Journal of Experimental Botany
https://doi.org/10.1093/jxb/erae045 Advance Access Publication 19 February 2024

EXPERT VIEW

The circadian clock and thermal regulation in plants: novel insights into the role of positive circadian clock regulators in temperature responses

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Received 11 October 2023; Editorial decision 30 January 2024; Accepted 19 February 2024

Editor: Steve Penfield, John Innes Centre, UK

Abstract

The impact of rising global temperatures on crop yields is a serious concern, and the development of heat-resistant crop varieties is crucial for mitigating the effects of climate change on agriculture. To achieve this, a better understanding of the molecular basis of the thermal responses of plants is necessary. The circadian clock plays a central role in modulating plant biology in synchrony with environmental changes, including temperature fluctuations. Recent studies have uncovered the role of transcriptional activators of the core circadian network in plant temperature responses. This expert view highlights key novel findings regarding the role of the *RVE* and *LNK* gene families in controlling gene expression patterns and plant growth under different temperature conditions, ranging from regular diurnal oscillations to extreme stress temperatures. These findings reinforce the essential role of the circadian clock in plant adaptation to changing temperatures and provide a basis for future studies on crop improvement.

Keywords: Circadian clock, cold stress, heat stress, *NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED*, *REVEILLE*, temperature responses, thermomorphogenesis, thermoperiodic entrainment, transcriptional regulation.

Introduction

Global temperatures are set to reach a record of 1.5 °C above pre-industrial levels between 2030 and 2052, and this will affect crop yields (Prasad and Jagadish, 2015; Lee *et al.*, 2023). Understanding how plants respond to temperature change is crucial for developing heat-resistant crop varieties. The circadian clock is a master regulator of plant life and plays a central role in modulating plant responses to environmental changes. It ensures timely stress responses and synchronizes plant biology with environmental cues such as light and temperature (Seo and Mas, 2015; Creux and Harmer, 2019). The circadian clock controls various processes, including gene expression, flowering, and responses to stress, with the temperature response being a significant factor. The clock network is complex and involves multiple interconnected regulatory transcriptional feedback loops. This expert view focuses on the interplay between temperature responses and the families of circadian clock regulators that operate as transcriptional activators.

At the core of the circadian network is a relatively small number of genes encoding proteins of a central oscillator that comprises the circadian clock per se. These genes are expressed

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at different times of the day, and the proteins that they encode regulate the activity and expression of other members of the circadian network. While the majority of the components of the central oscillator are negative regulators of gene expression, members of two gene families, termed *REVEILLE 8* (*RVE8*) and *RVE 4*, along with *NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED GENE 1* (*LNK1*) and *LNK2*, form an activation loop that positively regulates the expression of other clock genes (Rawat *et al.*, 2011; Hsu *et al.*, 2013; Rugnone *et al.*, 2013; Xie *et al.*, 2014) (for a condensed model of this network, see Fig. 1).

Light is a critical factor for photosynthetic organisms, and extensive research has explored the connection between light signalling, the circadian clock, and light responses (Sanchez *et al.*, 2020). Although the role of temperature as a circadian input and that of the circadian clock in regulating thermal responses remain incompletely understood, there is a clear link between the circadian clock of plants and their perception and response to temperature. To better understand this connection, we distinguish between two scenarios:

- Mild ambient temperatures (12–28 °C): temperature fluctuations during the day and night, even without light cues, can entrain the core oscillator. This phenomenon is known as 'thermoperiodic entrainment' and helps maintain circadian rhythms in synchrony with the environment (Michael *et al.*, 2003; Salomé and McClung, 2005a; Avello *et al.*, 2019). In addition, another mechanism, called 'temperature compensation', ensures stable clock function throughout the seasons, maintaining a consistent ~24 h circadian period despite sustained alterations in the average ambient temperature (Gould *et al.*, 2006; Avello *et al.*, 2019).
- Extreme temperatures (<12 °C or >28 °C): beyond the normal range, temperature is a stress factor capable of disrupting plant function and survival. Under these conditions, plants activate various response pathways, some of which are regulated by the circadian clock. Consequently, plants display varying levels of resistance to thermal stress throughout the day due to 'circadian gating'. This regulation restricts peak responsiveness to specific times of day, aligning with the likelihood of stress occurrence while minimizing the negative impact of resource allocation on crucial processes such as growth (Seo and Mas, 2015). The gating of cold- and heat-stress responses has been previously reported in *Arabidopsis thaliana* and other plant species (Fowler *et al.*, 2005; Covington *et al.*, 2008; Thomashow, 2010; Artlip *et al.*, 2013; Rienth *et al.*, 2014; Grinevich *et al.*, 2019; Li *et al.*, 2019; Graham *et al.*, 2023).

