phase. This information will then allow the application of high-throughput technologies to measure changes in plant AS, as routinely as microarrays are currently used to measure transcript levels. Alternatively, splice junction, exon and tiled genome arrays have been used in animals to investigate global AS [2,7]. These approaches have advantages and disadvantages in terms of de novo discovery compared with reliance on annotated events, detection of different types of event, quantification of changes in AS, expense and computational requirements [15], but ultimately will benefit our understanding of the key role of AS in plants.

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References

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