Insights into the role of alternative splicing in plant temperature response

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**Highlight:**

Temperature-dependent alternative splicing is a fast-expanding field, providing new insights into plant temperature acclimation processes. Here we highlight its complexity and outline the necessity for further research.
Abstract

Alternative splicing occurs in all eukaryotic organisms. Since the first description of multiexon genes and the splicing machinery, the field has expanded rapidly, especially in animals and yeast. However, our knowledge about splicing in plants is still quite fragmented. Though eukaryotes show some similarity in the composition and dynamics of the splicing machinery, observations of unique plant traits are only starting to emerge. For instance, plant alternative splicing is closely linked to their ability to perceive various environmental stimuli. Due to their sessile lifestyle, temperature is a central source of information allowing plants to adjust their development to match current growth conditions. Hence, seasonal temperature fluctuations and day-night cycles can strongly influence plant morphology across developmental stages. Here we discuss the available data about temperature-dependent alternative splicing in plants. Given its fragmented state it is not always possible to fit specific observations into a coherent picture, yet it is sufficient to estimate the complexity of this field and the need of further research. Better understanding of alternative splicing as a part of plant temperature response and adaptation may also prove to be a powerful tool for both, fundamental and applied sciences.

Keywords: alternative splicing, Arabidopsis thaliana, cold acclimation, heat acclimation, splicing factor, temperature adaptation, temperature response
List of Abbreviations

*A. thaliana*  *Arabidopsis thaliana*

ABA  Abscisic Acid

AS  Alternative Splicing

IR  Intron retention

NMD  Nonsense mediated decay

P-bodies  processing bodies

pre-mRNA  precursor mRNA

PTC  Premature termination codon

PTM  Postranscriptional modification

SF  Splicing factor

SMN  Survival of Motor Neuron

snRNA  Small nuclear RNA

snRNP  Small nuclear ribonucleoproteins

SR protein  Serine/Arginine rich protein

SS  Splice site
Eukaryotic splicing in a nutshell

The central dogma of molecular biology states that information about the cell is encoded in DNA and unidirectionally transferred to proteins via mRNA. It is also well known that the diversity of proteins in eukaryote cells significantly exceeds the number of genes and does not correlate with organism complexity (van Straalen et al., 2013). This imbalance was explained in the end of 1970s with the discovery of the multiexon structure of genes in eukaryotes and the splicing process in which non-coding sections of the newly transcribed pre-mRNA, referred to as introns, are excised, and the coding parts, called exons, are joined together to form the mature mRNA (Chow et al., 1977; Berget, Moore, and Sharp, 1977; Gilbert, 1978). Today, these definitions are more generalized to also account for the exon-intron structure of non-coding RNA transcripts (Lodish et al., 2000; Fu, 2014; Yang, 2015). However, here we only address the splicing of protein coding RNA transcripts. This intricate reaction is carried out by a multimeric protein-RNA complex called the spliceosome (Shi, 2017).

Constitutive splicing always gives rise to a defined mRNA because exons are ligated in a uniform manner. However, constitutive splicing per se does not explain how the diversity of the proteome exceeds the number of genes contained within the genome. This apparent discrepancy can be explained by the alternative splicing (AS) process (Maki et al., 1981; Kelemen et al., 2013), in which the inclusion of exons and introns or even their boundaries in the resulting mRNA can be altered. Thus, AS gives rise to different mRNA transcript variants that originate from the same pre-mRNA.

AS is a process common for eukaryotes. It can be traced back to the last eukaryotic common ancestor and is likely to have evolved from self-catalytic group II introns after the endosymbiotic event (Irimia and Roy, 2014; Vosseberg and Snel, 2017).

There are two spliceosomal complexes found and described in eukaryotes: the major (U2) spliceosome, and the less abundant, or minor (U12) spliceosome (Kreivi and Lamond, 1996; Tarn and
Steitz, 1996; Sharp and Burge, 1997). The main structural difference is in the small nuclear RNAs (snRNAs) incorporated in their core, while the protein machinery is to a large extent identical between the two (Hall and Padgett, 1996; Patel and Steitz, 2003; López et al., 2008). From now on we will refer only to U2-mediated splicing and discuss the function and regulation of the major spliceosome.

The spliceosome is a dynamic ribonucleoprotein machine of high structural complexity (Wahl et al., 2009). It is composed of five core subcomplexes called the U1, U2, U4, U5, and U6 small ribonucleoproteins (snRNPs) as well as a huge number of other, more dynamic proteins associated with them, all of which are referred to as splicing factors (SFs) (Jurica and Moore, 2003; Wahl et al., 2009; Chen and Cheng, 2012). Each U snRNP is built around its corresponding snRNA and a conserved ring-shaped heteroheptamer complex composed of the SM-like family proteins (Lerner and Steitz, 1979; Veretnik et al., 2009). U1, U2, U4 and U5 snRNPs carry SM proteins (B/B’, D1, D2, D3, E, F and G), while the core of the U6 snRNP contains SM-LIKE (LSM2 to LSM8) proteins. These structures are referred to as the SM-ring and the LSM2-8-ring, respectively.

Eukaryotic splicing is defined by the presence of specific splicing signals in the multiexon gene structure. For the U2-type introns these are flanked by the 5’ splice site (SS) and 3’ SS, that are characterized by conserved (but not invariant) GU and AG sequences, respectively. Upstream of the 3’ SS are the polypyrimidine tract and the adenosine branch site (Schwartz et al., 2008; Baralle and Baralle, 2018).
snRNP assembly and splicing reaction

The assembly of the U1, U2, U4 and U5 snRNPs in eukaryotes begins in the cytosol and requires multiple factors (Fig. 1A) (Matera and Wang, 2014). First, snRNAs bind to the Survival of Motor Neuron (SMN) and GEM NUCLEAR ORGANELLE ASSOCIATED PROTEIN (GEMIN) proteins, which together form the SMN complex (Shpargel and Matera, 2005). Assembly of the SM-ring around the snRNA happens stepwise via formation of the smaller oligomers SmD1/D2, SmE/F/G and SmB/D3 which are delivered with the assistance of chaperone proteins to the snRNA where their simultaneous binding to the SM-site of the snRNA is initiated (Raker et al., 1996; Yong et al., 2004; Chari et al., 2008; Grimm et al., 2013). To increase their affinity to the SMN complex and ensure proper ring formation the SmB/B’, SmD1 and SmD3 proteins need to be methylated beforehand by the PROTEIN ARGinine METHYltransferases (PRMTs) complex, also known as the methylosome (Brahms et al., 2001; Friesen et al., 2001; Gonsalvez et al., 2007).

