

# 1 **Alternative Splicing Control of Abiotic Stress Responses**

2 Tom Laloum<sup>1</sup>, Guiomar Martín<sup>1</sup>, and Paula Duque\*,<sup>1</sup>

3 <sup>1</sup>*Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal*

4 \*Correspondence: duquep@igc.gulbenkian.pt

5 **Keywords:** abiotic stress, abscisic acid (ABA), alternative splicing, posttranscriptional  
6 regulation, RNA-binding protein.

## 7 Abstract

8 Alternative splicing, which generates multiple transcripts from the same gene, is an important  
9 modulator of gene expression that can increase proteome diversity and regulate mRNA levels.  
10 In plants, this posttranscriptional mechanism is markedly induced in response to environmental  
11 stress, with recent studies identifying alternative splicing events that allow quickly adjusting the  
12 abundance and function of key stress-response components. In agreement, plant mutants  
13 defective in splicing factors are severely impaired in their response to abiotic stress. Notably,  
14 mounting evidence indicates that alternative splicing regulates stress responses largely by  
15 targeting the abscisic acid (ABA) pathway. We review here the current understanding of  
16 posttranscriptional control of plant stress tolerance via alternative splicing and discuss research  
17 challenges for the near future.

## 18 Pre-mRNA Splicing in Plant Responses to Abiotic Stress

19 Under both natural and agricultural contexts, plants are constantly subjected to environmental  
20 conditions that adversely affect their growth and development and may threaten their survival.  
21 They have therefore evolved a wide spectrum of molecular programs to rapidly perceive  
22 changes in the environment and adapt accordingly. The phytohormone **abscisic acid** (ABA)  
23 plays a crucial role in plant responses to major forms of **abiotic stress**, such as drought, high  
24 salinity and extreme temperatures, all of which induce **osmotic stress** in plant cells. Although  
25 osmotic stress also triggers ABA-independent pathways, an adequate plant stress response  
26 involves endogenous accumulation of the hormone. Intracellular sensing and signal  
27 transduction of ABA results in the activation of downstream effectors, including transcription  
28 factors and ion channels, that implement important adaptive responses to withstand reduced  
29 water availability, such as stomatal closure, osmoprotectant synthesis and the induction of a  
30 broad range of stress-responsive genes (reviewed in [1,2]).

31 During the last decade, research has made efficient use of “omics” approaches to identify the  
32 transcriptional and translational changes associated with plant perception and responses to  
33 abiotic stress (reviewed in [3-6]). While the involvement of posttranscriptional mechanisms is  
34 still poorly documented, a new molecular layer regulating these processes has been unfolding.  
35 In this review, we discuss emerging evidence that **alternative splicing** is of central importance  
36 to plant abiotic stress tolerance, particularly to ABA-mediated responses, and put forward  
37 prospects for future research as well as potential new avenues to improve plant performance  
38 under adverse environments.

## 39 The Splicing Process

40 **Precursor-mRNA** (pre-mRNA) **splicing** plays a crucial role in the accurate expression of the  
41 information encoded in eukaryotic genomes. This process is carried out by the **spliceosome**, a  
42 large molecular complex that recognizes sequences in the pre-mRNA called splice sites to  
43 remove the noncoding introns and join the flanking exons (Figure 1), thus generating a mature  
44 transcript. The spliceosome core consists of five **small nuclear ribonucleoprotein** (snRNPs)  
45 and numerous spliceosome-associated proteins, which assemble at introns in a precise order

46 (reviewed in [7,8]). Splice site selection is determined not only by core spliceosomal  
47 components, but also to a large extent by other RNA-binding proteins, predominantly  
48 **serine/arginine-rich (SR) proteins** and **heterogeneous nuclear ribonucleoproteins**  
49 (hnRNPs), which bind *cis*-regulatory elements located in either introns or exons, thereby  
50 activating or repressing splicing (Figure 1). SR proteins share a multidomain structure  
51 characterized by one or two N-terminal RNA Recognition Motifs (RRMs), which bind the  
52 RNA targets, and a C-terminal arginine/serine-rich (RS) domain involved in protein-protein  
53 interactions that promote recruitment of core spliceosomal components to nearby splice sites.  
54 hnRNPs contain a prominent structure of RRM or K homology RNA-binding domains and  
55 auxiliary domains, such as glycine-rich motifs and the arginine-glycine-glycine box, and while  
56 frequently described as silencers of splice site selection, their effect appears to depend on the  
57 binding position. Both SR proteins (reviewed in [9-11]) and hnRNPs (reviewed in [12-14])  
58 have been implicated in various other steps of posttranscriptional regulation apart from splicing.

59 Alternative splicing occurs when splice sites are differentially recognized and more than one  
60 transcript, and potentially multiple proteins, are generated from the same pre-mRNA. This  
61 greatly enhances the coding capacity of a genome, and indeed alternative splicing in humans is  
62 known to expand the proteome by at least five times when compared to the number of protein-  
63 coding genes [15], while in plants the effects of alternative splicing have not yet been widely  
64 addressed at the proteome level. Different types of alternative splicing events can occur in  
65 eukaryotes (Box 1), with intron retention being predominant in plants and exon skipping the  
66 most frequent alternative splicing event in mammals. Protein isoforms arising from alternative  
67 splicing may vary in virtually all aspects of protein function, while specific mRNA splice  
68 variants can be targeted for degradation. Possible consequences of alternative splicing at the  
69 mRNA and protein levels are detailed in Box 1.

70 Alternative splicing regulation studies have focused primarily on RNA sequence elements and  
71 their associated splicing factors, but extensive work in mammals also points to key roles for  
72 chromatin structure, histone modifications and transcription rates (reviewed in [16-18]).  
73 Mechanisms of epigenetic control of alternative splicing are emerging in plant systems too  
74 ([19]).

## 75 **Alternative Splicing as a Means to Promote Plant Stress Tolerance**

76 The current estimate indicates that around 61% of intron-containing genes in the model plant  
77 arabidopsis (*Arabidopsis thaliana*) undergo alternative splicing [20]. This number is likely to  
78 increase as more transcriptome data for plants under different developmental and environmental  
79 conditions are evaluated and as more precise tools for identifying splice variants in high-  
80 throughput analyses are developed. Notably, RNA-seq data have been confirming previous  
81 indications that abiotic stress markedly alters alternative splicing in plants [21-29]. For  
82 example, recent studies show that salt stress changes the alternative splicing pattern of more  
83 than 6,000 arabidopsis genes [23], while high-temperature stress induces differential splicing of  
84 more than 1,000 genes in grape [27]. Moreover, stress imposed by high salinity or treatment  
85 with the ABA phytohormone was found to substantially promote the use of non-canonical  
86 splice sites [23,29], which could provide a specific means of diversifying the transcriptome in

87 response to stress. Although some of these stress-induced changes in plant alternative splicing  
88 patterns could result from alterations in other processes, such as turnover and transport of  
89 specific splice variants, or reflect a decreased ability of the spliceosome to accurately recognize  
90 splice sites under stress conditions, a body of available evidence supports a major role for  
91 alternative splicing in implementing adaptive responses to adverse environments. Indeed, plant  
92 genes encoding known stress response regulators are particularly prone to generating multiple  
93 transcripts via this mechanism [30] and, most importantly, several studies have demonstrated  
94 the functional significance of some of these alternative splicing events, which often act as  
95 modulators of the ratio between active and non-active isoforms, thereby fine-tuning gene  
96 expression in response to abiotic stress.

