The spliceosome assembly factor GEMIN2 attenuates the effects of temperature on alternative splicing and circadian rhythms

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The mechanisms by which poikilothermic organisms ensure that biological processes are robust to temperature changes are largely unknown. Temperature compensation, the ability of circadian rhythms to maintain a relatively constant period over the broad range of temperatures resulting from seasonal fluctuations in environmental conditions, is a defining property of circadian networks. Temperature affects the alternative splicing (AS) of several clock genes in fungi, plants, and flies, but the splicing factors that modulate these effects to ensure clock accuracy throughout the year remain to be identified. Here we show that GEMIN2, a spliceosomal small nuclear ribonucleoprotein assembly factor conserved from yeast to humans, modulates low temperature effects on a large subset of pre-mRNA splicing events. In particular, GEMIN2 controls the AS of several clock genes and attenuates the effects of temperature on the circadian period in *Arabidopsis thaliana*. We conclude that GEMIN2 is a key component of a posttranscriptional regulatory mechanism that ensures the appropriate acclimation of plants to daily and seasonal changes in temperature conditions.

spliceosome assembly | alternative splicing | circadian rhythms | *Arabidopsis* | GEMIN2

Circadian clocks allow organisms to coordinate physiological processes with periodic environmental changes. The core of all circadian systems, in organisms ranging from cyanobacteria to humans, is a network of multiple interlocked feedback loops that operate at the transcriptional, translational, and post-translational levels to sustain oscillations with a period of ∼24 h, even in the absence of environmental cues. An increasing body of evidence links alternative splicing (AS) with the regulation of circadian networks across eukaryotic organisms (1–3). The core clock genes *period* in *Drosophila*, *frequency* in *Neurospora*, and *BMAL2* in humans undergo AS to give rise to different mRNA isoforms (1, 2, 4). In *Arabidopsis*, several core clock genes, including *TIMING OF CAB EXPRESSION 1* (*TOC1*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), also undergo extensive AS (5–7).

Interestingly, many of the alternative mRNA isoforms associated with the *Arabidopsis* core clock genes are abundant or alter their abundance upon changes in environmental conditions, suggesting that they have important physiological roles (5–7). For example, there is strong evidence that temperature regulation of *CCA1* AS is critical for the proper functioning of circadian rhythms under cold conditions (8). Temperature also regulates the AS of *frequency* in *Neurospora* and *period* in *Drosophila* (1, 2), thereby promoting the proper functioning of circadian networks under the wide range of temperatures occurring throughout the seasons. Although our knowledge of the transcription factors that regulate clock function in different organisms has increased drastically over the last two decades, the splicing factors that modulate the AS patterns of core clock genes are only starting to be characterized (1). Splicing factors that mediate the effects of temperature on the AS of core clock genes are unknown.

Pre-mRNA splicing is catalyzed by the spliceosome, a large and dynamic molecular complex composed of five different small nuclear ribonucleoprotein (snRNP) particles (U1, U2, U4, U5, and U6 snRNPs) and over 150 additional proteins (9). Each snRNP consists of a specific small nuclear RNA and a number of core spliceosomal proteins. The regulation of AS has traditionally been associated with auxiliary splicing factors such as arginine–serine-rich (RS) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), which repress or enhance the recruitment of snRNP particles to specific splice sites. More recently, interactions between the transcriptional machinery, chromatin structure, and core spliceosomal factors have also been shown to regulate AS (10). Furthermore, a systems-based analysis of the network of proteins that interact to regulate AS in mammalian cells suggested that the efficiency and/or kinetics of spliceosome assembly play a key role in the regulation of AS (11).

To investigate if modulation of spliceosome assembly links the regulation of AS to the control of circadian networks in plants, we characterized mutants with defects in genes encoding the main components of the survival motor neuron (SMN) complex, which controls the spliceosomal snRNP core assembly in eukaryotes (12–14). We found that GEMIN2, the only component

Significance

RNA processing, an important step in the regulation of gene expression, is mediated by proteins and RNA molecules that are highly sensitive to variations in temperature conditions. Most organisms do not control their own body temperature. Therefore, molecular mechanisms must have evolved that ensure that biological processes are robust to temperature changes. Here we identify a protein that buffers the effect of temperature on biological timing by enhancing the assembly of the spliceosome, a large ribonucleoprotein complex involved in RNA processing in organisms ranging from yeast to humans, and thereby controlling the alternative splicing of clock genes.

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of the SMN complex that is conserved from yeast to humans, controls the pace of the circadian clock under standard growth conditions in *Arabidopsis* by controlling the AS of *TOC1* and other core clock genes. Furthermore, our results suggest that GEMIN2 attenuates the effects of temperature on the circadian period by modulating AS events associated with several core clock genes, most likely altering the overall balance required for proper temperature compensation of the clock.