This step is crucial for the assembly process, since the ring stabilizes the snRNA and initiates its 5’ cap hypermethylation by TRIMETHYLGUANOSINE SYNTHASE 1 (TGS1) (Plessel et al., 1994; Mouaikel et al., 2002). The later step is required for the active translocation of the snRNA-SM-ring subcomplex into the nucleus with the assistance of SMN, IMPORTIN β and SNURPORTIN1 (Palacios et al., 1997; Huber et al., 1998; Massenet et al., 2002). The final steps of assembly and maturation happen in the Cajal bodies (Jady et al., 2003). The actual splicing reaction has been shown to take place in the nucleus (Fig. 1B). More specifically, splicing has been suggested to occur in subnuclear regions, including non-membrane compartments called nuclear speckles (Lamond and Spector, 2003). It has been reported that in human cells 80% of splicing occurs co-transcriptionally and that active spliceosomes localize to the periphery or within nuclear speckles (Girard et al., 2012). In line with this observation, nuclear speckles have been reported to be enriched in Ser/Arg-rich (SR) proteins and snRNPs and are thus considered to act as a source of SFs for the splicing process (Lamond and
Spector, 2003; Hasenson and Shav-Tal, 2020). In addition, a recent model in humans proposed that upon maturation the snRNPs are translocated from Cajal bodies to the interface of nuclear speckles (Liao and Regev, 2021). However, it is important to note that splicing does not necessarily have to occur within or in close contact with nuclear speckles but can also occur in other sub-nuclear domains (Han et al., 2011).

In contrast to the assembly of U1, U2, U4 and U5 snRNPs, all steps of U6 snRNP formation happen in the nucleus (Patel and Bellini, 2008). Interestingly, the U6 snRNP is also the only snRNP which reassembles all associated proteins after each round of splicing (Didychuk et al., 2018). Though the biological significance of this feature is not clear, possibly it confers the U6 snRNP with a great amount of functional and regulatory flexibility.

The activation and catalysis of the splicing reaction are largely mediated by the NineTeen complex also called pre-mRNA processing (PRP) 19 in humans and yeast or MOS4-associated complex in Arabidopsis thaliana (A. thaliana) (Johnson et al., 2011; Chanarat and Sträßer, 2013). Binding of the NineTeen complex to the snRNP and pre-mRNA is pivotal since it induces necessary conformational changes during the splicing reaction and ensures correct spliceosome turnover (de Almeida and O’Keefe, 2015).

Additional proteins central in the assembly and function of the final splicing machinery include SRs and heterogeneous nuclear RNPs (hnRNPs). It has been shown that their binding to signal sequences on the pre-mRNA can promote or suppress SS choice (Long and Caceres, 2009; Huelga et al., 2012; Erkelenz et al., 2013; Sahebi et al., 2016). The activity and subcellular localization of SRs and hnRNPs can be strongly influenced by posttranscriptional modifications (PTMs) (Chaudhury et al., 2010; Twyffels et al., 2011; Zhou and Fu, 2013; Xu et al., 2019). For more detailed information on splicing see Box 1.
Even though the players of constitutive and alternative splicing are essentially the same, they take on different roles in the organism. While constitutive splicing ensures the production of one protein that performs its functions in given conditions, AS adjusts the proteome to the current needs of the organism (Reddy et al., 2013; Fiszbein and Kornblihtt, 2017), acting as a relay that redirects primary transcripts into two major pathways: either to protein synthesis in the cytoplasm or, if the spliced mRNA carries a premature termination codon (PTC), towards nonsense-mediated decay (NMD) (Kervestin and Jacobson, 2012). The result of the first AS pathway is proteome expansion and functional diversification by creation of distinct protein isoforms, while the NMD pathway, on the other hand, is considered a way to control gene expression and remove nonsense mRNA transcripts (Lewis et al., 2003). An essential part of the NMD pathway is the LSM1-7 ring which is almost identical in composition to the LSM2-8 ring. However, the minor substitution of LSM8 by LSM1 localizes the LSM1-7 ring to the cytoplasm where it functions in NMD (Montemayor et al., 2020).

The seemingly simple AS-mediated redistribution of the newly transcribed pre-mRNAs in response to various stimuli is complicated by various up- and downstream regulatory pathways. Among them are DNA modifications, epigenetic marks, PTMs of the protein isoforms, and the presence or absence of potential interacting partners, etc. (Buccitelli and Selbach, 2020). In addition, transcription elongation rate is modulated by environmental factors and can affect splice site accessibility and usage when AS occurs co-transcriptionally (Herzel et al., 2017; Godoy Herz and Kornblihtt, 2019; Godoy Herz et al., 2019). Emerging details about the crosstalk between histone modifications and the regulation of transcription kinetics to modulate AS illustrates how well coordinated all these processes are (Luco et al., 2011; Naftelberg et al., 2015).

Additional feedback loops in AS itself add another layer of complexity to this process. For example, it is well-known that SFs undergo AS themselves (Lareau and Brenner, 2015; Preußner et al., 2017) and there are indications that transcripts directed towards NMD may play a role in their own NMD regulation (Feng et al., 2015b).
Plant specific traits of alternative splicing

While the mechanisms underlying AS are largely conserved among eukaryotes, specific adjustments can be found among different phyla (McGuire et al., 2008; Frey and Pucker, 2020). The main model systems for studying splicing are yeast (Saccharomyces cerevisiae) and human cell cultures. Comparisons between their splicing machinery showed an overlap of around 80 proteins (Wahl et al., 2009) and the majority of yeast SFs (~85%) are conserved in humans (Fabrizio et al., 2009). The human spliceosome includes approximately one hundred SFs not detected in yeast, which suggests that metazoan splicing is a more complicated process.

Comparisons of human and A. thaliana genomes revealed that core SFs are conserved in both, which suggests that the basic processes are similar between animals and plants (Reddy et al., 2013). Unfortunately, very few structural studies on plant spliceosomal components and assembly have been reported to date and our current knowledge is to a large extent based on comparative bioinformatic analyses (Reddy et al., 2012b; Szczęśniak et al., 2013; Chaudhary et al., 2019; Ling et al., 2019).

There are several plant specific traits that shaped the evolution and properties of AS. Among them is the tendency towards polyploidization (Wendel, 2015), which drastically expanded the pool of AS-regulated genes and led to the diversification of AS events among plant species. This also at least partially explains the low level of AS conservation found between plant phyla (Severing et al., 2009; Mei et al., 2017). Analyses of the A. thaliana genome suggest that, as a consequence of genome duplication events specific to the green lineage, it has approximately double the amount of spliceosome proteins found in humans (Wang and Brendel, 2004; Reddy et al., 2013). For example, it is assumed that plants possess the highest diversity of SR proteins among all eukaryotes (Morton et al., 2019). Some authors even speculate that several SR proteins evolved as an adaptation to land...
colonization by early plant species (Melo et al., 2020). At the same time, certain central actors of the splicing reaction which are conserved in animals, seem to be missing in plants. For instance, only GEMIN2 and PRMT5, two homologues of individual components of the multimeric SMN and methylosome complexes, respectively, were described in A. thaliana (Pei et al., 2007; Deng et al., 2010; Schlaen et al., 2015). It remains to be elucidated if the remaining components have not yet been identified in plants or are in fact completely missing.