97 Tolerance to extreme heat, one of the most detrimental stresses affecting plant productivity,  
98 involves evolutionarily conserved heat shock transcription factors (HSFs), which in arabidopsis  
99 undergo extensive heat-shock induced alternative splicing events [31]. At least one of these  
100 events has been assigned biological impact, as a truncated splice form of HsfA2, but not the  
101 full-length **isoform**, can bind to the *HSFA2* promoter and activate its own transcription in a  
102 positive auto-regulatory loop [31]. Expression of *HSF* genes is under the control of the  
103 DEHYDRATION-RESPONSIVE ELEMENT BINDING 2 (DREB2) transcription factor,  
104 which is also regulated by alternative splicing in response to stress in many plant species [32-  
105 36]. Indeed, while under normal conditions the *DREB2B* gene predominantly produces a non-  
106 functional transcript, the levels of the full-length, functional transcript significantly increase in  
107 response to abiotic stress [35,37]. Interestingly, a putative exonic splicing enhancer that affects  
108 alternative splicing of the sheepgrass *DREB2* gene was shown to interact *in vitro* with several  
109 SR proteins [36].

110 High temperatures are also known in plants to enhance endoplasmic reticulum (ER) stress,  
111 which triggers the well-conserved unfolded protein response (UPR). ER stress promotes  
112 splicing of a small intron in *bZIP60*, encoding a key transcription factor in UPR, by two ER-  
113 located stress sensors, IRE1a and IRE1b [38,39]. While under normal conditions bZIP60  
114 localizes mostly in the ER membrane, the splice form accumulated during ER stress lacks the  
115 transmembrane domain and is therefore targeted to the nucleus [38], where it activates  
116 transcription of genes that aid in protein folding and degradation [40,41].

117 Alternative splicing events in genes encoding downstream effectors of plant stress tolerance  
118 have also proved important in regulating stress responses. For example, alternative 3' splice site  
119 selection gives rise to two splice forms of the arabidopsis Zinc-Induced Facilitator-Like 1  
120 (ZIFL1) transporter — while the full-length isoform is localized in the tonoplast of root cells  
121 and regulates transport of the auxin phytohormone, a truncated ZIFL1 variant is targeted to the  
122 plasma membrane of leaf stomatal guard cells and mediates drought tolerance [42]. On the  
123 other hand, Zinc-Induced Facilitator 2 (ZIF2) is a vacuolar membrane transporter that confers  
124 tolerance to zinc by promoting immobilization of the heavy metal in root cells [43].  
125 Remarkably, elevated zinc levels promote an intron retention event in the 5' untranslated region  
126 (5'UTR) of *ZIF2* that enhances its own translation [43], thus controlling cellular levels of the  
127 encoded protein and thereby plant zinc tolerance.

128 It is interesting to note that the vast majority of arabidopsis SR protein genes, which encode key  
129 splicing regulators and are extensively alternatively spliced themselves, have their splicing  
130 patterns changed by various environmental stresses [21,22,44,45]. Furthermore, Ding *et al.* [22]  
131 found that salt stress induces alternative 5' and 3' splice site selection and intron retention  
132 events that introduce premature termination codons (PTCs) in SR protein pre-mRNAs, with  
133 these changes being accompanied by alterations in the splicing pattern of 49% of all intron-  
134 containing genes. These findings point to an important mode of stress regulation of plant SR  
135 proteins, which could function as central coordinators of responses to environmental changes.

### 136 **Splicing Regulators as Key Mediators of Plant Stress Responses**

137 In addition to ascribing functional significance to individual alternative splicing events, genetic  
138 studies have uncovered major *in vivo* roles for splicing regulators in plant responses to abiotic  
139 stress. This is evidenced by the numerous mutations in spliceosomal components that severely  
140 affect plant stress tolerance. Moreover, transcriptome analyses of plants expressing altered  
141 levels of splicing regulators have identified potential functional targets of these RNA-binding  
142 proteins during the response to stress. Apart from stress-specific regulatory genes, gene  
143 ontology enrichment studies of these transcriptomic data have revealed potential alternative  
144 splicing feedback loops through the splicing regulation of various other RNA-binding proteins  
145 genes [24,46].

146 Besides the already mentioned posttranscriptional regulation, plant SR protein genes are also  
147 regulated by stress at the transcriptional ([45,47,48] and publicly available transcriptomic data)  
148 as well as translational levels [27,49], with different environmental cues controlling their  
149 phosphorylation status [50,51] and, consequently, subcellular distribution [52-54]. Furthermore,  
150 functional evidence is corroborating a role for SR proteins in plant abiotic stress responses. For  
151 example, loss of function of the *SR34b* gene, whose expression is upregulated by cadmium  
152 (Cd), causes enhanced accumulation of this metal ion and hypersensitivity to its toxicity in  
153 arabidopsis [48]. Interestingly, the *IRT1* gene, which encodes a putative Cd transporter, is  
154 misspliced in the *sr34b* mutant, providing mechanistic clues on Cd tolerance control by the SR  
155 protein [48]. In another study [55], the arabidopsis RS40 and RS41 were found to interact in  
156 nuclear speckles with HIGH OSMOTIC STRESS GENE EXPRESSION 5 (HOS5), a KH-  
157 domain RNA-binding protein, and FIERY2/CTD phosphatase-like 1 (FRY2/CPL1), a major  
158 player in the co-transcriptional processing of nascent transcripts. Knockout mutants for *HOS5*,  
159 *RS40* and *RS41*, all displayed salt and ABA hypersensitivity as well as significant intron  
160 retention in many stress-related genes, thus implicating two plant SR proteins in the regulation  
161 of alternative splicing under abiotic stress.

162 Several hnRNPs, particularly glycine-rich RNA-binding proteins (GRPs) whose molecular  
163 roles remain largely unknown in plants, have also been functionally implicated in plant abiotic  
164 stress responses [56-59]. In arabidopsis, the RZ-1a, a zinc finger-containing GRP, negatively  
165 regulates early development under salt and drought stress [56], while heterologous expression  
166 of the arabidopsis GRP2 and GRP7 in rice leads to higher grain yields under drought stress  
167 [59]. In arabidopsis, GRP7 appears to perform opposite functions in response to different  
168 abiotic factors, having a positive effect on stress tolerance under low temperatures and a

169 negative effect under salt or dehydration stress conditions, at the seed germination, seedling  
170 growth and stomatal movement levels [57]. Notably, using a high-resolution RT-PCR  
171 alternative splicing panel and RNA immunoprecipitation (RIP) analyses, Staiger and co-  
172 workers [60] showed that GRP7 directly binds mRNA, affecting particularly the choice of  
173 alternative 5' splice sites, a function partially shared with its close paralog, GRP8. Future GRP7  
174 studies under abiotic stress conditions should uncover the transcripts targeted by this RNA-  
175 binding protein to regulate plant stress tolerance.

176 The cap-binding proteins CBP20 and CBP80 (also called ABH1 for ABA HYPERSENSITIVE  
177 1) have been reported to modulate plant salt stress responses as well as ABA sensitivity during  
178 seed germination and stomatal closure, and to confer drought tolerance [61-64]. These proteins  
179 constitute the two subunits of the dimeric nuclear cap-binding complex (CBC), which binds the  
180 cap structure of RNA polymerase II transcripts [65] and was found in arabidopsis to influence  
181 alternative splicing of first introns, particularly at the 5' splice site [66].