Results

***Arabidopsis* GEMIN2 Is Required for Proper Biological Timing.** An evolutionary analysis of the SMN complex suggested that plants possess orthologs of both SMN and GEMIN2 (12–14). By conducting a more detailed phylogenetic analysis, we found that the *Arabidopsis* gene *AT1G54380* is indeed an ortholog of mammalian *GEMIN2* (Fig. S1*A*), but that the *Arabidopsis SMN-like* gene, *AT2G02570*, is more closely related to mammalian SPF30 than to SMN (Fig. S1*B*). Therefore, similar to *Saccharomyces cerevisiae* (budding yeast) (12), *Arabidopsis* lacks a true SMN ortholog and GEMIN2 is the only component of the mammalian SMN complex that is conserved from yeast to humans (9). *GEMIN2* is essential for viability in all multicellular organisms characterized to date (15, 16), except for *Arabidopsis* (Fig. 1). Two different mutant alleles of *GEMIN2* showed similarly mild growth and developmental alterations in *Arabidopsis* plants grown at 22 °C under long-day conditions (16 h light:8 h darkness), such as shorter petioles and smaller leaves, and these phenotypes disappeared when the mutant was complemented with a functional *GEMIN2* gene (Fig. 1 *A* and *B*). We then characterized clock-dependent phenotypes in these plants. Flowering time is regulated by the circadian clock in *Arabidopsis*, and we found that both mutant alleles displayed an early flowering phenotype (Fig. 1*C*). Consistent with a role for GEMIN2 in the regulation of the circadian clock, both mutant alleles displayed a short-period phenotype for the circadian rhythms of leaf movement and gene expression (Fig. 1 *D*–F and Fig. S2 *A* and *B*). *GEMIN2* expression cycled in wild-type plants under light/dark cycles, but circadian oscillations in *GEMIN2* mRNA levels were not robust in plants transferred to constant light and temperature conditions, indicating that GEMIN2 is not a core component of the transcriptional feedback loops controlling clock function in *Arabidopsis* (Fig. S2 *C* and *D*). The temporal pattern of expression of all of the clock genes analyzed was consistent with the short-period phenotype of the mutant (i.e., the time of peak expression progressively advanced under constant light and temperature conditions), but overall mRNA levels of these clock genes were not altered in *gemin2* mutants compared with wild-type plants, suggesting that the defects leading to period alterations most likely resulted from alterations at the posttranscriptional level (Fig. S2 *E*–*H*).

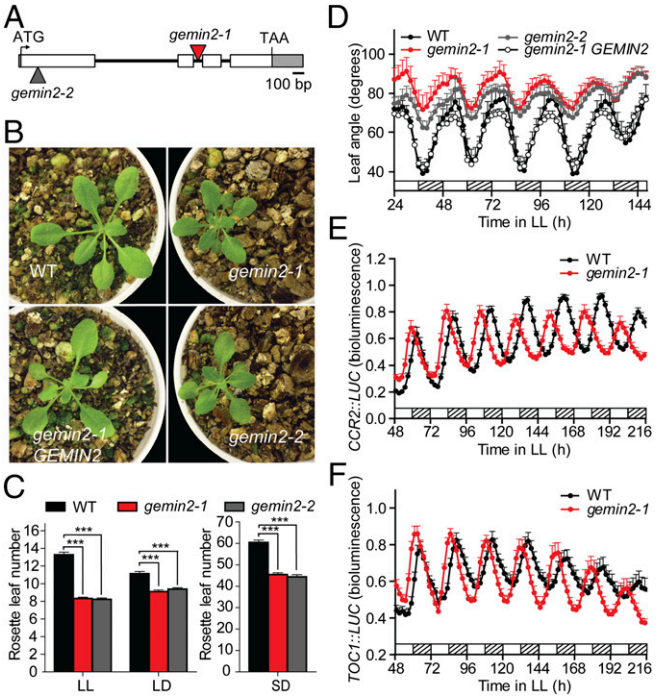

Fig. 1. A role for GEMIN2 in growth, development, and the circadian system of *Arabidopsis*. (*A*) Structure of *GEMIN2*. Introns are represented by lines and exons by boxes (white boxes indicate coding regions and the gray box represents the UTR). T-DNA insertions in *gemin2-1* and *gemin2-2* mutants are indicated. (*B*) Rosette phenotypes of wild-type (WT), *gemin2-1* and *gemin2-2* plants, and of the *gemin2-1* mutant complemented with *GEMIN2*. (*C*) Flowering time measured as the number of rosette leaves at bolting in constant light (LL), long day (LD), and short day (SD) conditions (ANOVA and Tukey's multiple comparison test, $***P < 0.001$, $n = 40–45$). (*D*) Circadian rhythm of leaf movement ($n = 8$). (*E* and *F*) Bioluminescence analysis of *CCR2::LUC* (*E*) or *TOC1::LUC* (*F*) expression ($n = 12$). In *D*–*F*, plants were entrained under LD cycles and then transferred to constant light and temperature conditions (22 °C). Data represent average + SEM. Open and hatched boxes indicate subjective day and night, respectively.