Until recently, most of the knowledge regarding clock–temperature cross-talk in plants has been limited to the role of negative regulators of the circadian central oscillator (Grundy *et al.*, 2015; Gil and Park, 2019; Mody *et al.*, 2020; Laosuntisuk and Doherty, 2022). In the past 2–3 years, several studies have uncovered relevant roles for two families of activators and coactivators of the clock: REVEILLE (RVE) and NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED (LNK). In this expert view, we summarize the most recent findings regarding the role of the families of circadian clock transcriptional (co-)activators in plant temperature responses.

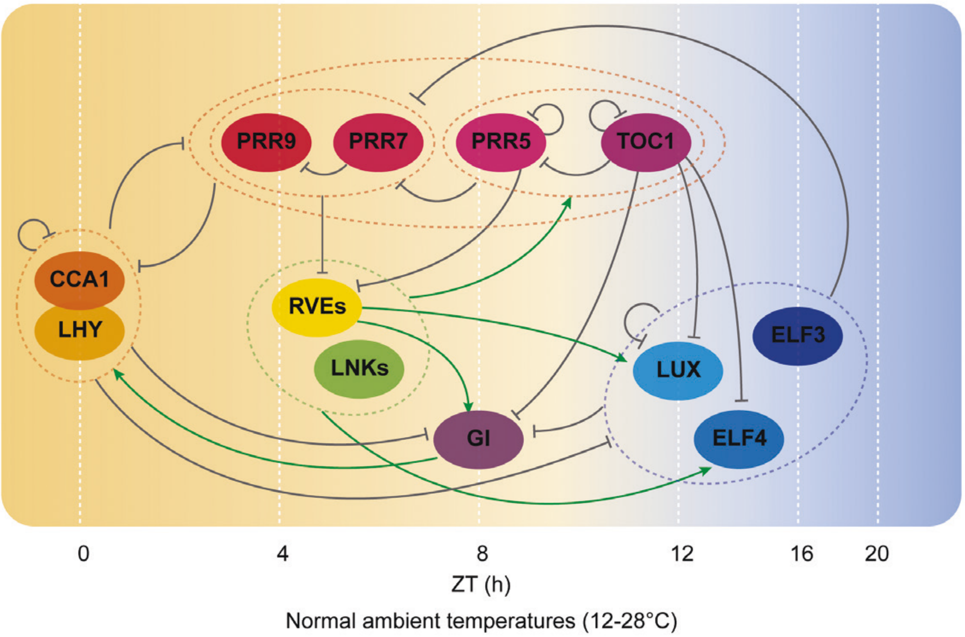

Fig. 1. Simplified model of the regulatory network that constitutes the central oscillator of the circadian clock in plants. From left to right, the sequential expression of each component throughout the day is represented, and the peak activity is expressed in hours from dawn. The orange and blue areas represent day and night, respectively. Grey lines represent repression and green lines represent transcription activation.

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Involvement of the LNK and RVE families in the integration of thermal information under non-stress conditions