182 Different arabidopsis spliceosomal components also influence the response to stress *in planta*.  
183 Loss-of-function mutations in the SNW/Ski-interacting protein (SKIP), which physically  
184 interacts with the SR-like protein SR45 and functions in alternative splicing through  
185 modulation of recognition or cleavage of 5' and 3' splice sites [67], led to plant oversensitivity  
186 to salt and osmotic stress [23]. Strikingly, RNA-seq analyses of wild-type and mutant plants  
187 under salt stress showed that SKIP controls over 86% of salt stress-induced alternative splicing  
188 events [23]. A component of the U6 snRNP LSm2-8 heptameric complex, LSm5, named  
189 SUPERSENSITIVE TO ABSCISIC ACID AND DROUGHT 1 (SAD1) as its loss of function  
190 causes exacerbated plant responses to ABA and osmotic stresses [68], also affects the splicing  
191 of transcripts associated with high salinity stress [69]. Indeed, overexpression of *SAD1*  
192 promotes the splicing accuracy and efficiency of stress-responsive genes and improves salt  
193 tolerance, while mutations in *SAD1* result in a global increase in alternative splicing [69]. It is  
194 noteworthy that a protein arginine methyltransferase (PRMT5, also known as SKB1) also  
195 affects salt stress-driven pre-mRNA splicing through methylation of another LSm protein,  
196 LSm4 [70]. On the other hand, an arabidopsis homolog of the human U5 snRNP-associated  
197 102-kD protein, STABILIZED1 (STA1), is a regulator of plant responses to not only high  
198 salinity, but also extreme temperatures and drought stress, controlling the alternative splicing of  
199 important stress-induced regulatory genes [71,72]. Finally, RDM16, a U4/U6 snRNP-associated  
200 protein as well as a component of the RNA-directed DNA methylation (RdDM) pathway, is  
201 required for an appropriate response to salt and ABA stress [73].

202 It is important to note that, because mRNA splicing is an essential cellular process, the  
203 identification of splicing regulators that exert stress-specific functions is rare. In fact, all of the  
204 loss-of-function mutants described above (listed in Table 1) show moderate to severe  
205 developmental defects, reflecting broader functions for the splicing factors in question. For  
206 instance, *SKIP* antisense transgenic lines show pleiotropic developmental phenotypes, including  
207 reduced inflorescence stems and smaller leaves [74], while a recessive *STA1* mutant exhibits  
208 several defects in leaf and inflorescence morphology [71], and mutations in *RDM16* result in  
209 dwarf stature as well as smaller leaves and siliques [73]. The identification of RNA-binding  
210 protein mutants displaying conditional stress phenotypes would allow the discovery of splicing

211 regulators involved specifically in plant responses to abiotic stress. SR proteins appear as  
212 promising candidates for such a specific role, as several individual knockout mutant lines for  
213 these splicing factors are impaired in the response to various stress cues, while showing no  
214 evident phenotypes when grown under normal conditions (our unpublished data). Larger and  
215 more diverse SR protein families in the plant kingdom (e.g. 18 SR proteins in arabidopsis [75]  
216 versus only 12 in humans [76]), resulting from genome amplification [77], could provide a  
217 plausible explanation for the occurrence of plant-specific functions, such as in the regulation of  
218 stress responses, for a subset of SR protein genes.

## 219 **An Emerging Link Between Splicing and ABA Signaling**

220 There is mounting evidence that the ABA phytohormone is a crucial mediator in  
221 posttranscriptional regulation of plant stress responses. In fact, most of the splicing regulators  
222 described above were also reported to affect ABA sensitivity [55,56,61,62,64,68,70,71,73],  
223 suggesting requirement of the hormone for control of abiotic stress responses by these RNA-  
224 binding proteins. In addition, the SR-like SR45 protein, which interacts with the U1-70K and  
225 U2AF35b to facilitate spliceosome assembly at the 5' and 3' splice sites [78], regulates sugar  
226 responses in arabidopsis by repressing both ABA signaling and glucose-induced accumulation  
227 of the hormone [79]. To achieve this, SR45 promotes proteasomal degradation of SnRK1 [46],  
228 a protein kinase that coordinates sugar and ABA signaling [80]. Importantly, RNA  
229 immunoprecipitation followed by high-throughput sequencing (RIP-seq) revealed that the  
230 SR45-bound transcripts are markedly enriched in ABA-signaling functions [81], providing  
231 mechanistic insight into the stress roles of SR45.

232 The involvement of pre-mRNA splicing in ABA stress responses is also corroborated by recent  
233 work making use of the splicing inhibitors pladienolide B (PB) and herboxidiene (GEX1a)  
234 known in mammalian cells to target the U2 snRNP component SF3b [82,83]. Two studies by  
235 Mahfouz and co-authors [84,85] have reported that treatment of arabidopsis plants with either  
236 of these compounds partially mimics stress signals, such as high salinity and drought, in a  
237 manner reminiscent of ABA, leading to activation of ABA-inducible promoters and stomatal  
238 closure. This splicing-dependent activation of stress signaling is substantiated by an increase in  
239 intron retention events, also in splice variants previously reported to be pivotal mediators of  
240 abiotic stress responses [85,86].

241 Upon its *de novo* biosynthesis in response to stress, ABA binds intracellular receptors,  
242 inhibiting PP2C phosphatases and thereby derepressing SnRK2 protein kinases [87] that  
243 phosphorylate downstream effectors to activate ABA-signaling (Figure 2). Of the few  
244 alternative-splicing events in main components of the ABA pathway characterized so far  
245 [86,88-90], key relevance in plant adaptation to abiotic stress has been reported for the HAB1  
246 PP2C [24,86], which negatively regulates ABA signaling by binding and dephosphorylating the  
247 SnRK2.6 protein kinase [91]. Remarkably, alternative splicing of the arabidopsis *HAB1* gene is  
248 regulated by ABA and appears to be crucial for switching ABA signal transduction on and off.  
249 Although the endogenous relative protein levels of the two HAB1 splice forms were not  
250 determined, results reported by Wang et al. [86] are consistent with an accumulation of the full-  
251 length protein under low ABA conditions, which would prevent SnRK2.6 activation, while high

252 ABA levels would promote retention of a *HAB1* intron and result in the production of a  
253 truncated splice form that binds but does not inhibit SnRK2.6, thereby activating ABA  
254 signaling.

255 ABA-regulated alternative splicing of the HAB1 PP2C is controlled by one of the sole plant  
256 RNA-binding proteins reported to fulfill stress-specific roles, RBM25 [24,86]. In fact, while  
257 arabidopsis knockout mutants for this human splicing factor homolog are severely affected in  
258 ABA, drought and salt stress responses, they display no obvious phenotypes under normal  
259 growth conditions [24,92]. Importantly, RBM25 is activated by ABA not only at the  
260 transcriptional level, but also posttranslationally through modulation of its phosphorylation  
261 levels [24]. Identification of the protein(s) controlling the phosphorylation of RBM25 would  
262 shed light on the upstream mechanisms underlying posttranscriptional control of the ABA  
263 pathway. Importantly, and consistent with a central role for pre-mRNA splicing in ABA-  
264 mediated stress responses, ABA signaling, SnRK2 kinases in particular, has been shown to  
265 regulate the phosphorylation status of several plant splicing factors [50,51,93]. The  
266 involvement of splicing regulators (Table 1) and their putative functional targets in ABA  
267 responses is summarized in Figure 2.

