Alternative Splicing: A Multilevel Regulation of Plant Abiotic Stress Responsive Genes

Review Article

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Abstract

في تعارض مع النظرية المعروفة "جين واحد، بروتين واحد"، تمسح عملية بدائل الربط والتي تعتبر آلية مهمة في مرحلة ما بعد عملية نسخ الجينات بإنتاج عدة صور من البروتينات كنتيجة لإنتاج عدد من الـ mRNA من جين ما والتي تختلف عن بعضها البعض رغم تشابهها. هذه العملية الجزيئية الفريدة و المنتشرة بشكل واسع في النباتات المختلفة تمثل آلية مهمة في زيادة التنوع البروتيني ولها أيضا وظيفة في التحكم الكمي للجينات. يتم إحداث هذه العملية بوضوح في النباتات كإستجابة للإجهادات البيئية. الدراسات الحديثة سمحت بالتعرف على صور لبدائل الربط والتى نتج عنها تعديل سريع في غزارة ووظيفة مكونات الإستجابة للإجهاد. الطريقة التقليدية لإختبارات الجينات الفردية التي أستخدمت لتحديد آلية بدائل الربط تم تحديثها بطرق حديثة معروفة دوليا. هذه الطرق تعد بكشف تفاصيل حول طبيعة وآلية عمل الشفرات الخلوية والتي تتكون من مجموعة من العناصر التنظيمية ذات العلاقة بمادة الـ pre-mRNA المبدئي والمكملات الخلوية للمكونات المنظمة لآلية الربط والتي تحدد معا مسارات الربط التي يتم تنظيمها. توجد عدة عوامل نسخ عُرفت بتنظيمها للإستجابة للبرودة، الملوحة والجفاف في النباتات. في هذه المراجعة، تم التركيز على بعض عوامل النسخ هذه مثل: بروتين الارتباط بعنصر الإستجابة لنقص الماء DREB2A والذي يرتبط بعناصر السيز للجينات التي لها دور في إستجابة النباتات لإنخفاض الحرارة و الجفاف، وعامل النسخ ICE2 (bHLH) ذو العلاقة بإستجابة النباتات لظروف المنخفضة ونقص الماء من خلال بدائل الربط ذات العلاقة بالإستجابة للاجهاد الغير الحيوي.

الكلمات المفتاحية: *بدائل الربط، الاجهاد الغير حيوي، عوامل النسخ، DREB2A ، ICE2، bHLH*

Infraction of the ‘one gene, one protein’ rule, alternative splicing (AS), as a post-transcriptional control mechanism allows individual genes to produce multiple polypeptide isoforms as a result of the production of different mRNAs from a certain gene. This distinct and widespread molecular process across plant species represents an important mechanism that lead to an increases in proteome complexity and also has a function in quantitative gene control. In plants, this post-transcriptional mechanism is markedly induced in response to environmental stresses. Recent studies have identified alternative splicing events that allow rapid adjustment of the abundance and function of key stress-response components. Traditional gene-by-gene investigations of alternative splicing mechanisms have been complemented by global approaches. These promise to reveal details of the nature and operation of cellular codes that are constituted by combinations of regulatory elements in pre-mRNA substrates and by cellular complements of splicing regulators, which together determine regulated splicing pathways. Several transcription factors are presently known to regulate the response to cold, salt and drought stress. In this review, I primarily focus on some of these transcription factors in plants; the dehydration-responsive element-binding protein 2A (DREB2A), that encodes a transcription factor which binds to DRE/CRT *cis*-elements (responsive to low-temperature and drought stress) and *ICE2*, which is a transcription factor of the bHLH family that participates in the response to deep freezing through the cold acclimation-dependent pathway. The involvement of these two TFs in abiotic stress response through AS in relation to CBFs is discussed too.

Keywords*: Alternative splicing, abiotic stress, transcription factors, DREB2A, ICE2, bHLH*

1. Introduction:

Alternative splicing (AS) represent an important molecular process that has a very substantial role in protein plasticity and diversity which is tightly compromised with the very complex molecular and biochemical activities that occur inside the living cell. As the AS process leads to the production a huge number of functional proteins from a limited number of genes, an organism is able to run its biological activities through a very complicated manner. In plants, alternative splicing (AS) affects all aspects of growth, development and responses to different abiotic and biotic stress (SIMPSON et al., 2008). For instance, AS in plants occurs to control flowering time, biological clock regulation, tolerance to diseases, activity of metabolic enzymes, and both transcription factors and splicing factors. Moreover, AS plays a role in mRNA stability and protein activity, and protein function in relation to the place of localization and protein-protein interaction. Also, AS is patterns of many genes that are regulated by different stress conditions and by altered levels of proteins which are involved in splicing.