The role of LNK *genes in thermoperiodic entrainment*

The LNK proteins serve as coactivators of RVE proteins in the positive regulation of the circadian clock of plants (Fig. 1). The LNK family comprises four closely related members (LNK1, LNK2, LNK3, and LNK4), each of which has distinctive and overlapping functions (de Leone *et al.*, 2018, 2020). LNK1 and LNK2 interact with RVE4 and RVE8, recruiting the transcriptional machinery to the promoters of target genes and thereby inducing transcription (Rugnone *et al.*, 2013; Xie *et al.*, 2014; Perez-Garcia *et al.*, 2015). *LNK1* and *LNK2* are more strongly induced by light in the middle of the night, when the clock is most responsive to this signal. This integration of early light signals with temporal information provided by core oscillator components allows the control of gene expression in the afternoon (Rugnone *et al.*, 2013), enabling plants to track seasonal changes in day length. Similarly, LNK1 and LNK2 also appear to play an important role in the integration of thermal cues under non-stress conditions (Box 1). In a recent investigation, Sorkin *et al.* (2023) identified a novel role of LNK1 and LNK2 in entraining the circadian central oscillator in response to temperature cycles. Under free-running conditions (continuous white light), the double mutant *lnk1;2* exhibited a characteristic long-period phenotype, leading to delayed expression profiles of core clock genes such as *CCA1*. However, under light cycles, the wild-type phenotype was restored because of the accurate integration of the temporal light cues. In contrast, when the daily cycles were defined by subtle temperature variations (e.g. 'putative day' = 12 h at 22 °C and 'putative night' = 12 h at 20 °C), plants lacking LNK1 and/or LNK2 were unable to resynchronize. This defect was mitigated when the temperature difference between the minimum and maximum values was changed from 2 °C to 4 °C. LNK3 and LNK4 do not appear to be involved in this process, as the double mutant *lnk3;4* resembles the wild type regardless of whether they are grown in light or temperature cycles. Data obtained from affinity purification coupled with mass spectrometry (APMS) assays suggest that the role of LNK1 and LNK2 in the temperature entrainment of the clock could be related to their interaction with multiple components of temperature-response pathways, such as COR27 and COR28 (Li *et al.*, 2016; Wang *et al.*, 2017; Sorkin *et al.*, 2023). Whether RVEs also play a role in this process remains to be determined.

The role of RVE *genes in thermomorphogenesis*

Plants perceive sustained changes in temperature as environmental cues that trigger adaptive responses. Continuous warm temperatures initiate a series of physiological and developmental changes, including hypocotyl/petiole elongation, leaf hyponasty, and accelerated flowering, in a process collectively known as thermomorphogenesis. Many clock components have been implicated in the regulation of growth responses associated with this process (Zhang *et al.*, 2021). Two studies suggested that two members of the RVE family are involved in the regulation of hypocotyl elongation during thermomorphogenic responses. *RVE* genes belong to the MYB transcription factor family, which includes other well-characterized clock components such as CCA1 and LHY (Rawat *et al.*, 2009; Kidokoro *et al.*, 2022). The RVE family comprises eight *RVE* genes, designated *RVE1* to *RVE8*. Among the *RVE* genes, *RVE8* functions partially redundantly with *RVE4* and *RVE6* and plays the most prominent role in regulating circadian rhythms (Hsu *et al.*, 2013). In contrast, other members of this family, such as RVE5 and RVE7, have only a minor effect on circadian regulation (Rawat *et al.*, 2009; Gray *et al.*, 2017).

In one study, a relevant yet contrasting role was discovered for RVE5 and RVE7 in the regulation of warm-induced hypocotyl elongation, mediated by the control of ELF4. ELF4, in conjunction with ELF3 and LUX ARRHYTHMO (LUX), forms the evening complex (EC) and plays a pivotal role in regulating the circadian clock (Nusinow *et al.*, 2011). ELF3 has been proposed as a significant thermosensor, acting in both an EC-dependent and an EC-independent manner; its

Box 1. Key novel advances in the regulation of circadian function and circadian clock outputs in response to non-stressing temperature shifts

- LNK1 and LNK2 have been identified as essential for proper entrainment of the circadian clock in response to subtle temperature changes in a process known as thermoperiodic entrainment. A possible explanation for this could be related to the identification of the direct interaction between LNK and COR27 and COR28 proteins (Sorkin *et al.*, 2023).
- Two recent studies found further evidence that clock components and clock auxiliary genes play a central role in the coordination of growth and development in synchrony with external thermal changes. Consequently, contrasting roles of RVE5 and RVE7 as negative and positive regulators of thermomorphogenesis, respectively, have been proposed (Li *et al.*, 2023; Tian *et al.*, 2022.