Multiple studies agree that the prevailing type of AS in flowering plants is intron retention (IR) (Marquez et al., 2012; Chamala et al., 2015; Mei et al., 2017; Song et al., 2019) which contrasts with humans where exon skipping is more common (Sammeth et al., 2008). This difference is considered an outcome of divergent evolution of AS in the animal and plant lineages and reflect their differing developmental strategies (Barbosa-Morais et al., 2012; Ling et al., 2019). While exon skipping events result in proteome expansion during the differentiation processes in human organ development (Wang et al., 2008; Merkin et al., 2012), IR often introduces PTCs and directs transcripts towards NMD (Kervestin and Jacobson, 2012). It has been proposed that, by the removal of truncated transcripts, plants avoid unnecessary energy losses in stress conditions (Chaudhary et al., 2019). On the other hand, it has been shown that IR can induce transcript retention in the nucleus, providing a molecular mechanism to escape NMD and delay the splicing of stored transcripts. This specific mechanism of post-transcriptional splicing has been suggested to be involved in the rapid response to environmental stresses (Jia et al., 2020). However, a recent study reevaluates the contribution of IR to the regulation of A. thaliana development and suggest that other types of AS can take a lead role in plant development and stress response (Martin et al., 2021).
Alternative splicing in plant temperature response

Plants are constantly exposed to environmental perturbations and experience diverse biotic and abiotic stresses (Lamers et al., 2020). Interestingly, genes involved in stress responses have been shown to be more likely to undergo AS (Laloum et al., 2018). Hence, it has been suggested that AS in extant plants may be an outcome of their adaptation to their new environment upon land colonization (Mastrangelo et al., 2012).

Since plants exhibit only a limited capacity of thermoregulation, one of the most important environmental stimuli for them is temperature. For the plant model species A. thaliana, temperatures above 27 °C and below 16 °C are often considered sub-optimal as they can induce symptoms of heat shock and cold stress, respectively (Quint et al., 2016; Guo et al., 2018; Hayes et al., 2021). For more detailed information on temperature perception and signaling see Box 2.

Deviations from the temperature optimum have severe effects on plant growth, development, and health (Eremina et al., 2016; Dai Vu et al., 2019; Wang et al., 2020). Therefore, it is not surprising that temperature responses in plants involve systemic changes, including chromatin modifications (Bäurle and Trindade, 2020) and metabolic adjustments (Yang et al., 2018; Fürtauer et al., 2019; Serrano et al., 2019). Such adjustments not only improve plant fitness but also create a stress memory, a process referred to as thermopriming (Serrano et al., 2019; Leuendorf et al., 2020). Interestingly, it has been shown that thermopriming not only changes chromatin marks and metabolic programs but also affects AS (Ling et al., 2018; Sanyal et al., 2018), which can in part be attributed to the modulation of RNA polymerase II activity by temperature (Kindgren et al., 2020). In addition, several transcriptome analyses of plant temperature acclimation and stress tolerance show an enrichment of functional categories linked to RNA processing (Calixto et al., 2018; Kannan et al., 2018; Walden et al., 2019). The specific role of the spliceosomal complexes and their components in
temperature sensing and response modulation, however, is only starting to emerge. Therefore, and due to space limitations, in the following sections we will focus on the core splicing machinery and closely associated splicing factors.

**LSM5 is necessary for heat stress tolerance**

LSM5, also known as SUPERSENSITIVE TO ABA AND DROUGHT1 (Xiong et al., 2001), is an integral part of the LSM1-7 and LSM2-8 rings and participates in the regulation of RNA degradation and splicing, respectively. Despite the large number of SM and LSM proteins, so far only LSM5 has been shown to modulate AS in elevated temperatures.

The lsm5 mutant displays a dwarfed phenotype with round leaves under control conditions and a higher sensitivity to ABA and NaCl treatments compared to wildtype plants (Xiong et al., 2001). A more recent study has shown that LSM5 is also pivotal for heat stress tolerance in *A. thaliana*, and that after exposure to elevated temperatures it localizes to cytoplasmic processing bodies (P-bodies) (Okamoto et al., 2016), which play a role in mRNA degradation (Maldonado-Bonilla, 2014). lsm5 mutants display increased tissue bleaching in elevated temperatures, presumably due to the mis-splicing of two heat stress components, *HEAT SHOCK TRANSCRIPTION FACTOR3A* (*HSFA3*) and *AT1G72416*, a chaperone DnaJ-domain superfamily protein (Okamoto et al., 2016). Supporting this observation, the expression of *AT1G72416* has been shown to be regulated by the hnRNP-like protein OLGOURIDYLATE-BINDING PROTEIN 1B which is involved in heat shock response (Nguyen et al., 2016).

An in-depth transcriptome analysis has shown that LSM5 plays an essential role in SS selection. Its loss leads to an increase in AS events and a higher abundance of transcripts with IR as compared to wildtype plants, while overexpression of *LSM5* enhances SS choice, hence impeding AS (Cui et al., 2014). Interestingly, under salt stress conditions, mis-spliced genes, such as *CALCIUM EXCHANGER1,*
and RARE-COLD-INDUCIBLE 2A (RCI2A), could be largely associated to the Gene Ontology terms related to ABA signaling, salt stress and response to cold temperature (Cui et al., 2014). In addition CALCIUM EXCHANGER1 and RCI2A have been shown to play an important role in drought stress response and cold acclimation (Nylander et al., 2001; Catalá et al., 2003). Furthermore, LSM5 also regulates the splicing of A. thaliana clock genes, including TIMING OF CAB EXPRESSION1 (Perez-Santángelo et al., 2014), which undergoes AS under low temperatures (James et al., 2012). Taken together, these results suggest that LSM5 plays a central role in plant abiotic stress response, especially in salt and heat tolerance. Future analysis will show if, and to which extent, LSM5 may also contribute to the cold acclimation of plants.

It is worthwhile to note that LSM3A, LSM3B and LSM4, which like LSM5 are components of both the LSM1-7 and the LSM2-8 rings, have been implicated in the regulation of RNA degradation under heat stress conditions (Perea-Resa et al., 2012). However, their role in temperature-dependent AS remains to be established.

**SR and hnRNP localization affect splicing**

SR proteins and hnRNPs are known regulators of abiotic stress responses and are presumed to modulate the splicing process (Laloum et al., 2018). Interestingly, this regulation depends on the AS of the transcripts themselves (Palusa et al., 2007; James et al., 2018). In A. thaliana it was shown that the amount of differentially expressed and alternatively spliced SR isoforms by far exceeds the actual number of SR genes, which can be largely explained by the effects of temperature (Palusa et al., 2007; Pajoro et al., 2017; Ling et al., 2018; Neumann et al., 2020). Furthermore, it was shown that hnRNP subcellular localization is modulated by temperature (Fig. 2 and Table 1) (Weber et al., 2008; Nguyen et al., 2016).
The most comprehensive data to exemplify the importance and complexity of temperature-dependent regulation of SR proteins is available for SR45. As stated before, the localization of SR proteins to specific regions in the nucleus is strongly linked to their availability for the splicing reaction. Interestingly, there are indications that the transition of SR45 to different nuclear compartments is temperature-dependent. For example, at control conditions, SR45 is evenly distributed in the nucleoplasm and in nuclear speckles. However, high temperatures cause SR45 to predominantly localize to irregularly shaped nuclear speckles, and cold temperatures induce a complete localization to the nucleoplasm (Ali et al., 2003). In general, this translocation is based on the phosphorylation status of the SR proteins. It is currently hypothesized, that nuclear speckles accumulate hypophosphorylated SR proteins, which are inactive and unavailable for the splicing reaction (Reddy et al., 2012a). Thus, it is intriguing to draw a link between the availability of SR45 for splicing reactions and temperature fluctuations. The fact that SR45 RNA binding is enriched in genes associated with cold signaling (Xing et al., 2015) further supports this hypothesis. Moreover, it has been suggested that SR45’s translocation correlates with temperature fluctuations and affects the alternative splicing of the circadian rhythm component CIRCADIAN CLOCK ASSOCIATED1 (Filichkin et al., 2015). In response to temperature stress, the isoform of CIRCADIAN CLOCK ASSOCIATED1 with a retained 4th intron is accumulated in the nucleus, and since SR45 specifically recognizes this intron, it has been argued that such binding could induce pre-mRNA sequestration and splicing delay (Filichkin et al., 2015).