In general, alternative splicing (AS) is common and essential for eukaryotic to control gene expression by which a single gene produces more than one transcript to affect the production and function of proteins. Therefore, the AS process represents an important regulatory step in post-transcriptional gene expression. Thus, AS is regulated in a cell type-, tissue- and developmental stage-specific pattern (MARTÍN et al., 2020; SUN et al., 2018; THATCHER et al., 2014; TIAN et al., 2019) in addition to stress and growth signals. Splicing is also regulated by a variety of upstream effectors that feed signaling information in relation to stress and growth to the transcriptional and post-transcriptional regulatory machinery. Generally, more than 60% of intron-containing genes undergo alternative splicing in plants (SYED et al., 2012). In humans, approximately 95% of multi-exon genes undergo alternative splicing making it a key mechanism in expanding a relatively limited number of genes into very complex transcriptomes and proteomes. It has been estimated that at any moment of time, each cell in the human body is engaged in splicing 20,000 introns. Also, the majority of protein-coding genes in plants and animals contain introns that must be removed from precursor mRNAs to produce mature mRNAs.

In the model plant; Arabidopsis, mapping transcriptome at single-base resolution using the Illumina platform for ultrahigh-throughput RNA sequencing (RNA-seq) and Deep transcriptome sequencing confirmed a majority of annotated introns and identified thousands of novel alternatively spliced mRNA isoforms (CALIXTO et al., 2018). This analysis suggests that at least approximately 42% of intron-containing genes in Arabidopsis are alternatively spliced; this is significantly higher than previous estimates based on cDNA/expressed sequence tag sequencing. In Tea, it was found that more than 41% representing about 14,103 genes underwent AS events (LI et al., 2020). This percent is increasable when different tissues, developmental stages, and

environmental conditions are investigated. It has been reported the possible existence of 11 types of AS events, including: intron retention (IR); the most common and predominant mechanism in plants (WEI et al., 2017; ZHANG and XIAO, 2018), exon skipping (ES); which represent a small fraction of AS genes, alternative 5′ splice site (A5SS), alternative 3′ splice site (A3SS) (LI et al., 2020), alternative first exon (AFE) and alternative last exon (ALE) which were found more abundant than IR in Arabidopsis (ZHU et al., 2017). The number of AS events increase rapidly during cold treatment, but decrease significantly after de-acclimation (LI et al., 2020), also the pattern of AS is changeable under cold stress (LEVIATAN et al., 2013). In general, many studies revealed several features of pre-mRNA splicing in plants under abiotic stress: (i) the majority of stress-induced splicing variants are associated to intron retention, (ii) the level of expression of these variants is not strongly relative to that of the main splicing variant(s), and (iii) a considerable number of stress-responsive genes are exposed to AS (CUI and XIONG, 2015). Given the low expression level of stress-induced AS variants, it is likely that the majority of AS events could be attributed to splicing mistakes as a result of decreased efficacy or precision of pre-mRNA splicing under abiotic stress, rather than of active regulation (CUI and XIONG, 2015).

Considering the environmental fluctuations, AS process takes place quickly in the cell life's real time and strongly linked to the plant response to changes in environmental stresses (CUI and XIONG, 2015). Recent genome-wide research have shown that a large number of plant genes extensively undergo AS under stress conditions (DING et al., 2020; MARTÍN et al., 2020). In relation to salt, the global changes in pre-mRNA splicing under different concentrations of NaCl have been analyzed (DING et al., 2014). It has been detected that about 49% of all intron-containing genes were alternatively spliced under salt stress, 10% of which run into a differential alternative splicing (DAS) (ZHU et al., 2018). The most studied 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.

In relation to cold, the initial response of Arabidopsis rosettes showed massive and rapid waves of both transcriptional and AS activity. Also, the AS of some long non-coding RNAs (lncRNAs) found to be highly sensitive to even slight changes in temperature suggesting tight regulation of expression which reveals the association to cold stress response (CALIXTO et al., 2019). In relation to drought stress, a considerable percentage of intron retained transcripts suggested to participate in drought tolerance in plants (MUTHUSAMY et al., 2018; THATCHER et al., 2014).