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functions have been extensively reviewed elsewhere (Zhang *et al.*, 2021; Laosuntisuk and Doherty, 2022). ELF4 has also been suggested as a modulator of thermomorphogenesis; however, the mechanisms governing ELF4 activity in plants during thermomorphogenesis remain poorly understood. Tian *et al.* (2022) found that in response to warm temperatures, the expression of the *RVE7* gene is up-regulated. *RVE7* encodes a protein that directly binds to the evening element (EE) in the promoter of *ELF4*, thereby repressing *ELF4* expression. This leads to a reduction in the influence of the EC on its target genes, including *PIF4*, a master positive regulator of thermomorphogenesis (Tian *et al.*, 2022). Consequently, when the temperature increases, RVE7 acts as a positive regulator of the adaptive responses. Interestingly, the same research group found that RVE5 plays the opposite role in thermoresponsive growth. Li *et al.* (2023) proposed a mechanism by which RVE5 fine-tunes *ELF4* expression by competing for a *cis*-element with the stronger transcriptional repressor CCA1. Binding of RVE5 to the EE in the *ELF4* promoter under warm temperatures attenuates the repression exerted by CCA1, leading to increased *ELF4* transcript levels. Consequently, RVE5 promotes the assembly of the EC and the concomitant repression of its target genes. Thus, RVE5 indirectly represses *PIF4* and its downstream genes, inhibiting growth responses under warm conditions. These novel findings, along with those of previous studies, reinforce the idea that the circadian clock plays a central role in plant growth regulation under warm temperatures. In line with this, future studies should take into account the interplay between light signalling and circadian regulation, along with growth-related hormones such as auxins, in order to fully comprehend the complexity of this process.

Circadian regulation of responses to temperature stress

Circadian gating of transcriptional and translational responses to heat stress

Heat stress rapidly affects gene expression (Li *et al.*, 2019; He *et al.*, 2023), and transcriptomic approaches in numerous crop species, including rice, tomato, barley, maize, wheat, soybean, *Brassica* spp., and grape, have successfully identified relevant genes and gene families associated with heat responses (Janni *et al.*, 2020). Historically, heat-stress studies have been conducted at single time points, but work considering different times of day revealed distinct responses, such as maximum heat tolerance in Arabidopsis seedlings at dusk (Grinevich *et al.*, 2019; Li *et al.*, 2019).

Circadian regulation of transcription is common and affects more than 30% of the transcriptome in various plant species (Grundy *et al.*, 2015; Romanowski *et al.*, 2020). In Arabidopsis, up to 50% of stress-responsive genes exhibit circadian regulation (Harmer *et al.*, 2000; Hotta *et al.*, 2007; Covington *et al.*, 2008), and transcriptional responses to heat stress vary throughout the day, with approximately 70% of heat-responsive genes showing temporal variations at dawn and dusk (Michael *et al.*, 2008; Blair *et al.*, 2019; Grinevich *et al.*, 2019). The involvement of the central oscillator in this regulation becomes evident when analysing transcriptional responses under free-running conditions (Grinevich *et al.*, 2019), and when comparing wild-type and core clock mutant plants (Blair *et al.*, 2019). As a result, previous studies have identified several roles for clock components in response to elevated temperatures. These cover a wide range of regulatory processes that span from the involvement of CCA1/LHY and PRR7/PRR9 in the transcriptional regulation of stress-related genes (Blair *et al.*, 2019) to a role for ZTL and GI in the post-translational regulation of heat responses (Cha *et al.*, 2017; Gil *et al.*, 2017).

In a recent study by Bonnot and Nagel (2021), the circadian transcriptional regulation of heat responses was further assessed, as the authors explored the effect of a 1 h heat shock at 37 °C at several time points (every 3 h in the course of one whole subjective day) (Box 2). Moreover, by employing translating ribosome affinity purification (TRAP) sequencing in conjunction with RNA sequencing, the authors were able to gain a complete insight into the dynamic regulation of gene expression at growth-limiting temperatures (Zanetti *et al.*, 2005). Under normal growth conditions at 22 °C, 8028 transcripts (total mRNA) cycled, and 10 657 transcripts displayed circadian oscillations at the translatome level (TRAP mRNAs). Among these transcripts, 667 TRAP-specific cycling transcripts were previously not associated with circadian regulation at the transcriptional level, suggesting a specific rhythmic control at the level of the translatome. This result serves as a novel example of the intricate, multilayered regulation of circadian gene expression.