Though the current knowledge on the regulation of SR proteins is based on temperature fluctuations, there is some data about the specific modulation of SR proteins and hnRNP activity by cold stress. SR proteins, such as RSZ22, SCL30 and RS40, are crucial for cold acclimation and acquisition of freezing tolerance, hypothetically due to their interaction with the cold induced LAMMER KINASE AME3, the loss of which, intriguingly, leads to cold sensitivity (Rosembert, 2017).
It remains to be clarified, however, to what extent temperature cues impact the dynamics of individual SR proteins and hnRNPs, as well as their splice isoforms, through protein modifications. Approaching this scientific question, a detailed proteomic study on grape vines under heat stress was conducted and an overall tendency of increased SR protein phosphorylation was identified (Liu et al., 2019). More studies like the aforementioned one will most likely further corroborate the biological significance of the temperature specific modifications of these proteins.

**STA1 ensures appropriate and rapid heat shock response**

The previous sections have shown that the protein composition of the core spliceosome and other SFs, such as SR proteins are essential to the modulation of plant stress responses. Another layer of complexity is added to this through the binding of different accessory proteins to the snRNPs.

STABILIZED1 (STA1) is the plant homolog of yeast PRP6, which, among other functions, aids in U4/U6.U5 tri-snRNP assembly as well as activation of the spliceosome (Lee et al., 2006; Ben Chaabane et al., 2013; Dou et al., 2013; Bertram et al., 2017). Interestingly, the expression of STA1 is induced by both low and elevated ambient temperatures (Lee et al., 2006; Yu et al., 2016; Kim et al., 2017). In several heat treatments with different setups, sta1-1 mutants showed sensitivity to high temperature stress, depicting a dwarfed phenotype, shoot bleaching and overall increased lethality rates (Kim et al., 2017, 2018). The observed developmental defects could be connected to the mis-splicing of HSFA3 which was, presumably, not the only affected heat shock factor. Based on these results it was suggested that STA1 is an important factor in the establishment of heat stress tolerance in A. thaliana and is necessary for the high temperature induced splicing response (Kim et al., 2017, 2018). Though this indicates that STA1 is a putative component of the heat shock response, it was shown that some HSPs are spliced independently of STA1 (Kim et al., 2018). In the future it should be deciphered which other SFs are necessary for the splicing of heat responsive
genes in the absence of STA1. Furthermore, it is an interesting observation that both heat sensitive splicing mutants, *lsm5* and *sta1-1*, converge in their splicing defects of *HSFA3*, which could indicate a joint regulation of high temperature-dependent AS.

**GEMIN2 stabilizes the circadian clock in cold stress**

As described in the introduction, the full composition of the plant SMN complex remains yet to be identified. The splicing of GEMIN2, the only known component of the assembly complex in *A. thaliana* (Schlaen *et al.*, 2015), is temperature controlled: In control conditions a truncated isoform is produced, which is directed towards NMD, while the functional transcript is preferentially produced at cold temperatures (Neumann *et al.*, 2020). This is in line with the observation that *GEMIN2* expression is induced by cold ambient temperatures (Schlaen *et al.*, 2015). GEMIN2 has also been implicated with the regulation of the circadian clock: When the effects of temperature on the circadian rhythm in *gemin2* and wildtype plants were compared, it was shown that the reaction of the circadian clock to cold temperature is strongly enhanced in the mutant (Schlaen *et al.*, 2015). In wildtype plants, the circadian clock output is stably maintained despite temperature fluctuations, a process which is termed temperature compensation (Gil and Park, 2019). Thus, the obtained results indicate that GEMIN2 is necessary for cold temperature compensation, likely through the AS of core clock genes, like *TIMING OF CAB EXPRESSION1* or *PSEUDO-RESPONSE REGULATOR9 (PRR9)*. Furthermore, *gemin2* mutants are severely sensitive to cold ambient temperatures with increased lethality rates at 10 °C in comparison to wildtype (Schlaen *et al.*, 2015).

Interestingly, the transcriptome of wildtype plants grown in 10 °C overlaps strongly with the *gemin2-1* transcriptome under control conditions (Schlaen *et al.*, 2015). This raises the question of whether there are further regulatory elements controlling the GEMIN2-mediated cold response which are thus far unknown.
Temperature regulates the methylation of splicing proteins

A second essential component for correct snRNP assembly is the methylosome. So far, the only identified component of the methylosome in plants is PRMT5 (Pei et al., 2007), also referred to as SHK1 BINDING PROTEIN 1 or CALCIUM UNDERACCUMULATION 1.

PRMT5 plays an important role in several cellular processes, including the control of splicing under different environmental conditions (Sanchez et al., 2010; Ueda and Seki, 2020). For instance, it has been shown that PRMT5 regulates the splicing of PRR9 (Sanchez et al., 2010), which might constitute a possible link between GEMIN2 and PRMT5, to jointly regulate the splicing of core clock genes.

There are several indications that PRMT5 may affect processes involved in thermotolerance (Fu et al., 2013, 2018), for example calcium signaling and proline accumulation (Szabados and Savouré, 2010; Szepesi and Szőllősi, 2018; Yuan et al., 2018). For instance it has been shown that PRMT5 regulates the expression of CALCIUM SENSING RECEPTOR, which is important for drought tolerance (Fu et al., 2013) and presumably also cold temperature tolerance in Thellungiella (Wong et al., 2006). PRMT5 also negatively regulates the expression of the A. thaliana transcription factor ANAC055 under drought stress (Fu et al., 2018). ANAC055 is necessary for the induction of P5CS1 (Fu et al., 2018), one of two genes coding for the proline biosynthetic enzyme P5CS, which is strongly induced under cold stress in A. thaliana (Ren et al., 2018). Intriguingly, it has been shown that the AS of P5CS1 is mostly associated with diurnal temperature variations (Kesari et al., 2012), further stressing on the importance of its regulation by temperature-dependent AS.

Furthermore, PRMT5 has been proven to play a significant role in flowering time control: prmt5 mutants are late flowering, due to reduced histone methylation at the FLOWERING LOCUS C (FLC) promoter, resulting in its upregulation (Pei et al., 2007; Wang et al., 2007; Schmitz et al., 2008; Hernando et al., 2015). Even after vernalization, the epigenetic silencing of FLC is not stable in the
*prmt5* mutant, further indicating the importance of PRMT5 for temperature-dependent developmental transitions (Schmitz *et al.*, 2008).