## 268 **Concluding Remarks and Future Perspectives**

269 Alternative splicing regulation represents an important means of fine-tuning gene expression  
270 that may save the time required for changes in transcriptional activation and pre-mRNA  
271 accumulation, thus allowing rapid plant adaptation to adverse environmental conditions.  
272 Ultimately, the effects of alternative splicing on mRNAs encoding effectors and modulators of  
273 abiotic stress responses are determined by the levels and/or activity of the splicing factors  
274 regulating this process. To date, very few studies have addressed the upstream regulatory  
275 mechanisms dictating the activity of splicing factors during the response to stress. In mammals,  
276 reversible phosphorylation by specific kinases and phosphatases is crucial in the regulation of  
277 splicing factor activity [94,95], while in plants the phosphorylation status of several plant SR  
278 proteins has been shown to promote their subcellular relocalization to specific nuclear  
279 compartments where they are known to control splicing [52-54,96]. Uncovering the upstream  
280 components modulating posttranslational modification of plant splicing factors could provide  
281 key insight into how environmental signals activate alternative splicing to regulate plant stress  
282 tolerance (see Outstanding Questions). Clearly, despite the extensive functional data gathered  
283 so far, elucidation of the precise mode of action of splicing regulators controlling plant stress  
284 responses will require the identification of their direct mRNA targets. The optimization of state-  
285 of-the-art techniques still challenging in plants, such as individual-nucleotide resolution  
286 crosslinking and immunoprecipitation (iCLIP, [97]), should help pinpoint the *bona fide*  
287 physiological targets as well as consensus RNA sequences recognized by these splicing  
288 regulators. Remarkably, strong evidence is emerging that ABA signaling is widely regulated at  
289 the alternative splicing level. Future identification of new splicing factors and their target  
290 mRNAs acting in this pathway should improve our understanding of how alternative splicing  
291 modulates plant abiotic stress responses, thus paving the way for new strategies to improve  
292 plant productivity under unfavorable environments.



## 293 Acknowledgments

294 We apologize to the many authors whose work was not cited due to space limitations. T.L. and  
295 G.M. are supported by a Marie Skłodowska-Curie Individual Fellowship (MSCA-IF-2015;  
296 grant 706274) and an EMBO Long-Term Fellowship (ALTF 1576-2016), respectively.  
297 Research in our laboratory is currently funded by Fundação para a Ciência e a Tecnologia  
298 (FCT) under grant PTDC/BIA-PLA/1084/2014. Funding from the GREEN-it research unit  
299 (UID/Multi/04551/2013) is also acknowledged.

## 300 References

- 301 1. Cutler, S.R. *et al.* (2010) Abscisic acid: emergence of a core signaling network. *Annu Rev*  
302 *Plant Biol* 61, 651-79
- 303 2. Hubbard, K.E. *et al.* (2010) Early abscisic acid signal transduction mechanisms: newly  
304 discovered components and newly emerging questions. *Genes Dev* 24, 1695-708
- 305 3. Imadi, S.R. *et al.* (2015) Plant transcriptomics and responses to environmental stress: an  
306 overview. *J Genet* 94, 525-37
- 307 4. Janmohammadi, M. *et al.* (2015) Low temperature tolerance in plants: Changes at the protein  
308 level. *Phytochemistry* 117, 76-89
- 309 5. Wang, W.Q. *et al.* (2015) Proteomics of seed development, desiccation tolerance,  
310 germination and vigor. *Plant Physiol Biochem* 86, 1-15
- 311 6. Yoshida, T. *et al.* (2015) Omics Approaches Toward Defining the Comprehensive Abscisic  
312 Acid Signaling Network in Plants. *Plant Cell Physiol* 56, 1043-52
- 313 7. Chen, W. and Moore, M.J. (2015) Spliceosomes. *Curr Biol* 25, R181-3
- 314 8. Koncz, C. *et al.* (2012) The spliceosome-activating complex: molecular mechanisms  
315 underlying the function of a pleiotropic regulator. *Front Plant Sci* 3, 9
- 316 9. Long, J.C. and Caceres, J.F. (2009) The SR protein family of splicing factors: master  
317 regulators of gene expression. *Biochem J* 417, 15-27
- 318 10. Duque, P. (2011) A role for SR proteins in plant stress responses. *Plant Signal Behav* 6, 49-  
319 54
- 320 11. Howard, J.M. and Sanford, J.R. (2015) The RNAissance family: SR proteins as  
321 multifaceted regulators of gene expression. *Wiley Interdiscip Rev RNA* 6, 93-110
- 322 12. Yeap, W.C. *et al.* (2014) HnRNP-like proteins as post-transcriptional regulators. *Plant Sci*  
323 227, 90-100
- 324 13. Han, S.P. *et al.* (2010) Functional diversity of the hnRNPs: past, present and perspectives.  
325 *Biochem J* 430, 379-92
- 326 14. Wachter, A. *et al.* (2012) The Role of Polypyrimidine Tract-Binding Proteins and Other  
327 hnRNP Proteins in Plant Splicing Regulation. *Front Plant Sci* 3, 81
- 328 15. Nilsen, T.W. and Graveley, B.R. (2010) Expansion of the eukaryotic proteome by  
329 alternative splicing. *Nature* 463, 457-63
- 330 16. Luco, R.F. *et al.* (2011) Epigenetics in alternative pre-mRNA splicing. *Cell* 144, 16-26
- 331 17. Naftelberg, S. *et al.* (2015) Regulation of alternative splicing through coupling with  
332 transcription and chromatin structure. *Annu Rev Biochem* 84, 165-98
- 333 18. Nieto Moreno, N. *et al.* (2015) Chromatin, DNA structure and alternative splicing. *FEBS*  
334 *Lett* 589, 3370-8
- 335 19. Pajoro, A. *et al.* (2017) Histone H3 lysine 36 methylation affects temperature-induced  
336 alternative splicing and flowering in plants. *Genome Biol* 18, 102