In response to heat stress, 3289 transcripts (total mRNA) and 4524 transcripts (TRAP mRNAs) responded differently at one or more times of the day, representing an overall number of 5445 circadian differentially regulated genes (DRGs). Core clock components show alterations in total and TRAP mRNA transcript levels in response to heat. In most cases, the magnitude of the response varies throughout the day. The dynamic responses of *LNK1*, *LNK3*, and *LNK4* are clear examples (Fig. 2). As seen for the *LNK* genes, among the most up-regulated DRGs, the highest up-regulation response is achieved when heat is applied at times other than the peak expression time. In contrast, for a high proportion (43%) of down-regulated DRGs, significant down-regulation occurred during the peak mRNA accumulation. The authors proposed that when stress occurs at the lowest expression levels, many genes involved in heat responses are selectively transcribed and translated, whereas genes that show peak expression at that time, but are not related to stress responses, are not. Thus, it appears that transcriptional and translational priorities are redefined under heat stress. Among the transcription factors that seem to have priority access to ribosomes under heat stress (i.e. up-regulated differentially throughout the day), more than half are direct

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Box 2. Key recent developments in the study of circadian gating of transcriptional and translational responses to temperature stress

Novel high-throughput sequencing studies have revealed circadian gating of the genome-wide transcriptional response to abiotic stress generated by high (Bonnot and Nagel, 2021) and low (Graham *et al.*, 2023) temperatures in *Arabidopsis thaliana* and bread wheat (*Triticum aestivum*), respectively.