Intriguingly, under salt stress PRMT5 dissociates from the *FLC* locus, thus allowing its expression, and instead mediates the methylation of LSM4, constituting a link between transcription control and RNA processing. Curiously, the *lsm4* mutant, much like the *prmt5* mutant, is salt sensitive (Zhang *et al.*, 2011).

Another putative component of the plant methylosome, AtICln, has been reported, based on the findings of a chemical genetics suppressor screen of *chilling sensitive 3* (*chs3*). In this screen, a potent suppressor molecule of the *chs3* phenotype was identified. The cold sensitive phenotype was restored by the loss of *aticln* in the *chs3* background (Huang *et al.*, 2016b). Since AtICln is the homologue of the human pICln, a known component of the methylosome, its interaction with the plant counterparts of this complex was tested. The result of a co-immunoprecipitation assay showed that AtICln forms a complex with PRMT5 and SmD3b, thus providing an indirect indication of its association to the methylosome (Huang *et al.*, 2016b).

Furthermore, a more recent study has shown that AtICln is important for plant tolerance to osmotic stress, in addition to also being central to nitrate ion accumulation (Chu *et al.*, 2021). According to the observations of both studies, AtICln subcellular localization and the molecular function seem to diverge depending on the environmental conditions (Huang *et al.*, 2016b; Chu *et al.*, 2021), however further research and experimental data is required to further clarify the function of *AtICln* with the hopes of confirming its role in the plant methylosome.

As explained in the introduction, snRNP assembly is initiated in the cytosol before the complex is transported to the nucleus where it performs its function (Fig. 1). This import process depends on snRNA 5’ cap hypermethylation by TGS1 and presumably on nuclear import by AtKPNB1, the homologue of the human *IMPORTIN SUBUNIT BETA-1* (Ohtani, 2018). Interestingly, TGS1 and
AtKPBN1 have been reported to play a role in cold tolerance, and response to ABA and drought tolerance, respectively (Luo et al., 2013; Gao et al., 2017). Together these results suggest that the nuclear import of the snRNPs may be affected by temperature, thus indicating a putative additional layer of regulation.

**SKIP regulates the splicing of clock genes**

As described above, the MOS4-associated complex plays a central role in the splicing reaction. It encompasses a large number of proteins which are also implicated in other biological processes, however, due to the lack of data, it is difficult to differentiate the functions of the complex from those of its separate protein parts.

MAC3A and MAC3B (MOS4-associated complex 3A/B) are functionally redundant plant proteins which have so far mostly been studied for their role in miRNA biogenesis and plant immunity (Monaghan et al., 2009; Li et al., 2018). They co-immunoprecipitate with both, SmB, and the evolutionary conserved SNW/SKI INTERACTING PROTEIN (SKIP) that is associated to the spliceosome through its interaction with SR45 (Wang et al., 2012; Li et al., 2016, 2019; Cao and Ma, 2019).

SKIP appears to have retained its function as a temperature regulator in various organisms. In rice it has been shown that SKIP is induced by cold ambient temperatures, drought, and high salinity (Hou et al., 2009) whereas in yeast, the loss of the SKIP homolog PRP45 causes a high temperature-sensitive phenotype (Gahura et al., 2009). In A. thaliana, SKIP was shown to play a role in the temperature-dependent regulation of the circadian rhythm, as well as tolerance to salt and drought stress (Wang et al., 2012; Feng et al., 2015a). The observed salt and drought sensitivity of SKIP mutants was linked to the mis-splicing of several salt-stress responsive genes, including CALCINEURIN B–like, P5CS1 and RC12A (Feng et al., 2015a), which have been implicated in cold and freezing tolerance (Medina et al., 2001; Cheong et al., 2003; Sivankalyani et al., 2015; Ren et al.,...
Intriguingly, the expression of P5CS1 is also regulated by PRMT5, and RC12A is mis-spliced in the Ism5 mutant, suggesting that these genes are strongly controlled by temperature-dependent AS.

Interestingly, skip, mac3a and mac3b all exhibit a period lengthening phenotype which could potentially be explained by the mis-splicing of the clock genes PRR7 and PRR9 (Li et al., 2019), which are essential for the temperature compensation of the circadian clock (Salomé and McClung, 2005; Calixto et al., 2016). Additionally, the skip-1 allele displays an early flowering phenotype under different photoperiod and temperature conditions (Wang et al., 2012). This is presumably caused by a reduced expression of SERRATED LEAVES AND EARLY FLOWERING, an important component of chromatin remodeling (March-Díaz et al., 2007; Cui et al., 2017), which would result in fewer activating histone marks being deposited at the FLC locus (Cui et al., 2017).

SM-like family proteins assure appropriate cold stress response

As explained in the introduction, SM- and LSM proteins are core components of the spliceosome and are crucial for snRNP assembly and stability. Recently, the SmE1 protein, also referred to as PORCUPINE, has been implicated in cold temperature splicing (Capovilla et al., 2018; Huertas et al., 2019). Upon exposure to cold ambient temperatures, SME1 expression is induced, and the protein localizes to the nucleus (Huertas et al., 2019). The production of a functional SmE1 protein under cold temperatures is required for appropriate U1, U2, U4 and U5 snRNA accumulation (Huertas et al., 2019), and the loss of SME1 causes severe developmental defects at low ambient temperatures (Capovilla et al., 2018; Huertas et al., 2019). Interestingly, it seems that minimal deviations from the optimal growth temperature are sufficient to trigger a SmE1-dependent temperature response: While at 23 °C, the sme1 mutants look essentially like wildtype plants (Capovilla et al., 2018), at 20 °C the mutants develop smaller rosettes, twisted leaves, shorter roots and are early flowering (Huertas et al., 2019). The early flowering phenotype may be associated with the accumulation of
the class I isoform of COOLAIR, a long non-coding antisense RNA involved in FLC transcription (Huertas et al., 2019).

The SME1 homologue in two tomato cultivars is induced by cold temperatures (Chechanovsky et al., 2019). Likewise, the mutation of the yeast SME causes high temperature-sensitivity, and impairs the accumulation of U4 and U5 snRNAs (Camasses et al., 1998), indicating the evolutionary conservation of SmE1 function in temperature response.

Recently it has been shown that the LSM complex, and more specifically LSM8, plays an important role in plant cold acclimation and salt stress tolerance by maintaining splicing accuracy of specific stress responsive genes (Carrasco-López et al., 2017). The expression of LSM8 is induced by exposure to cold ambient temperatures, and its mutation causes a cold sensitive phenotype, with splicing defects enriched in IR (Carrasco-López et al., 2017). Moreover, the GC content and length of LSM8-targeted introns differ greatly between abiotic stress conditions (Carrasco-López et al., 2017; Catalá et al., 2019). A current hypothesis is that GC content and intron length could alter the secondary RNA structure, this way determining splice site accessibility after cold acclimation (Carrasco-López et al., 2017; Catalá et al., 2019).