- 337 20. Marquez, Y. *et al.* (2012) Transcriptome survey reveals increased complexity of the  
338 alternative splicing landscape in Arabidopsis. *Genome Res* 22, 1184-95
- 339 21. Filichkin, S.A. *et al.* (2010) Genome-wide mapping of alternative splicing in Arabidopsis  
340 thaliana. *Genome Res* 20, 45-58
- 341 22. Ding, F. *et al.* (2014) Genome-wide analysis of alternative splicing of pre-mRNA under salt  
342 stress in Arabidopsis. *BMC Genomics* 15, 431
- 343 23. Feng, J. *et al.* (2015) SKIP Confers Osmotic Tolerance during Salt Stress by Controlling  
344 Alternative Gene Splicing in Arabidopsis. *Mol Plant* 8, 1038-52
- 345 24. Zhan, X. *et al.* (2015) An Arabidopsis PWI and RRM motif-containing protein is critical for  
346 pre-mRNA splicing and ABA responses. *Nat Commun* 6, 8139
- 347 25. Thatcher, S.R. *et al.* (2016) Genome-Wide Analysis of Alternative Splicing during  
348 Development and Drought Stress in Maize. *Plant Physiol* 170, 586-99
- 349 26. Zhang, F. *et al.* (2016) Genetic regulation of salt stress tolerance revealed by RNA-Seq in  
350 cotton diploid wild species, *Gossypium davidsonii*. *Sci Rep* 6, 20582
- 351 27. Jiang, J. *et al.* (2017) Integrating Omics and Alternative Splicing Reveals Insights into  
352 Grape Response to High Temperature. *Plant Physiol* 173, 1502-1518
- 353 28. Keller, M. *et al.* (2017) Alternative splicing in tomato pollen in response to heat  
354 stress(aEuro). *DNA Research* 24, 205-217
- 355 29. Zhu, F.Y. *et al.* (2017) Proteogenomic analysis reveals alternative splicing and translation  
356 as part of the abscisic acid response in Arabidopsis seedlings. *Plant J* 91, 518-533
- 357 30. Ner-Gaon, H. *et al.* (2004) Intron retention is a major phenomenon in alternative splicing in  
358 Arabidopsis. *Plant J* 39, 877-85
- 359 31. Liu, J. *et al.* (2013) An autoregulatory loop controlling Arabidopsis HsfA2 expression: role  
360 of heat shock-induced alternative splicing. *Plant Physiol* 162, 512-21
- 361 32. Xue, G.P. and Loveridge, C.W. (2004) HvDRF1 is involved in abscisic acid-mediated gene  
362 regulation in barley and produces two forms of AP2 transcriptional activators, interacting  
363 preferably with a CT-rich element. *Plant J* 37, 326-39
- 364 33. Egawa, C. *et al.* (2006) Differential regulation of transcript accumulation and alternative  
365 splicing of a DREB2 homolog under abiotic stress conditions in common wheat. *Genes*  
366 *Genet Syst* 81, 77-91
- 367 34. Qin, F. *et al.* (2007) Regulation and functional analysis of ZmDREB2A in response to  
368 drought and heat stresses in *Zea mays* L. *Plant J* 50, 54-69
- 369 35. Matsukura, S. *et al.* (2010) Comprehensive analysis of rice DREB2-type genes that encode  
370 transcription factors involved in the expression of abiotic stress-responsive genes. *Mol*  
371 *Genet Genomics* 283, 185-96
- 372 36. Liu, Z. *et al.* (2017) Identified of a novel cis-element regulating the alternative splicing of  
373 LcDREB2. *Sci Rep* 7, 46106
- 374 37. Peng, X.J. *et al.* (2013) The transcriptional factor LcDREB2 cooperates with LcSAMDC2  
375 to contribute to salt tolerance in *Leymus chinensis*. *Plant Cell Tissue and Organ Culture*  
376 113, 245-256
- 377 38. Deng, Y. *et al.* (2011) Heat induces the splicing by IRE1 of a mRNA encoding a  
378 transcription factor involved in the unfolded protein response in Arabidopsis. *Proc Natl*  
379 *Acad Sci U S A* 108, 7247-52
- 380 39. Nagashima, Y. *et al.* (2011) Arabidopsis IRE1 catalyses unconventional splicing of bZIP60  
381 mRNA to produce the active transcription factor. *Sci Rep* 1, 29
- 382 40. Iwata, Y. *et al.* (2008) Arabidopsis bZIP60 is a proteolysis-activated transcription factor  
383 involved in the endoplasmic reticulum stress response. *Plant Cell* 20, 3107-21

- 384 41. Wang, B. *et al.* (2017) BhbZIP60 from Resurrection Plant *Boea hygrometrica* Is an mRNA  
385 Splicing-Activated Endoplasmic Reticulum Stress Regulator Involved in Drought  
386 Tolerance. *Front Plant Sci* 8, 245
- 387 42. Remy, E. *et al.* (2013) A major facilitator superfamily transporter plays a dual role in polar  
388 auxin transport and drought stress tolerance in *Arabidopsis*. *Plant Cell* 25, 901-26
- 389 43. Remy, E. *et al.* (2014) Intron retention in the 5'UTR of the novel ZIF2 transporter enhances  
390 translation to promote zinc tolerance in *Arabidopsis*. *PLoS Genet* 10, e1004375
- 391 44. Palusa, S.G. *et al.* (2007) Alternative splicing of pre-mRNAs of *Arabidopsis*  
392 serine/arginine-rich proteins: regulation by hormones and stresses. *Plant J* 49, 1091-107
- 393 45. Tanabe, N. *et al.* (2007) Differential expression of alternatively spliced mRNAs of  
394 *Arabidopsis* SR protein homologs, atSR30 and atSR45a, in response to environmental  
395 stress. *Plant Cell Physiol* 48, 1036-49
- 396 46. Carvalho, R.F. *et al.* (2016) The *Arabidopsis* SR45 Splicing Factor, a Negative Regulator of  
397 Sugar Signaling, Modulates SNF1-Related Protein Kinase 1 Stability. *Plant Cell* 28, 1910-  
398 25
- 399 47. Cruz, T.M. *et al.* (2014) Abscisic acid (ABA) regulation of *Arabidopsis* SR protein gene  
400 expression. *Int J Mol Sci* 15, 17541-64
- 401 48. Zhang, W. *et al.* (2014) Splicing factor SR34b mutation reduces cadmium tolerance in  
402 *Arabidopsis* by regulating iron-regulated transporter 1 gene. *Biochem Biophys Res*  
403 *Commun* 455, 312-7
- 404 49. Palusa, S.G. and Reddy, A.S. (2015) Differential recruitment of splice variants from SR  
405 pre-mRNAs to polysomes during development and in response to stresses. *Plant Cell*  
406 *Physiol* 56, 421-7
- 407 50. Umezawa, T. *et al.* (2013) Genetics and phosphoproteomics reveal a protein  
408 phosphorylation network in the abscisic acid signaling pathway in *Arabidopsis thaliana*. *Sci*  
409 *Signal* 6, rs8
- 410 51. Wang, P. *et al.* (2013) Quantitative phosphoproteomics identifies SnRK2 protein kinase  
411 substrates and reveals the effectors of abscisic acid action. *Proc Natl Acad Sci U S A* 110,  
412 11205-10
- 413 52. Ali, G.S. *et al.* (2003) Nuclear localization and in vivo dynamics of a plant-specific  
414 serine/arginine-rich protein. *Plant J* 36, 883-93
- 415 53. Tillemans, V. *et al.* (2006) Insights into nuclear organization in plants as revealed by the  
416 dynamic distribution of *Arabidopsis* SR splicing factors. *Plant Cell* 18, 3218-34
- 417 54. Rausin, G. *et al.* (2010) Dynamic nucleocytoplasmic shuttling of an *Arabidopsis* SR  
418 splicing factor: role of the RNA-binding domains. *Plant Physiol* 153, 273-84
- 419 55. Chen, T. *et al.* (2013) A KH-domain RNA-binding protein interacts with FIERY2/CTD  
420 phosphatase-like 1 and splicing factors and is important for pre-mRNA splicing in  
421 *Arabidopsis*. *PLoS Genet* 9, e1003875
- 422 56. Kim, Y.O. *et al.* (2007) A zinc finger-containing glycine-rich RNA-binding protein, atRZ-  
423 1a, has a negative impact on seed germination and seedling growth of *Arabidopsis thaliana*  
424 under salt or drought stress conditions. *Plant Cell Physiol* 48, 1170-81
- 425 57. Kim, J.S. *et al.* (2008) Glycine-rich RNA-binding protein 7 affects abiotic stress responses  
426 by regulating stomata opening and closing in *Arabidopsis thaliana*. *Plant J* 55, 455-66
- 427 58. Yeap, W.C. *et al.* (2012) EgRBP42 encoding an hnRNP-like RNA-binding protein from  
428 *Elaeis guineensis* Jacq. is responsive to abiotic stresses. *Plant Cell Rep* 31, 1829-43
- 429 59. Yang, D.H. *et al.* (2014) Expression of *Arabidopsis* glycine-rich RNA-binding protein  
430 AtGRP2 or AtGRP7 improves grain yield of rice (*Oryza sativa*) under drought stress  
431 conditions. *Plant Sci* 214, 106-12