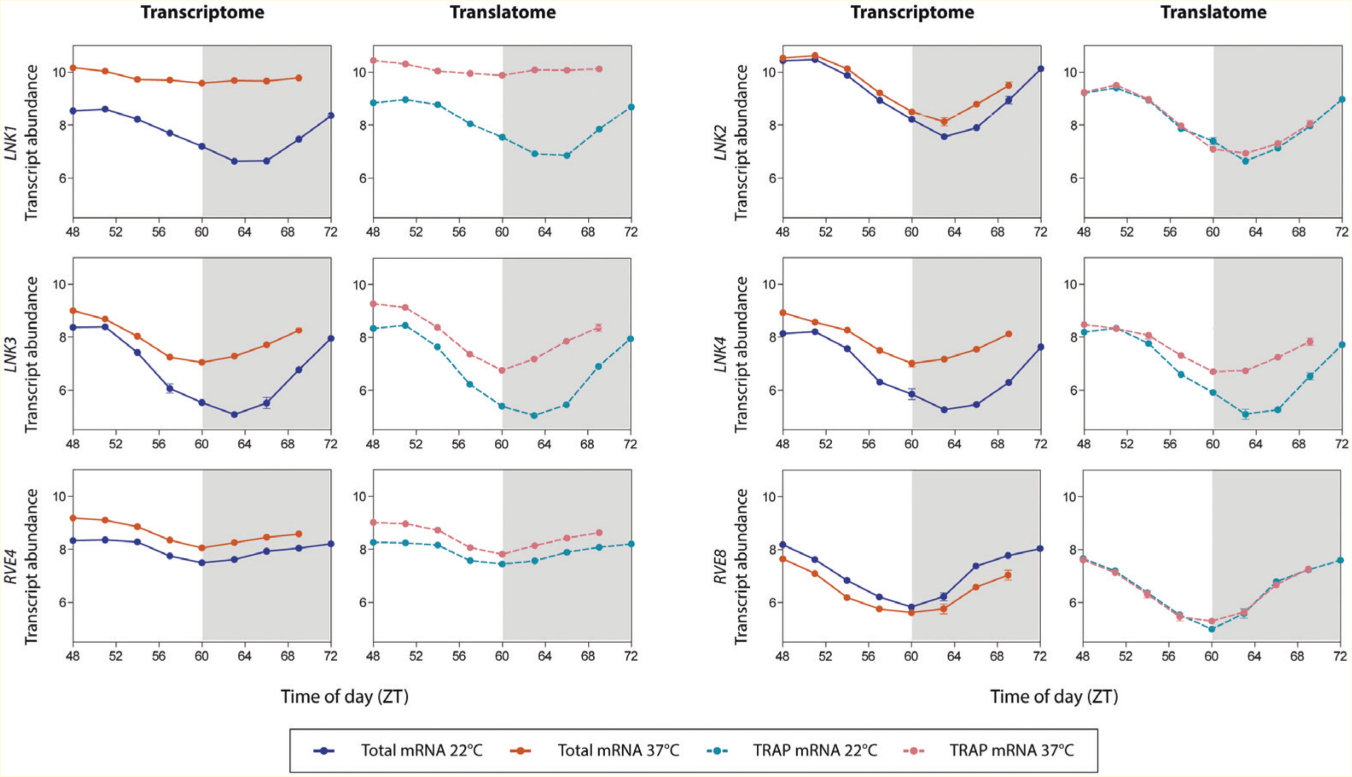

Fig. 2. Transcript abundance of the whole *LNK* gene family and *RVE4* and RVE*8* in total and TRAP mRNAs at 37 °C and 22 °C, as reported by Bonnot and Nagel (2021).

clock targets, according to previous chromatin immunoprecipitation and sequencing (ChIP-seq) analysis. These include the CDF, BBX, and Myb-related families of transcription factors. Similar to other well-described master regulators, such as DREBs and HSFs, these transcription factors are also involved in other stress responses, positioning them as potential regulatory hubs in the coordination of external signals with the endogenous developmental state throughout the 24 h of the day (Ohama *et al.*, 2017).

Circadian gating of transcriptional and post-transcriptional responses to cold stress

Dynamic changes in the regulation of gene expression and transcript levels also occur during cold acclimation. Previous studies in *A. thaliana* have suggested that within the first few hours of cold exposure, a reconfiguration in the expression and alternative splicing of cold-response pathways, such as the signalling cascade involving the *C-REPEAT BINDING FACTOR/DEHYDRATION RESPONSE ELEMENT BINDING PROTEIN* (*CBF/DREB*) and clock components, might be involved in initial responses to cold and regular 24 h day/night temperature cycles (Calixto *et al.*, 2018). Circadian gating of cold responses has been reported for specific Arabidopsis genes (Fowler *et al.*, 2005; Bieniawska *et al.*, 2008; Mikkelsen and Thomashow, 2009). These include genes in the CBF pathway, which has been proposed as a circadian clock output positively regulated by CCA1/LHY (Dong *et al.*, 2011) and negatively regulated by PRR5/PRR7/TOC1 (Nakamichi *et al.*, 2009, 2012; Keily *et al.*, 2013; Liu *et al.*, 2013), which in turn acts as a temperature input for the circadian clock through the transcriptional regulation of LUX (Chow *et al.*, 2014). However, until recently, there was a lack of knowledge regarding the circadian gating of cold-temperature responses at a genome-wide

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scale in plants. Graham *et al.* (2023) performed the first high-throughput RNA-seq analysis of hexaploid bread wheat (*Triticum aestivum*) seedlings exposed to 3 h treatments with cold temperature (4 °C) at sequential time points under free-running conditions. The authors identified a varying number of differentially expressed genes at each time point and found a stronger transcriptional response around the middle of the subjective day (ZT32) and towards the end of the subjective night (ZT48). Some transcripts were responsive only at a certain time of the day, whereas others responded at various time points. For some transcripts, the peak of cold responsiveness was aligned with the underlying circadian rhythm under controlled-temperature conditions; however, in others, it was not. To quantify the extent of circadian gating of the cold-responsive genome, the authors measured the magnitude of the response for each gene at each time point and identified fluctuations over 24 h in the sensitivity of 1677 transcripts that showed circadian oscillations in their transcriptional response. This is the case for, for example, the *CBF* ortholog (*TaCBFIVc-B14*), which shows circadian expression under control conditions and a circadian gating of cold responsiveness. Furthermore, it was found that for 882 transcripts, stabilization of transcript abundance during cold treatment at each time point resulted in a displacement of the oscillation of the transcript. This was the case for several clock components. However, the effect of cold treatment was not consistent for all wheat oscillator components, and in some cases cold treatment did not equally affect all cycling homologs for each gene. These results reflect the complexity of the interplay between the circadian clock and cold responses at the transcriptional level. Future work on this matter could unveil whether this effect can act as a *zeitgeber* for the wheat circadian clock in a similar way to the way in which moderate temperature changes shift the circadian phase in *A. thaliana* (Salomé and McClung, 2005b).