Recently it was described that the function of LSM8 depends on its interaction with PREFOLDIN4 (PFD4), a putative adaptor between LSM8 and other proteins (Esteve-Bruna et al., 2020). Intriguingly, cold temperatures induce the expression of PFD4 as well as its localization to the nucleus, where it attenuates the accumulation of ELONGATED HYPOCOTYL5 (Perea-Resa et al., 2017), a key regulator of plant cold acclimation (Catalá et al., 2011). Furthermore, it was found that PFD4 is essential for functional splicing in cold temperatures, but not in control conditions, and that the splicing defects in pfd4 overlap with those found in lsm8-1 mutants (Esteve-Bruna et al., 2020).

Curiously, however, the loss of all of the above-mentioned factors induces increased freezing tolerance after cold acclimation (Perea-Resa et al., 2017; Carrasco-López et al., 2017; Huertas et al.,
Taken together this suggests that these proteins negatively regulate the plant cold acclimation response, and that at least PFD4 and LSM8 directly interact (Esteve-Bruna et al., 2020). This cold-induced binding of PFD4 to LSM8 also exemplifies the necessity to identify more interaction partners of the LSM family proteins to fully understand the complexity of temperature-dependent AS.

snRNP associated proteins are modulators of cold stress response

LETHAL UNLESS CBC7 (LUC7) is a U1 snRNP component, that carries a Ser/Arg-rich domain (Fortes et al., 1999). The A. thaliana genome codes for three LUC7 genes and loss of all copies results in salt and cold sensitivity (de Francisco Amorim et al., 2018). It has been shown that there are genes with LUC7-dependent introns, and that the splicing of these introns can be modulated by different abiotic stresses. Curiously, the loss of LUC7-mediated intron processing leads to the accumulation of IR transcripts, which could allow them to theoretically escape NMD through retention in the nucleus (de Francisco Amorim et al., 2018).

Since yeast luc7 mutants exhibit a high temperature-sensitive phenotype (Fortes et al., 1999) and the expression of the tomato homologue is induced in cold temperatures (Chechanovsky et al., 2019), it is reasonable to assume that LUC7 plays a conserved role in temperature response. Therefore, the biological function LUC7-dependent transcripts play in plant stress response in general, and temperature response in particular, should be investigated. Furthermore, a study of what functions the nuclear retained transcripts take on and how their turnover is regulated should also be conducted.

The RNA SPLICING FACTOR1 (SF1) is another protein that regulates the positioning of the spliceosome on the pre-mRNA. It recognizes the branch point and interacts with U2AF proteins, which recruits the U2 snRNP (Zhu et al., 2020). The interaction of SF1 with U2AF65A is noteworthy
(Zhu et al., 2020), since it exhibits temperature-dependent AS (Pajoro et al., 2017; Verhage et al., 2017; Cavallari et al., 2018; James et al., 2018). This mechanism seems to be evolutionarily conserved, as the orthologous gene in cauliflower is also alternatively spliced upon exposure to elevated temperatures (Verhage et al., 2017).

The sf1 mutant is dwarfed and exhibits paling leaves under cold stress (Zhu et al., 2020). Additionally, it was found that the sf1-2 allele exhibits an early flowering phenotype, irrespective of the growth temperature (16 °C, 23 °C or 27°C), which could be linked to the significantly decreased FLOWERING LOCUS M-β (FLM-β) expression observed (Lee et al., 2020). FLM-β, the predominant splice form at low ambient temperatures, has been determined to play a major role in the control of floral transition (Posé et al., 2013; Lee et al., 2013). Intriguingly, an amino acid substitution at position 104 in yeast SF1 also leads to cold temperature-sensitivity (Zhu et al., 2020).

As mentioned before, U4/U6.U5 tri-snRNP assembly is regulated by STA1 at elevated temperatures. Intriguingly, expression of STA1, which is required for the appropriate splicing of COLD REGULATED 15A (COR15A), is induced gradually within 72h under chilling conditions (Yu et al., 2016), and sta1-1 seedlings die after prolonged exposure to chilling stress (Lee et al., 2006). STA1 also interacts with PRP31, an additional factor for U4/U6.U5 tri-snRNP assembly. Recently it was shown that PRP31 plays an important role in the pre-mRNA splicing and gene expression of cold responsive genes, and that the loss of PRP31 leads to severe chilling stress sensitivity (Du et al., 2015).

In summary, these reports indicate that splice site recognition, as well as snRNP complex assembly, are temperature-regulated and needed for correct pre-mRNA processing under stress conditions (Fig. 2). In the future it will be interesting to assess how these SFs contribute to temperature sensing and stress tolerance establishment.
Conclusions and future perspectives

Various studies have shown that alternative splicing is a central mechanism of stress responses in plants, ensuring physiological flexibility and mediating the integration of various environmental stimuli (Fig. 2). We are, however, very far from fully understanding the underlying principles of this network. Though some detailed studies about temperature-dependent regulation of splicing have been discussed in this review, they hardly begin to capture the full complexity of temperature-regulated alternative splicing in plants. In this review we focused on discussing the role of core components and selected associated factors of the splicing machinery in regulating temperature-dependent plant growth and development. However, it is important to note that numerous other proteins have been implicated in temperature-dependent AS. Details regarding the role of these factors in mediating AS in response to temperature are often lacking, and we therefore, and due to space limitations, refrained from discussing them in detail. In summary it can be concluded that, while numerous reports on individual factors demonstrate the importance of temperature-dependent AS, systematic studies of AS and its role in plant acclimation and phenotypic plasticity are lacking. Clearly, a concerted effort is needed to incorporate the existing data into a more complete picture of the temperature-regulated AS process and to fill the gaps in our knowledge.

While the currently available data indicates that alternative splicing overlaps, at least partially, with known regulators of temperature signaling pathways, this information is insufficient to identify the core nodes which draw these two processes together. This leaves the question whether temperature responsiveness is a general characteristic of certain splicing-related protein families or rather of individual splicing factors unanswered. Addressing this question is not a trivial matter, since splicing factors can associate with multiple different protein complexes depending on their localization and environmental stimuli. Thus, it is necessary to uncover the dynamical spliceosome composition by
collecting data about the splicing interactome, for example through stress specific gene and protein interaction networks.

Moreover, it is not possible to understand the alternative splicing process and its role in plant temperature response without taking evolutionary aspects into account. For instance, several studies have already given an indication for a link between alternative splicing in different A. thaliana accessions and their ecological distribution (Kesari et al., 2012; Wang et al., 2018; Hanemian et al., 2020). Hence, temperature-dependent alternative splicing seems to be strongly associated to plant fitness. Future studies will be of great value in improving our understanding of plant temperature response with potential applications in improving crop yields as well as mitigating climate change.
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Author contributions