- 432 60. Streitner, C. *et al.* (2012) An hnRNP-like RNA-binding protein affects alternative splicing  
433 by in vivo interaction with transcripts in *Arabidopsis thaliana*. *Nucleic Acids Res* 40,  
434 11240-55
- 435 61. Hugouvieux, V. *et al.* (2001) An mRNA cap binding protein, ABH1, modulates early  
436 abscisic acid signal transduction in *Arabidopsis*. *Cell* 106, 477-87
- 437 62. Papp, I. *et al.* (2004) A mutation in the Cap Binding Protein 20 gene confers drought  
438 tolerance to *Arabidopsis*. *Plant Mol Biol* 55, 679-86
- 439 63. Kong, X. *et al.* (2014) Quantitative proteomics analysis reveals that the nuclear cap-binding  
440 complex proteins *Arabidopsis* CBP20 and CBP80 modulate the salt stress response. *J*  
441 *Proteome Res* 13, 2495-510
- 442 64. Daszkowska-Golec, A. *et al.* (2017) Mutation in HvCBP20 (Cap Binding Protein 20)  
443 Adapts Barley to Drought Stress at Phenotypic and Transcriptomic Levels. *Front Plant Sci*  
444 8, 942
- 445 65. Izaurralde, E. *et al.* (1994) A nuclear cap binding protein complex involved in pre-mRNA  
446 splicing. *Cell* 78, 657-68
- 447 66. Raczynska, K.D. *et al.* (2010) Involvement of the nuclear cap-binding protein complex in  
448 alternative splicing in *Arabidopsis thaliana*. *Nucleic Acids Res* 38, 265-78
- 449 67. Wang, X. *et al.* (2012) SKIP is a component of the spliceosome linking alternative splicing  
450 and the circadian clock in *Arabidopsis*. *Plant Cell* 24, 3278-95
- 451 68. Xiong, L. *et al.* (2001) Modulation of abscisic acid signal transduction and biosynthesis by  
452 an Sm-like protein in *Arabidopsis*. *Dev Cell* 1, 771-81
- 453 69. Cui, P. *et al.* (2014) Dynamic regulation of genome-wide pre-mRNA splicing and stress  
454 tolerance by the Sm-like protein LSm5 in *Arabidopsis*. *Genome Biol* 15, R1
- 455 70. Zhang, Z. *et al.* (2011) *Arabidopsis* floral initiator SKB1 confers high salt tolerance by  
456 regulating transcription and pre-mRNA splicing through altering histone H4R3 and small  
457 nuclear ribonucleoprotein LSM4 methylation. *Plant Cell* 23, 396-411
- 458 71. Lee, B.H. *et al.* (2006) STABILIZED1, a stress-upregulated nuclear protein, is required for  
459 pre-mRNA splicing, mRNA turnover, and stress tolerance in *Arabidopsis*. *Plant Cell* 18,  
460 1736-49
- 461 72. Kim, G.D. *et al.* (2017) STABILIZED1 Modulates Pre-mRNA Splicing for  
462 Thermotolerance. *Plant Physiol* 173, 2370-2382
- 463 73. Huang, C.F. *et al.* (2013) A Pre-mRNA-splicing factor is required for RNA-directed DNA  
464 methylation in *Arabidopsis*. *PLoS Genet* 9, e1003779
- 465 74. Lim, G.H. *et al.* (2010) A putative novel transcription factor, AtSKIP, is involved in  
466 abscisic acid signalling and confers salt and osmotic tolerance in *Arabidopsis*. *New Phytol*  
467 185, 103-13
- 468 75. Barta, A. *et al.* (2010) Implementing a rational and consistent nomenclature for  
469 serine/arginine-rich protein splicing factors (SR proteins) in plants. *Plant Cell* 22, 2926-9
- 470 76. Manley, J.L. and Krainer, A.R. (2010) A rational nomenclature for serine/arginine-rich  
471 protein splicing factors (SR proteins). *Genes Dev* 24, 1073-4
- 472 77. Kalyna, M. and Barta, A. (2004) A plethora of plant serine/arginine-rich proteins:  
473 redundancy or evolution of novel gene functions? *Biochem Soc Trans* 32, 561-4
- 474 78. Day, I.S. *et al.* (2012) Interactions of SR45, an SR-like protein, with spliceosomal proteins  
475 and an intronic sequence: insights into regulated splicing. *Plant J* 71, 936-47
- 476 79. Carvalho, R.F. *et al.* (2010) The plant-specific SR45 protein negatively regulates glucose  
477 and ABA signaling during early seedling development in *Arabidopsis*. *Plant Physiol* 154,  
478 772-83