Role of the RVE and LNK families of clock (co-)activators in heat responses

Heat shock factors (HSFs) are well-characterized master transcriptional regulators involved in modulating plant transcriptional responses. HSFs, such as HSFA1s, bind to heat shock regulatory elements in the promoters of heat shock response genes, thereby regulating their expression and promoting thermotolerance (Liu *et al.*, 2011; Yoshida *et al.*, 2011). In parallel, other pathways independent of HSFs also play a significant role in activating heat shock responses (Box 3). One of these pathways is mediated by core clock components and members of the REVEILLE family of transcriptional activators, specifically RVE4 and RVE8 (Li *et al.*, 2019). Accordingly, many heat-responsive genes are still induced in the *hsfa1-qk* quadruple mutant, but not in the *rve4;8* double mutant, which shows impaired basal and acquired thermotolerance. The EE motif, which is bound by RVE4 and RVE8, is enriched among heat-responsive genes, and RVE8 binds to heat-responsive gene promoters only under heat shock conditions, unlike its temperature-independent binding to clock targets such as *PRR5* (Li et al., 2019). Additionally, the overexpression of another RVE8 circadian target, *TOC1*, reduces acquired heat tolerance, whereas a *prr5toc1* double mutant exhibits enhanced thermotolerance (Rawat *et al.*, 2011; Zhu *et al.*, 2016). This suggests that the involvement of RVE8 in the heat response may be independent of its role in clock regulation. *RVE4* and *RVE8* are circadian-regulated (Rawat *et al.*, 2011), and a brief heat shock (<15 min) does not appear to alter their expression levels (Li *et al.*, 2019). However, longer treatments (>30 min for *RVE8* and 1 h for *RVE4*) affect their expression profiles (Bonnot and Nagel, 2021) (Fig. 2). Heat shock experiments conducted with the *rve4;8* mutant revealed that these genes are essential for survival even after a brief treatment of just 10 min (Li *et al.*, 2019). Hence, there must be mechanisms other than transcriptional regulation that explain the activation of these proteins during the early stages of the heat response. Heat stress triggers the relocation of RVE4 and RVE8 from the cytosol to the nucleus (Kidokoro *et al.*, 2021). However, interactions with other proteins may also account for the RVE-mediated temperature responses.

Similar to what happens with *LNK1* in the response to a light stimulus, the expression of this gene is more strongly induced by heat at night (Mizuno *et al.*, 2014; Bonnot and Nagel, 2021). The regulation of *LNK1* expression in the response to temperature changes appears to be mediated by the EC. According to the model proposed by Mizuno *et al.* (2014), warm temperatures antagonize EC repressor activity, releasing *LNK1* repression, whereas cool temperatures stimulate it (Fig. 3). Consequently, the simultaneous induction or repression of *LNK1* expression, along with that of other EC-regulated clock components, such as *PRR9*, *PRR7*, *LUX*, and *GI*, could serve as a thermosensing mechanism that allows temperature cues to be input into the circadian network (Mizuno *et al.*, 2014).

Kidokoro *et al.* (2023) further characterized the role of the *LNK* genes in heat responses, confirming their significant involvement in the RVE-mediated response pathway. LNK1 and LNK2 appear to be necessary for the induction of RVE-activated heat-responsive genes such as *ERF53* and *ERF54*. In line with this, subjecting null mutants for multiple *LNK* and *RVE* components (*rve4;8*, *rve3;4;5;6;8*, *lnk1/2*, and *lnk1;2;3;4*) to a strong heat treatment (45 °C) following an acclimation process (37 °C) severely affects growth. Notably, although the negative impact on chlorophyll content is significant, it is not as severe as that observed in mutants affecting other heat shock response regulators, such as *hsfa1abd*. This observation suggests that the roles of these gene families are more related to promoting growth under extreme conditions than survival (Kidokoro *et al.*, 2023).