VD, NEA and MS devised the review. VD and NEA wrote the main manuscript with contributions from MS. VD and NEA created the summary Fig. 2. DG, SMN and NRM and provided comments on the manuscript. DG, SMN and NRM devised and created Fig. 1, the boxes and the summary table, with contributions from VD and NEA. VD, NEA and MS reviewed and edited the final version of the manuscript.
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| Gene Name                                                | Gene Acronym | AGI Code  | Low Temperature References | High Temperature References |
|---------------------------------------------------------|--------------|-----------|----------------------------|-----------------------------|
| ARGinine/Serine-rich splicing factor 31                 | RS31         | AT3G 61860| Paluska et al., 2007       | Neumann et al., 2020        |
| ARGinine/Serine-rich splicing factor 31a                | RS31a        | AT2G 46610|                             |                             |
| ARGinine/Serine-rich splicing factor 40                 | RS40         | AT4G 25500| Paluska et al., 2007, Rosembert, 2017; Neumann et al., 2020 | Paluska et al., 2007, Pajoro et al., 2017 |
| ARGinine/Serine-rich splicing factor 41                 | RS41         | AT5G 52040| Neumann et al., 2020       |                             |
| Calcium exchanger 1                                     | CAX1         | AT2G 38170| Catalá et al., 2003, Cui et al., 2014 |                             |
| Chaperone DNAJ-domain superfamily protein               | AT1G724      | AT1G 72416| Filichkin et al., 2015     |                             |
| Cold regulated 15A                                      | COR15A       | AT2G 42540| Lee et al., 2006           |                             |
| CoolAIR                                                 | COOLAIR      | AT5G 01675| Swiezewski et al., 2009, Marquardt et al., 2014 |                             |
| DEAD-box ATP-dependent RNA helicase 3                   | RH3          | AT5G 26742| Gu et al., 2014            |                             |
| DEAD-box ATP-dependent RNA helicase 7                   | RH7          | AT5G 62190| Huang et al., 2016a, Liu et al., 2016 |                             |
| Delta1-Pyrroline-5-carboxylate synthase 1               | P5CS1        | AT2G 39800| Kesari et al., 2012, Ren et al., 2018 |                             |
| Elongated hypocotyl 5                                  | HY5          | AT5G 11260| Perera-Resa et al., 2017   |                             |
| Enhanced silencing phenotype 3                         | ESP3         | AT1G 32400| Howles et al., 2016        |                             |
| Flowering locus C                                       | FLC          | AT5G 10140| Schmitz et al., 2008       |                             |
| Flowering locus M                                       | FLM          | AT1G 77080| Posé et al., 2013, Lee et al., 2013; Capovilla et al., 2017; Lee et al., 2020 | Posé et al., 2013; Capovilla et al., 2017; Lee et al., 2020 |
| Gemin 2                                                 | GEMIN2       | AT1G 54380| Schlaen et al., 2015, Neumann et al., 2020 |                             |
| Glucine-rich protein 7                                  | GRP7         | AT2G 21660| Kim et al., 2008, Wiernkop et al., 2008, Czolphinska and Rurek, 2018 |                             |
| Heat shock transcription factor 3A                      | HSFA3        | AT5G 03720| Okamoto et al., 2016, Kim et al., 2017, 2018 |                             |
| LAMmer kinase A3                                        | AME3         | AT4G 32660| Rosembert, 2017            |                             |
| Lethal unless CBC7 A                                    | LUC7A        | AT3G 02340| de Francisco Amorim et al., 2018 |                             |
| Lethal unless CBC7 B                                    | LUC7B        | AT5G 17440| de Francisco Amorim et al., 2018 |                             |
| Lethal unless CBC7 RL                                   | LUC7RL       | AT5G 51410| de Francisco Amorim et al., 2018 |                             |
| Oligouridylic binding protein 1B                        | UBP1B        | AT1G 17370| Weber et al., 2008, Nguyen et al., 2016 |                             |
| Phytotriheme interacting factor 4                       | PIF4         | AT2G 43010| Jin et al., 2020           |                             |
| pre-mRNA-splicing helicase BRR2B                        | BRR2B        | AT2G 42270| Guan et al., 2013          |                             |
| PolyPyrimidine tract-binding protein 1                  | PTB1         | AT3G 01150| James et al., 2018         |                             |
| PolyPyrimidine tract-binding protein 2                  | PTB2         | AT5G 53180| James et al., 2018         |                             |
| PolyPyrimidine tract-binding protein 3                  | PTB3         | AT1G 43190| James et al., 2018         |                             |
| Porcupine                                              | PCP          | AT2G 18740| Capovilla et al., 2018, Huertas et al., 2019 |                             |
| pre-mRNA-processing factor 31 HOMolog                   | PRP31        | AT1G 60170| Du et al., 2015            |                             |
| Prefoldin 4                                             | PFD4         | AT1G 08780| Perera-Resa et al., 2017, Esteve-Bruna et al., 2020 |                             |
| Protein Name                                      | Accession | Reference                           |
|--------------------------------------------------|-----------|-------------------------------------|
| Proline-rich protein Sickle                      | AT4G24500 | Marshall et al., 2016              |
| Putative U2A65 splicing factor A                 | AT4G36690 | Verhaeghe et al., 2017; James et al., 2018; Cavallari et al., 2018; Pajoro et al., 2017; Cavallari et al., 2018 |
| Rare-cold-inducible 2A                          | AT3G05880 | Nylander et al., 2001; Cui et al., 2014 |
| Regulator of CBF gene expression 1              | AT1G20920 | Guan et al., 2013                  |
| Root initiation defective 1                      | AT1G26370 | Ohtani et al., 2013                |
| RS-containing zinc finger protein 21             | AT1G23960 | Neumann et al., 2020               |
| RS-containing zinc finger protein 22             | AT4G31580 | Rosembert, 2017; Neumann et al., 2020 |
| RS-containing zinc finger protein 22a            | AT2G24590 | Neumann et al., 2020               |
| RS-containing zinc finger protein 32             | AT3G53500 | Neumann et al., 2020               |
| RS-containing zinc finger protein 33             | AT2G37340 | Palusa et al., 2007                |
| SC35-like splicing factor                        | AT5G64200 | Neumann et al., 2020               |
| SC35-like splicing factor 28                      | AT5G18810 | Neumann et al., 2020               |
| SC35-like splicing factor 30                      | AT3G55460 | Neumann et al., 2020               |
| SC35-like splicing factor 30a                    | AT3G13570 | Neumann et al., 2020               |
| Serine/arginine rich protein splicing factor 45  | AT1G16810 | Ali et al., 2003; Xing et al., 2015 |
| Serine/arginine rich-like protein splicing factor 45a | AT1G07350 | Ling et al., 2018                  |
| Serine/arginine-rich-like protein splicing factor 30 | AT1G09140 | Palusa et al., 2007; Ling et al., 2018; Neumann et al., 2020 |
| Serine/arginine-rich-like protein splicing factor 33/SC35-like splicing factor 33 | AT1G55310 | Palusa et al., 2007; Palusa et al., 2007; Palusa et al., 2007; Pajoro et al., 2017; Neumann et al., 2020 |
| Serine/arginine-rich-like protein splicing factor 34 | AT1G02840 | Palusa et al., 2007; Neumann et al., 2020 |
| Serine/arginine-rich-like protein splicing factor 34a | AT1G48430 | Neumann et al., 2020               |
| Serine/arginine-rich-like protein splicing factor 34b | AT1G02430 | Palusa et al., 2007; Neumann et al., 2020 |
| Shoot redifferentiation defective 2              | AT1G28560 | Yasutani et al., 1994; Sugiyama et al., 2003; Ohtani et al., 2013 |
| SM-like protein 3A                                | AT1G21190 | Perea-Resa et al., 2012            |
| SM-like protein 3B                                | AT1G72960 | Perea-Resa et al., 2012            |
| SM-like protein 4                                 | AT1G27720 | Perea-Resa et al., 2012            |
| SM-like protein 5/SuperSensitive to ABA and drought1 | AT5G48870 | Okamoto et al., 2016               |
| SM-like protein 8                                | AT1G62790 | Carrasco-López et al., 2017; Esteve-Bruna et al., 2020 |
| Splicing factor 1                                | AT1G51300 | Zhu et al., 2020; Lee et al., 2020 |
| Stabilized 1                                     | AT4G03430 | Lee et al., 2006; Yu et al., 2016; Kim et al., 2017, 2018 |
| Timing of CAB expression 1                       | AT5G61380 | James et al., 2012                 |
| Trimethylguanosine synthase 1                    | AT1G45231 | Gao et al., 2017                   |
Boxes