- 479 80. Rodrigues, A. *et al.* (2013) ABI1 and PP2CA phosphatases are negative regulators of Snf1-  
480 related protein kinase1 signaling in Arabidopsis. *Plant Cell* 25, 3871-84
- 481 81. Xing, D. *et al.* (2015) Transcriptome-Wide Identification of RNA Targets of Arabidopsis  
482 SERINE/ARGININE-RICH45 Uncovers the Unexpected Roles of This RNA Binding  
483 Protein in RNA Processing. *Plant Cell* 27, 3294-308
- 484 82. Kotake, Y. *et al.* (2007) Splicing factor SF3b as a target of the antitumor natural product  
485 pladienolide. *Nat Chem Biol* 3, 570-5
- 486 83. Hasegawa, M. *et al.* (2011) Identification of SAP155 as the target of GEX1A  
487 (Herboxidiene), an antitumor natural product. *ACS Chem Biol* 6, 229-33
- 488 84. AlShareef, S. *et al.* (2017) Herboxidiene triggers splicing repression and abiotic stress  
489 responses in plants. *BMC Genomics* 18, 260
- 490 85. Ling, Y. *et al.* (2017) Pre-mRNA splicing repression triggers abiotic stress signaling in  
491 plants. *Plant J* 89, 291-309
- 492 86. Wang, Z. *et al.* (2015) ABA signalling is fine-tuned by antagonistic HAB1 variants. *Nat*  
493 *Commun* 6, 8138
- 494 87. Umezawa, T. *et al.* (2009) Type 2C protein phosphatases directly regulate abscisic acid-  
495 activated protein kinases in Arabidopsis. *Proc Natl Acad Sci U S A* 106, 17588-93
- 496 88. Sugliani, M. *et al.* (2010) The conserved splicing factor SUA controls alternative splicing of  
497 the developmental regulator ABI3 in Arabidopsis. *Plant Cell* 22, 1936-46
- 498 89. Gao, Y. *et al.* (2013) Functional characterization of two alternatively spliced transcripts of  
499 tomato ABSCISIC ACID INSENSITIVE3 (ABI3) gene. *Plant Mol Biol* 82, 131-45
- 500 90. Wang, M. *et al.* (2013) BolOST1, an ortholog of Open Stomata 1 with alternative splicing  
501 products in Brassica oleracea, positively modulates drought responses in plants. *Biochem*  
502 *Biophys Res Commun* 442, 214-20
- 503 91. Vlad, F. *et al.* (2009) Protein phosphatases 2C regulate the activation of the Snf1-related  
504 kinase OST1 by abscisic acid in Arabidopsis. *Plant Cell* 21, 3170-84
- 505 92. Cheng, C. *et al.* (2017) RBM25 Mediates Abiotic Responses in Plants. *Front Plant Sci* 8,  
506 292
- 507 93. Li, J. *et al.* (2002) Modulation of an RNA-binding protein by abscisic-acid-activated protein  
508 kinase. *Nature* 418, 793-7
- 509 94. Stamm, S. (2008) Regulation of alternative splicing by reversible protein phosphorylation. *J*  
510 *Biol Chem* 283, 1223-7
- 511 95. Zhou, Z. and Fu, X.D. (2013) Regulation of splicing by SR proteins and SR protein-specific  
512 kinases. *Chromosoma* 122, 191-207
- 513 96. Stankovic, N. *et al.* (2016) Dynamic Distribution and Interaction of the Arabidopsis SRSF1  
514 Subfamily Splicing Factors. *Plant Physiol* 170, 1000-13
- 515 97. Huppertz, I. *et al.* (2014) iCLIP: protein-RNA interactions at nucleotide resolution. *Methods*  
516 65, 274-87

## 517 **Figure Legends and Boxes**

### 518 **Box 1. Alternative Splicing: Different Types and Molecular Outcomes**

519 Splicing of a nascent pre-mRNA occurs in the nucleus, mostly cotranscriptionally, when the  
520 spliceosome recognizes nucleotide sequences defining exon/intron boundaries called splice  
521 sites to remove the introns, join the exons, and generate a mature mRNA molecule.  
522 Importantly, detection of splice sites by the spliceosome can vary, thereby producing alternative  
523 mature transcripts from the same pre-mRNA. This mechanism, termed alternative splicing, can

524 change the structure of transcripts in different ways and have multiple molecular consequences.  
525 For example, intron retention occurs when both the 5' and 3' ends of a specific intron are not  
526 recognized, splicing fails, and a mature mRNA that includes the intronic sequence is produced  
527 (see Figure I). Also, depending on the correct recognition of either or both splice sites flanking  
528 an exon, the latter can fail to be included in the final transcript, and selection of alternative 5' or  
529 3' splice sites results in the inclusion of different stretches of an exon. The insertion or deletion  
530 of alternative fragments in an mRNA can generate proteins with altered domains, and thereby  
531 affect key aspects of their molecular function, such as enzymatic activity, subcellular  
532 localization, stability, binding properties or posttranslational modifications. By contrast, when  
533 the alternatively-spliced sequence affects noncoding regions such as untranslated regions  
534 (UTRs) or introns, the stability, nuclear export, localization and/or translation of the mRNA  
535 may be affected. Importantly, when alternative splicing causes a change in the open reading  
536 frame (ORF), a premature termination codon (PTC) is often introduced in the mRNA. Specific  
537 proteins in the cell can then recognize this PTC during the first round of translation and target  
538 the transcript for degradation through a cytoplasmic mRNA surveillance system known as  
539 nonsense-mediated decay (NMD).

#### 540 **Figure I. Schematic Representation of Different Alternative Splicing Types**

541 **Figure 1. Schematic Representation of the Splicing Process.** Canonical sequences in the  
542 precursor mRNA (pre-mRNA) define the splice sites (5' splice site, branch point,  
543 polypyrimidine tract, and 3' splice site), while additional *cis*-regulatory elements in introns  
544 (ISRs) or exons (ESRs) modulate the recognition of splice sites by the spliceosome. This  
545 multimegadalton complex is composed of five snRNPs, named U1, U2, U4, U5 and U6, and a  
546 range of spliceosome-associated non-snRNP proteins, such as the U2 auxiliary factor (U2AF),  
547 required for the binding of snRNPs to the pre-mRNA. In addition, SR proteins and hnRNPs  
548 regulate the efficiency of splice site recognition, with the former generally binding ESRs to  
549 enhance splicing and the latter antagonizing this effect by binding ISRs and repressing splicing,  
550 though several exceptions to these effects have been reported. Variations in the selection of  
551 splice sites result in the production of different mRNA molecules from the same pre-mRNA  
552 through alternative splicing — in the example shown, an hnRNP recognizing an intronic  
553 silencer sequence inhibits splicing of an intron, leading to its retention in one of the two  
554 alternative transcripts produced. Abbreviations: 5' SS, 5' splice site; BP, branch point; (Y)<sub>n</sub>,  
555 polypyrimidine tract; 3' SS, 3' splice site; ISR, intronic splicing regulator; ESR, exonic splicing  
556 regulator; snRNP, small nuclear ribonucleoprotein; SR, serine/arginine-rich (SR) protein;  
557 hnRNP, heterogeneous ribonucleoprotein.

558 **Figure 2. Interplay Between Alternative Splicing and ABA Signaling.** ABA is a major  
559 mediator of plant responses to abiotic stress. Drought, high salinity and extreme temperatures  
560 all induce osmotic stress in plant cells, triggering ABA biosynthesis. Binding of the hormone to  
561 its intracellular receptors inhibits PP2C phosphatases, thereby derepressing SnRK2 protein  
562 kinases that through phosphorylation activate proteins with different molecular functions, thus  
563 implementing the diverse physiological and developmental responses that allow plants to  
564 withstand environmental stress. An increasing number of splicing factors is being reported to  
565 bind and regulate the processing of mRNAs encoding ABA signaling components. The best-  
566 characterized example of a single alternative splicing event impacting ABA physiological

567 responses is that of the RBM25 splicing factor [24,86], which under abiotic stress is activated  
 568 by ABA and binds the *HABI* PP2C pre-mRNA to ensure it is spliced correctly and generates a  
 569 functional protein. In the absence of ABA and therefore RBM25 activation, the last intron of  
 570 *HABI* is retained, producing a non-functional protein that is unable to transduce the stress  
 571 signal via the ABA pathway. Finally, increased complexity in the interplay between ABA  
 572 signaling (shown in green) and pre-mRNA splicing (shown in pink) is underscored by evidence  
 573 that ABA or related stresses can regulate splicing factor levels and/or activity. This regulation  
 574 includes transcriptional, posttranscriptional and posttranslational mechanisms. Interestingly,  
 575 some data also suggest that central players in the ABA signaling pathway could directly affect  
 576 the activity of splicing factors by regulating their phosphorylation status [50,51]. Abbreviations:  
 577 SF, splicing factor; TF, transcription factor.