Mutations in *GEMIN2* Affect snRNP Levels and a Specific Subset of Splicing Events. GEMIN2 is known to modulate the assembly of U1–U5 snRNPs (9, 13, 14, 17), each of which is composed of a common heptameric ring of seven Sm proteins, a specific snRNA, and several specific accessory factors. Defects in snRNP assembly destabilize uridine-rich snRNAs and, consistent with a role for *Arabidopsis* GEMIN2 in snRNP assembly, *gemin2* mutants had severely reduced levels of U1 snRNA. We also observed slightly increased levels of U2, U4, and U5 snRNAs, as well as reduced levels of U6 snRNA (Fig. 2*A*). Similar alterations in snRNA stoichiometry have been reported in SMN-deficient mammalian cell lines, which correlate with tissue-specific defects in pre-mRNA splicing of a subset of splicing events in *SMN*-deficient mice (18).

We then conducted a genome-wide characterization of GEMIN2 effects on alternative and constitutive splicing, combining different experimental approaches such as tiling arrays, high-resolution RT-PCR panels, and RNA sequencing (RNA-seq) (Datasets S1–S3). We observed that GEMIN2 had a greater effect on alternative than on constitutive splicing, a phenomenon that has already been reported for several core snRNP components, such as SmB (19), LSm4 (20), or U1C (21) (Fig. 2*B*). Defects in AS were enriched for intron retention events, the most common AS event in *Arabidopsis*, but also included changes in exon skipping, as well as alternative donor and acceptor splice sites (Fig. 2*C*, Fig. S3, and Datasets S1–S3).

***GEMIN2* Defects Alter the AS of Core Clock Genes.** Among the AS events detected with all three techniques, we found that *gemin2* mutants displayed increased retention of the alternatively spliced intron 4 of the core clock gene *TOC1* (Fig. 2*D* and Fig. S4*A*). Plants with mutations in the *TOC1* gene have a short period phenotype (22), so we tested whether the *gemin2* phenotype could be partially explained by altered levels of *TOC1* functional mRNA by analyzing the effect of GEMIN2 on clock function in a null *toc1* mutant background. Indeed, we found that, whereas *gemin2* mutants showed a short-period phenotype when the mutation was present in an otherwise wild-type background, no additional effect on circadian rhythms was observed when *gemin2* was in a *toc1* mutant background (Fig. 2 *E* and *F* and Fig. S4 *B* and *C*). Thus, the *gemin2* circadian phenotype requires functional *TOC1* mRNA, suggesting that the aberrant phenotype could be due, at least in part, to increased retention of intron 4 of *TOC1*.

In addition to alterations in the AS of *TOC1*, we also found alterations in the AS of *CCA1* and other core clock genes in

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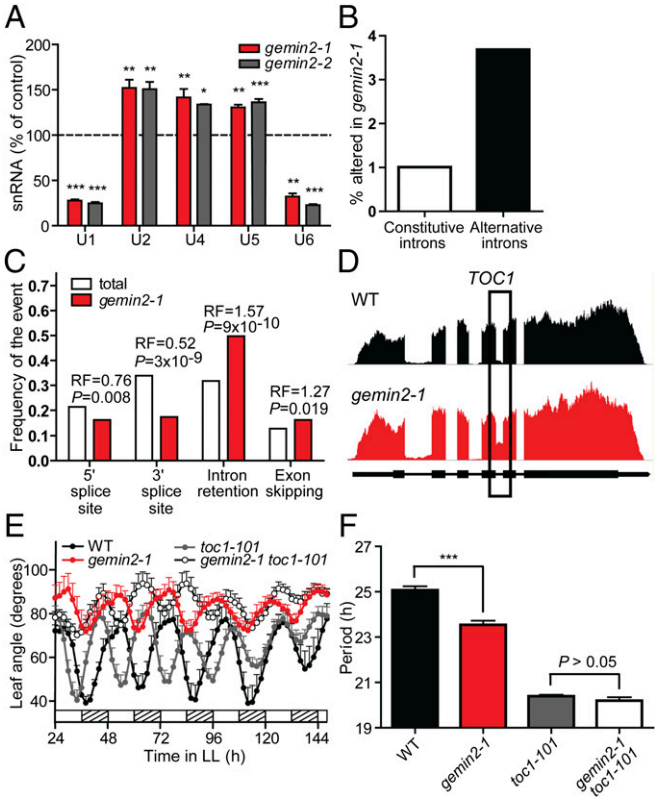