Regarding environmental cope, plants must mount appropriate responses to overcome changes in environmental conditions. This is acquired by altering their growth and development behavior via specialized metabolism, modifications in morphology, or changes in their life style. Organizing these responses is fulfilled through multilevel regulatory processes by the alteration of gene expression. Alternative splicing or differential splicing is a regulated process during gene

expression that generates multiple transcripts from a single gene which leads to increase proteome diversity and regulate mRNA levels. This quick post-transcriptional mechanism in plants is clearly induced in response to various environmental stresses. Recent studies have identified alternative splicing events that allow rapid adjustment of the abundance and function of key stress-response components. Therefore, alternative splicing leads to differential gene regulations depending on the type of inappropriate environmental factors plants face.

2. Mechanism of Alternative Splicing:

When a gene is switched on, the DNA sequence is copied into a molecule called messenger RNA (mRNA) in a process named as gene expression, which is then translated in a specific protein. Initially, the mature mRNA formation is preceded by what is called mRNA processing through which structural introns are removed prior to capping and polyadynelation. So, to generate the mature mRNA, these introns must be precisely excised and the exons joined together. The excision mechanism of the introns from the pre-mRNA is controlled by splicesomes; a highly dynamic, megadaltonsized complex machinery composed of small nuclear ribonucleoproteins (snRNPs) and hundreds of associated proteins leading to AS; through which a set mRNAs is produced from the same precursor messenger RNA (pre-mRNA), giving rise to functionally different proteins (CHAUDHARY et al., 2019). This post-transcriptional control of a gene represents the most important mechanism in the gene expression and regulation.

With the side by side production of a large amount of stress-inducible pre-mRNAs in response to abiotic stress, plant cells would need to instantly increase the recruitment of splicing and other factors for their co-transcriptional processing. This substantial increase may overwork splicing machinery leading to a substantial portion of these transcripts to be improperly processed, especially when the splicing machinery is compromised. On the other hand, there have been some natural products that are synthesized inside the live cell that work as inhibitors to trigger the AS process; one of which is macrolide pladienolide B (PB). The research showed that PB interrupts the splicing machinery leading to a sharp increase in intron retention in parallel with a reduction in other splicing forms, confirming the role of PB to inhibit and modulate the splicing and AS, and to mimic of abiotic stress signaling in plants through the activation of abiotic stress- and ABA-responsive reporter genes (LING et al., 2017).

Alternative splicing produces multiple proteins which have different structural domain compositions from a single transcription factor gene. Recent studies have shown that alternative splicing of some transcription factor genes generates small interfering peptides (siPEPs) that negatively regulate the target transcription factors via peptide interference (PEPi), constituting self-regulatory circuits in plant response to cold. A number of splicing factors, which are involved in RNA binding, splice site selection, and spliceosome assembly, are also affected by temperature

fluctuations, supporting the close association of alternative splicing of transcription factors with plant responses to low temperatures (SEO et al., 2013).

It is well-known that transcription factors have a critical role in the regulatory networks of genes to mediate various aspects of plant growth and environmental variation responses. Thus, their activities are accurately regulated at multi-level steps. There are clear evidences show that post-transcriptional control of mRNA metabolism is a key molecular platform that alters the activities of the transcription factors in plant in response to temperature fluctuation. New approaches to analyze histone methylation have revealed that temperature-dependent spliced genes are enriched in histone H3 lysine 36 tri-methylation and the reduction in the level of this histone precipitation led to a modulation in the temperature-induced splicing (PAJORO et al., 2017).

In plants, relatively little is known about the factors regulating AS or the consequences of AS on mRNA levels and protein function. The identification of *cis*-elements related to AS has later attracted the attention of researchers. These *cis*-elements are found to be recognized by specific factors (proteins families) that regulate AS which called *trans*-acting factors; from which is the SR (Ser/Arg-rich) and hnRNA (heterogeneous nuclear ribonucleoprotein). This regulation process takes place by recognizing the *cis*-elements in exons or introns of specific gene and by promoting or blocking the assembly of the slpiceosome at adjacent splice site. Therefore, a novel *cis*-element that affects the AS of *LcDREB2* was identified as Exonic Splicing Enhancer 1 (ESE1). Also, five serine-arginine rich (SR) proteins were confirmed to interact with ESE1 in Sheepgrass (LIU et al., 2017). Other investigations showed and confirmed a main function of SR in drought stress response through an alteration process in the alternative splicing of a key stress inducible genes; one of which is DREB2A in *Brassica rapa* (MUTHUSAMY et al., 2020).