578 **Table 1. Splicing Factors Involved In Plant Abiotic Stress Responses**

Splicing factor		Abiotic stress under which <i>in vivo</i> role was reported						References
		ABA	drought	salt	cold	heat	cadmium	
SR proteins	SR45	✓	✗	✗	✗	✗	✗	[46,81]
	SR34b	✗	✗	✗	✗	✗	✓	[48]
	RS40	✓	✗	✓	✗	✗	✗	[55]
	RS41	✓	✗	✓	✗	✗	✗	[55]
GRPs	GRP2	✗	✓	✗	✗	✗	✗	[59]
	GRP7	✗	✓	✓	✓	✗	✗	[57,59]
	RZ-1a	✓	✓	✓	✗	✗	✗	[56]
CBPs	CBP20	✓	✓	✓	✗	✗	✗	[62-64]
	CBP80/ABH1	✓	✓	✓	✗	✗	✗	[61,63]
Spliceosomal components	SKIP	✗	✓	✓	✗	✗	✗	[23]
	SAD1	✓	✓	✓	✗	✗	✗	[68,69]
	LSm4	✓	✗	✓	✗	✗	✗	[70]
	RDM16	✓	✗	✓	✗	✗	✗	[73]
	STA1	✓	✓	✓	✓	✓	✗	[71]
	RBM25	✓	✓	✓	✗	✗	✗	[24,86,92]

579 **Glossary**

580 **Abiotic stress:** detrimental effect of environmental (nonliving) factors — *e.g.* extreme  
 581 temperatures, drought, flooding, toxic compounds — on living organisms such as plants.

582 **Abscisic acid (ABA):** an isoprenoid plant hormone involved in various developmental  
 583 processes — *e.g.* seed maturation and germination, seed and bud dormancy, floral transition —  
 584 and a major player in mediating plant responses to abiotic stress through regulation of stomatal  
 585 closure and induction of the expression of stress response genes.

586 **Alternative splicing:** occurs when splice sites are differentially recognized and multiple  
 587 transcripts are generated from the same pre-mRNA, greatly enhancing the coding capacity of  
 588 the genome and providing a means of regulating gene expression.

589 **Heterogeneous nuclear ribonucleoproteins (hnRNPs):** a large family of structurally diverse  
 590 RNA-binding proteins, usually consisting of several RNA-binding domains connected by linker

591 regions of varying length, involved in multiple aspects of nucleic acid metabolism, such as  
592 alternative splicing, mRNA stability, or transcriptional and translational regulation.

593 **Isoform:** a version of a protein showing a similar but not identical amino acid sequence that,  
594 when originating from the same pre-mRNA, often results from alternative splicing.

595 **Precursor-mRNA (pre-mRNA):** a single strand of messenger RNA (mRNA), produced by  
596 transcription of the genomic DNA, that has yet to be processed or has been processed  
597 incompletely.

598 **Pre-mRNA Splicing:** the stepwise process by which introns are excised from the pre-mRNA  
599 and the exons joined to produce a mature mRNA molecule.

600 **Serine/arginine-rich (SR) proteins:** a conserved family of RNA-binding proteins involved  
601 mainly in pre-mRNA splicing — but also implicated in other posttranscriptional functions, such  
602 as mRNA export, stability or translation — characterized by the presence of one or two N-  
603 terminal RNA-recognition motifs (RRMs) and a C-terminal arginine/serine dipeptide-rich RS  
604 domain involved in protein interactions.

605 **small nuclear ribonucleoproteins (snRNPs):** RNA-protein complexes comprising small  
606 nuclear RNAs (snRNAs) and many nuclear proteins — the five snRNPs that form the  
607 spliceosome, called U1, U2, U4, U5, and U6, are all essential for the removal of introns from  
608 pre-mRNAs.

609 **Spliceosome:** a large and complex molecular apparatus, composed of five snRNPs and  
610 numerous spliceosome-associated proteins, that carries out the splicing reaction.

611 **Osmotic stress:** negative impact of a sudden change in solute concentration, causing a rapid  
612 passage of water or another solvent across a membrane by osmosis, which in living cells can  
613 result in cell lysis (rupture of the plasma membrane).



## 1 Trends

2 Alternative splicing, which generates multiple transcripts and potentially more than one  
3 protein from the same gene, is markedly changed by environmental stresses that  
4 negatively impact plant growth and development.

5 Plant stress-related genes are particularly prone to alternative splicing events, which often  
6 modulate the ratio between active and non-active isoforms in response to abiotic stress,  
7 thus fine-tuning the expression of key stress regulators.

8 Recent genetic and transcriptomic analyses identified important roles for numerous  
9 splicing factors in the control of plant abiotic stress responses.

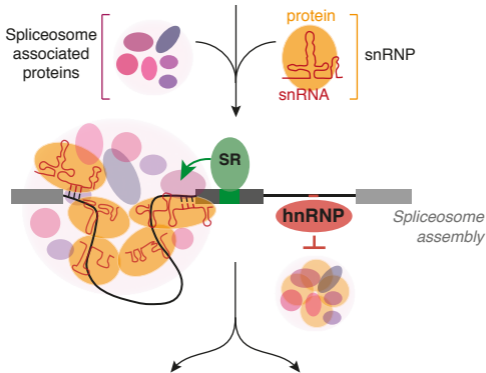
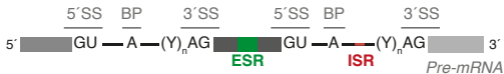
10 Emerging evidence indicates that splicing factors modulate stress responses by targeting  
11 components of the ABA pathway, unveiling a novel regulatory layer in plant stress  
12 tolerance.

## 1 Outstanding Questions

2 Which are the *bona fide* physiological targets of splicing factors under abiotic stress?  
3 And which RNA consensus sequences do they bind? Though challenging, the  
4 optimization of techniques such as CLIP or improvements of this protocol in plant  
5 systems holds much promise for the identification of the binding motifs and mRNAs  
6 targeted directly by splicing factors to regulate plant abiotic stress tolerance.

7 How do splicing factors integrate environmental signals to regulate alternative splicing?  
8 While large-scale transcriptomic data revealing marked splicing changes in response to  
9 stress are accumulating at a fast pace, the upstream regulatory mechanisms dictating the  
10 activity of splicing regulators under stress conditions remain elusive. Unraveling these  
11 mechanisms will require in-depth studies of posttranslational modifications, particularly  
12 phosphorylation, and of the splicing factor protein interactors essential to their function.

13 How and to what extent does alternative splicing control ABA signaling during the  
14 response to abiotic stress? What are the components of the ABA pathway targeted by  
15 splicing regulators? Is the activity of these splicing factors controlled in an ABA-  
16 dependent manner? Answering these questions will provide crucial insight into how  
17 alternative splicing modulates plant stress responses and contribute new approaches to  
18 improve crop tolerance under environmental stress.



### Constitutive splicing



### Intron retention



### Exon skipping



### Alternative 5' splice sites



### Alternative 3' splice sites



Salt Heat  
Drought Cold

osmotic sensor

ABA receptor

ABA

PP2C

P

SnRK2  
2/3/6

P

P

P

TF

enzyme

ion channel

Stress tolerance

SF

Splicing pattern

ABA → RBM25

HAB1

HAB1

HAB1

Not functional

snRK2

SF

Splicing pattern

Gene expression

SF

Splicing pattern

SF SF

Protein levels or activity

SF P

Splicing pattern