Fig. 2. GEMIN2 affects a specific subset of alternative splicing events, including retention of intron 4 of the clock gene *TOC1*. (*A*) Levels of snRNAs measured by qPCR (ANOVA and Tukey's multiple comparison test, $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, $n = 3$). (*B*) Percentage of introns showing increased retention in *gemin2-1* mutants relative to wild-type plants among alternatively or "constitutively" spliced introns. (*C*) Relative frequency of each type of annotated AS event among those present in the RNA-seq dataset of wild-type plants (total) or among those AS events altered in *gemin2-1*. The representation factor (RF) is the frequency of the event type among those altered in *gemin2-1* divided by its frequency among all of the events evaluated in wild-type plants. (*D*) Read density map of *TOC1* visualized with the Integrated Genome Browser. Intron 4 is highlighted with a box. (*E* and *F*) Circadian rhythm of leaf movement in constant light (LL) at 22 °C (*E*) and estimated circadian periods (ANOVA and Tukey's multiple comparison test, $***P < 0.001$, $n = 8$) (*F*). Data represent average + SEM.

gemin2, some of which might also contribute to the circadian phenotype of this mutant (Fig. S4 *E*–*G*). Indeed, we found that *gemin2* enhances the retention of intron 6 of pseudo-response regulator 9 (*PRR9*), an alteration that should cause period lengthening. Consistent with this possibility, we found that the period-shortening effect of the *gemin2* mutation was enhanced in the *prr9 prr7* mutant background (Fig. S4 *H* and *I*), suggesting that the *gemin2* circadian phenotype is most likely the result of integrating alterations in the splicing of several core clock genes.

Similar AS Patterns Are Observed in *gemin2* Mutants Grown at 22 °C and in Wild-Type Plants Exposed to Cold Conditions. Strikingly, it was previously reported that both retention of *TOC1* intron 4 and exclusion of *CCA1* intron 4 are AS patterns promoted by low temperature conditions in wild-type plants (5, 6) (Fig. S5). We therefore examined in more detail the overlap between GEMIN2 and the effects of low temperature on AS. For this examination, we first visually compared the effects of *gemin2* on AS with those found in a publicly available RNA-seq dataset of plants exposed for 4 d to low temperature conditions (23). We found several AS events, including intron retention (IR), exon skipping (ES) and alternative 5′ splice sites (A5′SS), that were similarly affected by *gemin2* at 22 °C and by low temperature conditions in wild-type plants (Fig. 3 *A* and *B*). Lack of replicates of this particular low temperature transcriptome prompted us to obtain an independent dataset with three biological replicates. For this purpose, we compared the transcriptome of plants grown for 9 d at 22 °C with that of plants grown at 22 °C for the 9 d and exposed at the end of this period for 1 or 24 h to 10 °C. Approximately 80% of a set of genes previously reported to be either induced or repressed in response to cold conditions in *Arabidopsis* also showed large and significant changes in expression in our experiment, validating our cold RNA-seq dataset (Fig. S6). We found a strong enrichment in genes associated with ribosome biogenesis, protein translation, and RNA processing and splicing among the genes up-regulated in response to cold conditions in wild-type plants (Datasets S4 and S5), supporting the notion that transcriptional regulation of the RNA processing machinery is an important component of the cold acclimation mechanism in plants. Interestingly, we also observed a strong overlap between the effects of cold conditions on pre-mRNA splicing in wild-type plants and the effects of GEMIN2 on pre-mRNA splicing at 22 °C, an overlap that was much larger than that expected to occur simply by chance (Fig. 3*C*). In particular, this extensive overlap was more significant for pre-mRNA splicing events affected in similar directions (i.e., increased inclusion or exclusion) by the mutation in *GEMIN2* and exposure to cold stress (Fig. 3*C*).

GEMIN2 Maintains U1 snRNP Homeostasis and Buffers Low Temperature Effects on AS. Based on the extensive overlap between pre-mRNA splicing events affected by low temperature conditions in wild-type