Specifically, transcription factors have a modular structure consisting of distinct protein domains essential for DNA binding, dimerization, and transcriptional regulation (SEO et al., 2013). In the last few years, numerous transcription factors controlling various aspects of plant's life have been identified. For instance, the transcription factor DREB2A that interacts with *cis*-acting element (DRE/CRT) which had been identified to have a function in cold and drought stresses. Also, the ICE2; a factor of the bHLH (b-helix-loop-helix) family that has involvement in the freezing conditions response through the cold acclimation-dependent mechanism (FURSOVA et al., 2009). Both transcription factors were proved to undergo AS in response to low temperature in Arabidopsis. Two splice forms of the transcription factor DEHYDRATION RESPONSIVE ELEMENT BINDING 2A (DREB2A): *DREB2A.1* and *DREB2A.2* that were reported by TAIR and two splice forms of ICE2 that were reported by TAIR and MIPS were tested under cold and drought stresses (ELHAJ, 2009). Research show that a massive and rapid AS response occur within the few hours of exposing to low temperature recruiting hundreds of genes "early AS" genes including many novel low temperature-responsive transcription factors and splicing factors/RNA

binding proteins regulated only by AS (CALIXTO et al., 2018). This set of genes includes the above mentioned TFs; DREB2As and ICE2.

3. DREB2A Alternative Splicing:

Many research results showed that *DREB2A* encodes mainly for dehydration and salinity stresses (EGAWA et al., 2006; LIU et al., 1998; MATSUKURA et al., 2010; MORIMOTO et al., 2013; NAKASHIMA et al., 2000; SAKUMA et al., 2006), but others' findings indicate that DREB2A is regulated by cold too (AGARWAL et al., 2007; ALI, 2002; SAKUMA et al., 2006; VAEZI-K, 2007). In a comprehensive analysis of many DREB2As in rice, it is found that only two types (OsDREB2A and OsDREB2B) of these transcription factors showed abiotic stress involvement, and OsDREB2B had two forms of transcript; functional and non-functional and only the functional isoform increased under stress (MATSUKURA et al., 2010). Also, in the same study, the splicing mechanism analysis of OsDREB2B in transgenic rice expressing the non-functional transcript showed that the non-functional form was not the precursor of the functional one concluding that the stress-inducible alternative splicing of pre-mRNA is very important mechanism for the regulation of this transcription factor. Following to this, transgenic Arabidopsis which over-expressed OsDREB2B showed increased expression of DREB2A; thus drought and heat-shock stress tolerance was enhanced (MATSUKURA et al., 2010). In plants, alternative splicing can be manifested at different developmental stages, and is frequently associated with specific tissue types or environmental conditions such as abiotic stress. Under cold stress, DREB2A expression tends to be a tissue-specific. Mohamed (2006) indicated the expression of *DREB2A* in epidermis under cold and sucrose supplement. Moreover, this transcription factor was tested in Arabidopsis under cold and osmotic stress. The molecular analysis revealed that *DREB2A.1* was the only splice form to be expressed in Arabidopsis epidermal tissue under these conditions (ELHAJ, 2009). In wheat, the three isoforms of Wdreb2 that found to have high homolog to Arabidopsis and barley DREB2 were expressed under drought, cold and salt conditions. The three produced transcripts consisted from 4 exons and 3 introns (EGAWA et al., 2006; SAZEGARI and NIAZI, 2012). These results are constant with that for Arabidopsis and barley which indicate the similarity of dreb2 homologous in different plants grown under stress conditions.