Box 1. Splicing at a glance

The spliceosome is a complex protein-RNA machinery which excises introns from the pre-mRNA and joins exons to form the mature mRNA. The general turnover of the spliceosome requires a multitude of structural rearrangements which are mediated by several DExD/H-box RNA helicases in an ATP-dependent manner.

Splicing is initiated with the binding of the U1 snRNP to the 5′-splice site (SS), and the interaction of SPlicing FACTOR 1 with the U2 snRNP auxiliary factors (U2AFs), which together bind the 3′-SS, the polypyrimidine tract and branch point sequence. Together, these proteins form the primary E complex and determine further positioning of the spliceosome. The U2AFs recruit the U2 snRNP to the pre-mRNA, giving rise to the A complex. Subsequently, a trimer complex of tightly bound U4/U6 snRNPs and U5 snRNP, called U4/U6.U5 tri-snRNP, is recruited to the A complex forming the B complex. The transition to the catalytically active B* complex is defined by the multiple inner rearrangements that lead to the release of U1 and U4 snRNPs and approximation of the two SSs. Completion of the first catalytic reaction marks the transition from the B* complex to the C complex, which performs all intron excision and exon ligation steps. Finally, the intron lariat as well as the snRNPs are released and the splicing proteins can be recruited to other pre-mRNAs (Fig. 1B) (Matera and Wang, 2014; Wilkinson et al., 2020).

The composition of the spliceosome can vary which causes its proteins to interact with different SSs. Besides “strong” SSs, which are constitutively spliced, alternative splicing (AS) can lead to inclusion or omission of “weak” SSs. SS choice is influenced by RNA-binding proteins, such as SR proteins or hnRNPs, RNA polymerase-II elongation rate, epigenetic modifications and nucleosome positioning (Luco et al., 2011). The combination of these factors gives rise to a plethora of different AS events, which may carry premature termination codons (PTCs). These transcripts do not encode functional
proteins and thus need to be directed towards degradation via the nonsense-mediated RNA decay (NMD) pathway, a necessary process to protect the cell from energy losses through the production of truncated proteins.

Box 2. Plant responses to temperature stress in a nutshell.

Temperature response in plants is a complex regulatory network that can conceptually be divided into three steps: signal perception, transduction, and cellular output. At the most basal level, temperature affects protein conformation, metabolic rates, molecular interactions, and membrane fluidity, which are considered primary sensors and cause various downstream responses. The most studied cellular response is Ca$^{2+}$ influx to the cell as a result of changes in membrane fluidity. Ca$^{2+}$ triggers phosphorylation and transcription factor cascades that ultimately result in a massive reprogramming of the cellular transcriptome.

Exposure of *A. thaliana* to non-lethal cold temperatures, for example, results in induction of C-REPEAT BINDING FACTORS/DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN1 (CBF/DREB1) transcription factors. The CBFs directly activate expression of COLD RESPONSIVE (COR) genes. However, inactivation of CBFs impairs freezing tolerance only partially, indicating the existence of parallel cold signaling pathways. Continuous exposure to cold leads to reduced and delayed growth, wilting and chlorosis. Major changes on the organism level are tissue dehydration and membrane damage. Ultimately, cold acclimation endows the plant with increased freezing tolerance.

In contrast, during thermomorphogenesis plants respond to warm temperatures through morphological changes including deeper roots, leaf petiole elongation, up-ward growth of leaves to reduce exposure to light, and induction of flowering. Heat-stress response involves crosstalk between transcription factors, heat-stress responsive genes and epigenetic modifications that convey a primed-memory response to subsequent heat stresses. Heat stress transcription factors
(HSFs) play a key role in this process by controlling the expression of proteins with chaperone activity to help cope with heat-induced protein misfolding. One of the central regulators of plant thermomorphogenesis which integrates temperature and light quality cues with phytohormones signalling is PHYTOCHROME INTERACTING FACTOR4 (PIF4).

As highlighted in this review, cold and heat trigger alternative splicing (AS) of cognate stress response genes in plants. However, mechanistic understanding regarding the role of AS in a plant’s response to temperature stress is missing.

For detailed explanation on temperature perception and signaling please refer to the following reviews (Yadav, 2010; Mittler, Finka, and Goloubinoff, 2012; Ding, Shi, and Yang, 2019; Casal and Balasubramanian, 2019; Hayes et al., 2021).
Figure legends

Figure 1: Overview of the splicing machinery assembly and splicing process

A) Stepwise assembly of the SM-ring in the cytoplasm based on the proposed model in human cells. As described in the main text, the assembly of the SM-ring involves the methylosome and SMN complex. So far, the only two identified components of these complexes in plants are PRMT5 and GEMIN2, respectively. Clear evidence about the role of AtICln in the methylosome, as well as the existence of intermediate SM-ring complexes in plants is missing. Once the snRNP (U1, U2, U4 or U5) is assembled, the 5’ cap of the snRNA is methylated by TGS1 and presumably bound by AtKPNB1, the orthologue of the human IMPORTIN SUBUNIT BETA-1. This process gives rise to the import complex, which can be shuttled into the nucleus through nuclear pore channels (NPC).

B) Simplified visualization of the splicing reaction in the nucleus. The boxes list the splicing factors mentioned in the main text and position them accordingly along the splicing process.

Figure 2: Temperature-dependent alternative splicing adjusts the proteome to the plant’s needs.

Temperature regulates the expression and splicing of splicing-related and temperature-response genes. Temperature fluctuations influence the expression and splicing pattern of splicing-related genes, giving rise to a specific set of splicing proteins. Further on, these proteins can be modified, affecting their subcellular localization, activity and/or inclusion into the spliceosome. Together, these processes define a temperature-specific composition of the spliceosome, which modulates the splicing result of temperature-response pre-mRNAs and ultimately the plant’s response to the environment. Box lists splicing-related genes, which are regulated by temperature. Colors indicate the temperature stimulus (blue = cold, red = heat). Numbers indicate the known levels of regulation (1: expression, 2: AS, 3: AS of target genes, 4: PTM, 5: localization, 6: target methylation).
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Figure Box 1: Possible outcomes of AS

AS events can lead to exon skipping, usage of mutually exclusive exons (possibility to include/exclude functional domains), intron retention, or alternative SS choice. Constitutively spliced exons are in grey. Alternatively spliced exons are colored.