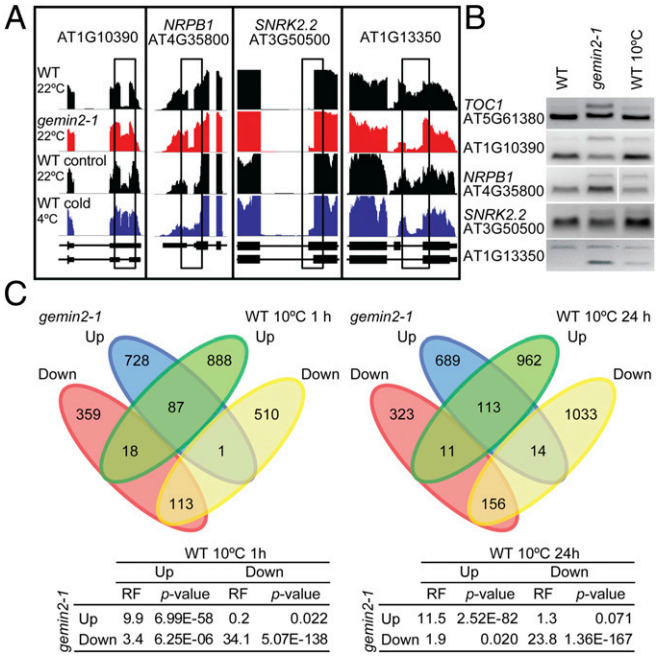

Fig. 3. Alternative splicing patterns of *gemin2* mutants at 22 °C mimic those of wild-type plants exposed to low temperature conditions. (*A*) AS events similarly affected by a mutation in *GEMIN2* at 22 °C and by exposure to 4 °C for 48 h in wild-type plants, visualized with the Integrated Genome Browser. Control and cold correspond to public RNA-seq datasets described in Gulledge et al. (23). Black boxes highlight the events of interest. (*B*) Affected events confirmed by RT-PCR. Cold-treated plants were subjected to 10 °C for 12 h. Space between lanes denotes that data were collected from noncontiguous lanes of the same gel. (*C*) Venn diagram showing the overlap between pre-mRNA splicing events affected in *gemin2-1* mutants at 22 °C and those altered in wild-type plants exposed to 10 °C for 1 or 24 h.

plants and those affected in *gemin2* at 22 °C, we hypothesized that cold exposure would inhibit U1 snRNP assembly. However, we found that U1 snRNA levels increased in wild-type plants exposed for 24 h to low temperatures (Fig. 4*A*). This finding is opposite to that expected if U1 snRNP assembly were compromised, because free U1 snRNA is unstable. Alternatively, cold conditions may reduce the functionality, rather than the levels, of U1 snRNP, and the increment observed in U1 snRNA levels may be required for a compensatory enhancement of U1 snRNP assembly. Interestingly, we found that a 24-h exposure to low temperatures increased *GEMIN2* expression by almost 50%, suggesting that this effect might be associated with the cold-mediated enhancement of U1 snRNA levels (Fig. 4*B*). In agreement with this possibility, U1 snRNA levels did not accumulate to higher levels in response to low temperatures in *gemin2* mutants.