4. bHLH Alternative Splicing:

Basic helix-loop-helix (bHLH), is a transcription factor that is involved in many abiotic stresses response such as cold (ELHAJ, 2009; FURSOVA et al., 2009) and drought stress (WEI and CHEN, 2018) in addition to the biosynthesis of flavonol and anthocyanins which are usually synthesized under stress conditions (WANG et al., 2018). The research results show that the structure of bHLH (locus At1g12860) which is named ICE2 is much complicated than was early expected (FURSOVA et al., 2004). Both the MIPS and RIKEN databases contain information on the experimentally

obtained full-length cDNA of the ICE2 gene but the published sequences do not contain the sequence from the first exon. In two different labs, the investigations indicated that bHLH splice form of only 4 exons (2, 3, 4 and 5) was expressed in Arabidopsis, confirming the absence of the expected exon 1 from the transcript (ELHAJ, 2009) and (FURSOVA et al., 2009) under cold stress in the wild type and was overexpressed in the *bhlh(ice2)* mutant (ELHAJ, 2009). This indicates that the ICE2 gene product is likely to be encoded by four rather than five exons excluding the first expected exon in the cold stressed mutant and wild type plants; which probably encodes the F-box domain (FURSOVA et al., 2004).

It is known that the resistance to cold stress is regulated by a group of genes but the signal pathways are still not completely identified. Three members; CBF1, CBF2 and CBF3 of the C-repeat-binding factor (CBF)/dehydration-responsive element-binding factor (DREB1) gene family are rapidly and transiently induced by cold stress. Previously, ICE1 was identified as the inducer of CBF3 expression which acts upstream of the CBFs in the cold-response pathway. Later, it has been detected that alterations in ICE2 expression result in changes to the transcription level of the CBF1 (DREB1B) gene but not for CBF3. The Expression of this gene increased many folds in the mutant lines (FURSOVA et al., 2009). Also, the results showed that ICE2 participates in the CBF dependent pathway of regulation of the response to cold stress and acclimation. Form the different disclosed results; it has been shown that the ICE2 gene product is likely to be encoded only from the second to the fifth exon of the At1g12860 locus in Arabidopsis. The first exon is either an adjacent gene or is removed during alternative splicing. This is antagonizes with the data published by TAIR for this gene which is been designated ICE2 to be consists of five exons. Therefore, bHLH (ICE2) is consisted from 4 exons and 3 introns as reported in MIBS cDNA database. There is no evidence shows that this splice from is produced under other environmental stresses such as salinity and heat stress. But under drought conditions, it is suggested that there is an exclude related to the response pathway that may be involved in a shared response mechanism between drought and cold.

5. The function of AS in modulating stress response

Alternative splicing (AS) plays a vital function in plant development; especially in stress responses. At the molecular level, alteration of the cellular proteome pool upon exposing to stress permit plants to bear environmental variations. Confirming the importance of splicing in plant stress adaptation, key players of stress signaling have been shown to encode alternative transcripts, while mutants that lack of splicing factors or associated components show a modified sensitivity and defective responses to abiotic fluctuations. There are escalating evidences indicate that alternative splicing regulates stress response through targeting the abscisic acid (ABA) pathway (LALOUM et al., 2018; WANG et al., 2015). In general, members of the serine/arginine-rich (SR) protein gene family are key players in the regulation of alternative splicing (DUQUE, 2011). The

expression of plant SR proteins is stress-regulated at multiple levels, with environmental signals controlling their own alternative splicing patterns. Most significant, functional links between these RNA-binding proteins and plant stress tolerance to be referred to the role in the regulation of abscisic acid (ABA) signaling (DUQUE, 2011). In addition, ABA was identified to affect the AS pattern (ZHU et al., 2017). Further on, it has been proved that several genes tend to accumulate novel splicing transcripts in a drought-induced manner, suggesting the contribution of an additional level of control for genetic improvement of drought resistance in rice (WEI et al., 2017)

There are many reports show that AS variants have been found to function in plant stress response and tolerance. 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, which are mostly long non-coding RNAs (lncRNAs), may be trap inside the nucleus and lack of the ability of exportation to cell pool and are subject to decay. In speculation, 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 (CUI and XIONG, 2015). In tropical maize, three splice variants had been identified and are predicted to encode three different protein isoforms, which retain the MYB domain. The expression level of these three protein isoforms was differentially affected by drought stress and also by other developmental and environmental factors (TIAN et al., 2019).

Overall, having protein diversity in plant's and other organism's live has a crucial role to cover all the very complex biochemical, physiological, genetic, and developmental requirements with a limited gene number in the whole genome. Thus, collecting enough information about the mechanism of producing slightly different (not identical) proteins from a certain mRNA, is a key player in understanding all life phenomena of any organism including the response to changeable environment, and the possible manipulation of the genetic material of any creature.

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