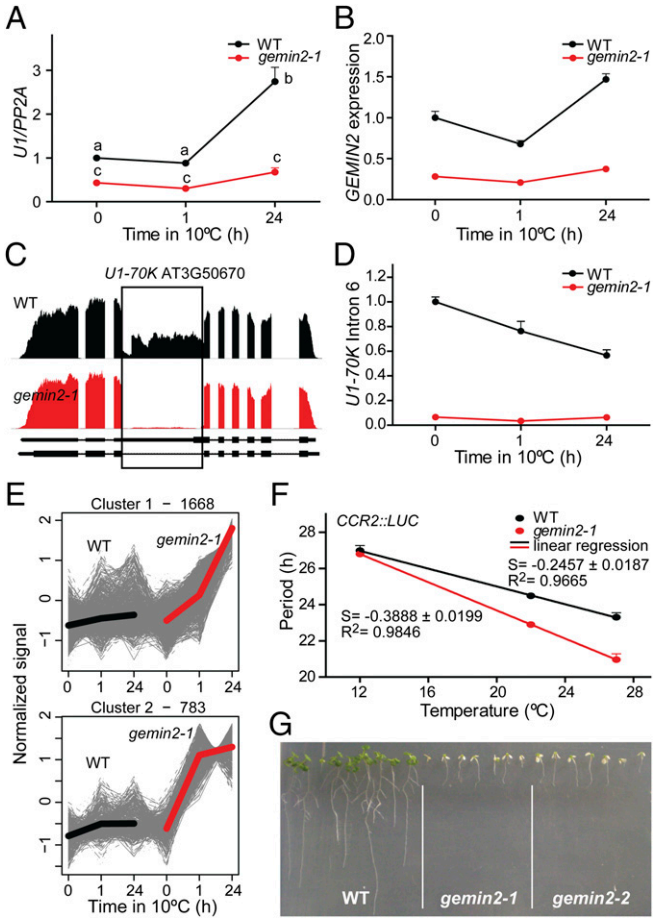

Fig. 4. GEMIN2 modulates low temperature effects on U1 snRNA levels, alternative splicing, circadian rhythm, and survival in *Arabidopsis*. (*A*) Levels of U1 snRNA measured by qPCR in wild-type and *gemin2* mutant plants exposed to cold conditions for 1 or 24 h. Statistically significant differences are indicated by different lowercase letters (ANOVA and Tukey's multiple comparison test, $P < 0.001$). (*B*) *GEMIN2* expression determined by RNA-seq in wild-type plants. In *A* and *B*, data represent average + SEM ($n = 3$) and were normalized to the value present in wild-type plants at 0 h. (*C*) Read density (RD) map of *U1-70K* visualized with the Integrated Genome Browser. The alternatively spliced intron is highlighted with a box. (*D*) Relative expression (RD intron/RD gene) of the alternatively spliced intron from *U1-70K*. Data were normalized to the value observed in wild-type plants at the beginning of the experiment. (*E*) Response patterns of the two largest clusters of pre-mRNA splicing events whose relative abundance (RD alternatively spliced region/RD gene) was altered in response to cold exposures in *gemin2-1* plants. Thin lines represent normalized values for individual alternatively spliced regions. Thick lines represent the average. The numbers appearing next to the cluster number represent the total number of splicing events present in that particular cluster. (*F*) Free-running periods of *CCR2::LUC* expression under constant light at different temperatures. Data represent the average of three independent experiments + SEM. (*G*) Five-day-old seedlings transferred to 10 °C for 4 wk.

Evidence suggesting reduced U1 snRNP functionality under cold conditions in wild-type plants was provided by the response to low temperatures of *U1-70K*, a key component of the U1 snRNP. Similar to recent findings in mammals, *U1-70K* mRNA is alternatively spliced in *Arabidopsis*, with one isoform encoding a functional U1-70K protein and another encoding a truncated protein (24). In mammals, compromising U1 snRNP function by interfering with another component of the U1 snRNP complex results in a compensatory increment in the functional *U1-70K* mRNA isoform (25). A strikingly similar situation is observed in *gemin2* mutant plants defective in U1 snRNP assembly, which showed a dramatic shift in AS, leading to the complete depletion of the nonfunctional mRNA and to full accumulation of the functional *U1-70K* mRNA isoform (Fig. 4*C*). A 24-h exposure to low temperature conditions reduced the levels of the nonfunctional *U1-70K* mRNA isoform in wild-type plants (Fig. 4*D*). Together, these results support the idea that U1 snRNP functionality is negatively affected by low temperature conditions in wild-type plants, and that GEMIN2 compensates for this defect by enhancing U1 snRNP assembly.

To further characterize the connection between GEMIN2 and AS responses to low temperatures in *Arabidopsis*, we conducted a comparative genome-wide analysis of the effect of cold on the transcriptome of wild-type and *gemin2* mutant plants. In agreement with the hypothesis that GEMIN2 attenuates the effects of low temperatures on pre-mRNA splicing, we found that most differences in splicing between *gemin2* and wild-type plants were observed in plants exposed to cold conditions for 24 h (Datasets S6 and S7). Interestingly, most of the pre-mRNA splicing events that were strongly responsive to cold exposure in *gemin2* were almost nonresponsive to low temperature in wild-type plants, indicating that GEMIN2 indeed mostly acts to buffer the effects of cold conditions on pre-mRNA splicing (Fig. 4*E* and Fig. S7). In addition, we found that most of the splicing events affected by cold exposure in wild-type plants showed a significantly altered response to low temperatures in *gemin2* mutants, indicating that GEMIN2 is a strong modulator of the effects of cold conditions for a large subset of pre-mRNA splicing events (Fig. S8).

GEMIN2 Is Required for the Acclimation of Physiological Processes to Low Temperatures. Finally, we tested whether the GEMIN2 and temperature-dependent effects on pre-mRNA splicing were associated with alterations in the temperature-mediated regulation of different physiological processes. Consistent with this idea, we found that the effects of temperature on circadian period were strongly enhanced in *gemin2* mutants, resulting in a disruption of temperature compensation (Fig. 4*F*). This defect could be due to temperature-dependent alterations in the overall balance of core clock components such as *PRR9*, *PRR7*, and *CCA1*, some of which undergo temperature-dependent changes in AS (5) and have been implicated in the temperature compensation of the *Arabidopsis* clock (26) (Fig. S9*A*). In addition, we observed that the survival rate of *gemin2* mutants, but not of wild-type plants, was reduced after keeping the plants at 10 °C for 4 wk (Fig. 4*G*). This reduction in survival was most likely due to severe alterations in the AS of a large subset of events, which resulted in almost full retention of several introns (Fig. S10).

Discussion

Posttranscriptional mechanisms, including AS, control circadian rhythms across eukaryotic organisms, and the factors linking these regulatory networks are starting to be identified. We previously showed that PRMT5, an arginine methyltransferase that methylates Sm and LSm spliceosomal proteins, is important for the proper regulation of circadian rhythms in *Arabidopsis* and flies (27). Other splicing factors known to control clock function in plants are LSm4 and LSm5 (core components of the U6

snRNP) (20) and STIPL (28), an *Arabidopsis* protein with homology to a splicing factor involved in spliceosome disassembly in humans and yeast. In addition, the *Arabidopsis* homolog of the mammalian SKI interacting protein (SKIP), a splicing factor present in the spliceosomal NineTeen complex, also regulates the circadian period length in plants (29). For all these genes encoding splicing factors or regulators, the circadian phenotypes exhibited by the corresponding mutants are associated with alterations in specific subsets of AS events involving a few core clock genes, rather than with global defects in pre-mRNA splicing. Interestingly, most of these AS events are affected by temperature changes, and some are important for keeping the clock running at a proper pace at different temperatures. However, the splicing factors or regulators that modulate the effects of temperature on the AS of core clock genes have not been identified in any eukaryotic organism. Here we showed that GEMIN2, a spliceosomal snRNP assembly factor that is conserved from yeast to humans, controls several AS events in *Arabidopsis* plants grown at warm temperatures and modulates the effects of cold temperatures on a large subset of AS events, including many associated with core clock genes. These molecular events are tightly linked to the physiological role we identified for GEMIN2 in regulating the circadian period at warm temperatures, as well as in buffering the effects of temperature on the pace of the circadian clock in *Arabidopsis*. GEMIN2 is also essential for acclimation and survival under extended exposure to mild cold conditions.

Recent work suggests that changes in the level and/or activity of previously considered core spliceosomal proteins may influence AS by altering the kinetics and/or order of recruitment of core spliceosomal proteins during spliceosome assembly (11). In agreement with the idea that spliceosome assembly plays a critical role in regulating AS, depletion or inactivation of SMN in mammals results predominantly in defects in AS rather than in constitutive pre-mRNA splicing (18). Whereas initial studies on the regulation of snRNP biogenesis focused mainly on the role of SMN, recent crystallographic studies suggest that GEMIN2 has a more prominent role in controlling this process (9). Interestingly, we found that *Arabidopsis* lacks a true ortholog of SMN and, therefore, GEMIN2 is the only member of the SMN complex that is present in all organisms from yeast to humans. We found that *gemin2 Arabidopsis* mutants are viable and fertile, strongly suggesting that neither SMN nor GEMIN2 are essential under normal growth conditions. We did observe, however, that *gemin2* has reduced levels of specific snRNAs, in particular U1, which strongly suggests that GEMIN2 is important for the assembly of normal levels of U1 snRNP, the most abundant of the snRNP particles. This defect in snRNP assembly correlates with significant defects in the pre-mRNA splicing of a number of genes in plants grown at 22 °C. Splicing defects are more frequent among AS events than among constitutively spliced introns, most of which are fully spliced, similar to what has been reported for SMN in mammals (18). Some of the AS events affected in *gemin2* mutants are associated with core clock genes such as *TOC1* and *PRR9* and, indeed, the circadian period of *gemin2* mutant results from the overall balance of its effects on several clock genes. Thus, GEMIN2 controls the assembly of some snRNP particles under normal growth conditions and thereby modulates several AS events and physiological processes.

Interestingly, the AS patterns of the *TOC1* and *CCA1* core clock genes in *gemin2* mutants resemble those observed for these genes in wild-type plants under low temperature conditions. Furthermore, global RNA-seq analysis revealed a large overlap between the AS defects present in *gemin2* plants grown at 22 °C and the AS changes induced by cold temperatures in wild-type plants. This overlap was not caused by reduced assembly of U1 snRNP under cold conditions in wild-type plants, because we observed increased rather than reduced U1 snRNA levels after 24 h at 10 °C. These observations suggest that U1 snRNP functionality might be limiting at low temperatures and plants may compensate for this by enhancing U1 snRNA synthesis and snRNP assembly.

Two different observations support the idea that spliceosomal function may be impaired at low temperatures in wild-type plants. First, we found that the changes in the AS of *U1-70K* in wild-type plants exposed to cold conditions resembled those found in mammalian cells with reduced functional U1 snRNP levels (25), as well as in *gemin2* mutant plants, which are impaired in U1 snRNP assembly. Indeed, in both mammals and *Arabidopsis* plants, the AS of *U1-70K* mRNA, a core component of the U1 snRNP, results in two different mRNAs, one encoding a fully functional protein and the other introducing a premature termination codon that targets the mRNA to NMD pathway. Depleting the levels of another U1 snRNP component in mammalian cells, U1C, triggers compensatory increases in the levels of the functional *U1-70K* mRNA isoform that result in increased U1-70K protein levels (25). Given the conservation of this homeostatic mechanism from plants to mammals, we expect that the increased levels of the functional U1-70K mRNA isoform observed in wild type plant at low temperatures will be associated with higher levels of U1-70K protein. Taken together, these observations suggest that wild-type plants exposed to cold conditions have limiting levels of functional U1 snRNPs and react to this deficit with changes in AS, leading to higher levels of the functional *U1-70K* isoform, which, together with the increased levels of U1 snRNA, result in higher levels of U1 snRNP particles. Second, the expression of several RNA processing factors, including some involved in pre-mRNA splicing, was strongly enhanced in plants exposed to cold conditions for 24 h. Similar observations have been made in zebrafish, where exposure to cold affects the expression and splicing of many genes, including core clock genes, and is associated with an enrichment in genes encoding spliceosomal proteins (30). Therefore, several parallel mechanisms appear to operate simultaneously to enhance spliceosomal activity under cold conditions and we propose that GEMIN2 is a component of one of these compensatory mechanisms. Indeed, in the absence of functional *GEMIN2*, cold conditions affect the AS pattern of many genes that do not normally show changes in AS patterns in response to cold conditions in wild-type plants, and *gemin2* mutants perish after being exposed to cold conditions for several weeks, whereas wild-type plants do not. Consistent with the idea that a key role of GEMIN2 is to enhance U1 snRNP assembly under cold conditions in eukaryotic organisms, the *GEMIN2* ortholog in yeast, known as Bad Refrigeration Response 1 (BRR1), was originally identified in a genetic screen for cold sensitive pre-mRNA splicing mutants (31), and later shown to encode a key regulator of U1 snRNP assembly (32).

The period of circadian rhythms is temperature compensated, i.e., shows only modest changes over a broad range of temperatures, allowing circadian clocks to function as time measuring devices throughout the year (33). By contrast, temperature compensation is disrupted in the *gemin2* mutant, resulting in significant period lengthening in response to cold conditions. Strikingly, GEMIN2 has similar effects on the AS of the clock gene *TOC1* at both 22 °C and 10 °C (Fig. S9*A*). If defective AS of *TOC1* were the main mechanism through which *GEMIN2* affected the clock, the short period phenotype of *gemin2* at 22 °C would also be observed at 12 °C. The finding that the circadian period is similar in *gemin2* and wild-type plants at 12 °C strongly suggests that, at low temperatures, *gemin2* affects the AS of other clock genes, which result in period-lengthening effects that balance the period-shortening effect associated with *TOC1* intron 4 retention (Fig. 4*F* and Fig. S9*B*). Indeed, increased intron retention in *PRR9* contributes to attenuate the period-shortening effect of *gemin2* at 22 °C (Fig. S4). However, *PRR9* and *PRR7* are unlikely to be the targets of *GEMIN2* that lengthen circadian period at low temperatures because their effects are larger at intermediate and high temperatures, but disappear at low temperatures (26). Thus, the clock genes that lengthen the period of *gemin2* at low temperatures remain to be identified. Finally, we

do not think that GEMIN2 is part of a specific molecular mechanism that evolved to modulate the effects of temperature on the circadian clock. Recent evidence indicates that temperature compensation in plants depends on the overall balance of temperature-dependent period-lengthening and -shortening effects (34). Here we propose that GEMIN2 acts as a global modulator of AS, particularly under cold conditions, and temperature compensation depends on GEMIN2 function because, in its absence, low temperatures drastically alter the AS of several clock genes, disrupting the proper balance of period-shortening and -lengthening effects.

Most organisms living on this planet are poikilothermic, i.e., they do not control their own body temperature, and buffering biological processes from daily and seasonal fluctuations in ambient temperature is essential for their survival. Splicing involves extensive remodeling of the interactions between spliceosomal snRNAs and pre-mRNAs (9). Low temperatures are expected to enhance these interactions, reducing the speed of the required rearrangements, and poikilothermic organisms must have mechanisms to compensate for the detrimental consequences of these effects. Our results indicate that GEMIN2 is a key component of one such mechanism that modulates the effects of low temperature on pre-mRNA splicing, helping plants to keep time accurately and survive the cold weather conditions they may face at different times of the day and year. These results are also consistent with the hypothesis that spliceosomal interactions with pre-mRNAs and the capacity to generate transcript variants may act as a thermometer that allows plants to adjust to changes in ambient temperature (35).

Materials and Methods

Plant Material. All of the *Arabidopsis* lines used in this work were of the Columbia (Col-0) accession. The *gemin2-1* (SALK_142993) and *gemin2-2* (SAIL_567_D05) mutants were obtained from the *Arabidopsis* Biological Resource Center. Genotypes were confirmed by PCR using oligonucleotides listed in Dataset S8.

Growth Conditions. Seeds were stratified for 4 d in the dark at 4 °C and then sown onto either soil or solid Murashige and Skoog medium containing 1% agarose. Seedlings were grown under different temperature and light regimes depending on the experiment.

Physiological Measurements. Detailed information is in *SI Materials and Methods*.

qRT–PCR and RNA-Seq Analysis. Detailed information is in *SI Materials and Methods*.

Full methods and any associated references are available in the *SI Materials and Methods*.

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