Role of the RVE and LNK families of clock (co-)activators in cold responses

The interplay between the RVE and LNK families in the temperature response extends beyond the heat response. Indeed,

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Box 3. Key recent findings identified *RVE* and *LNK* families as co-regulators of heat and cold responses

By implementing reverse genetic strategies involving multiple null mutants for members of the *RVE* and *LNK* gene families, Kidokoro *et al.* (2021, 2023) identified a role for these transcriptional activators in the regulation of temperature-stress responses. While RVE4 and RVE8 participate in both heat and cold responses, LNK1 and LNK2 have a prominent role at high temperatures, and LNK3 and LNK4 become more relevant at low temperatures.

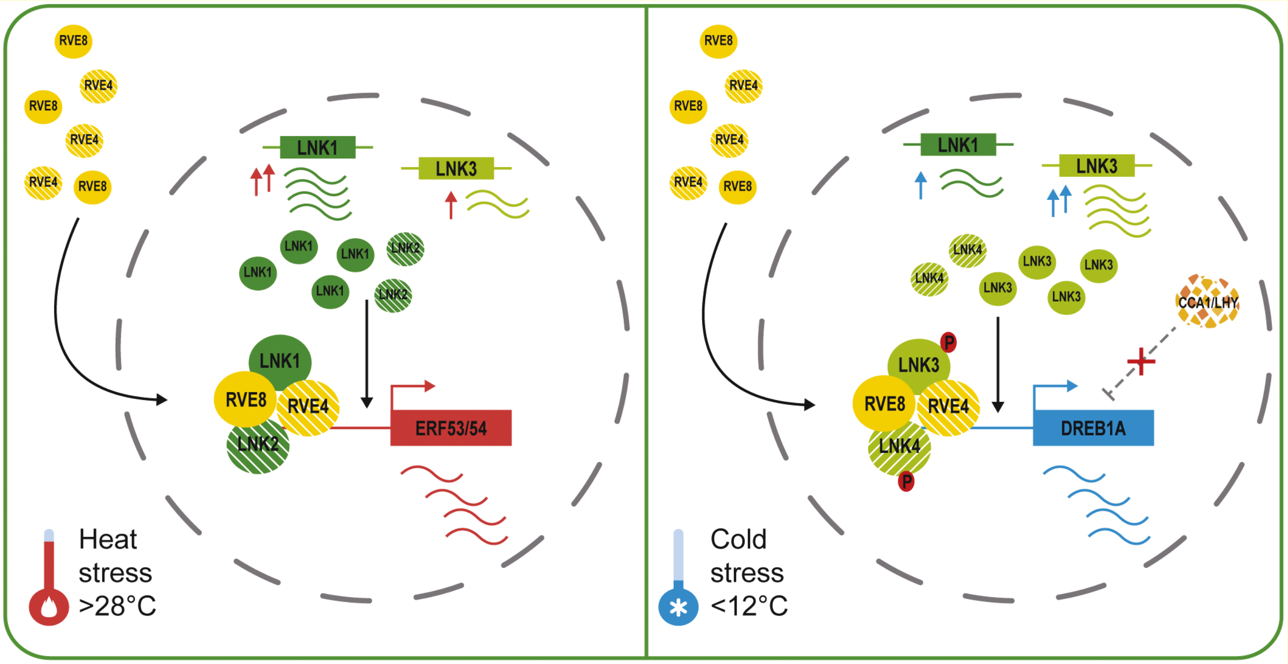

Fig. 3. Representation of the proposed model for the activation of *LNK* gene expression in response to high and low temperatures (Mizuno *et al.*, 2014; Bonnot and Nagel, 2021; Kidokoro *et al.*, 2023) and their consequent role as co-activators of the RVE proteins in the transcriptional activation of the thermal stress response (Kidokoro *et al.*, 2021, 2023).

dynamic interactions among members of each group may represent a mechanism that regulates specific target genes in response to varying temperature conditions.

The role of RVEs in the cold response has been previously documented. Kidokoro *et al.* (2021) discovered that RVE4 and RVE8 are essential for the activation of the cold master regulator *CBF*/*DREB* genes. These genes initiate the cold-responsive transcriptional cascade necessary for the cold-stress response and tolerance (Yamaguchi-Shinozaki and Shinozaki, 2006; Kidokoro *et al.*, 2022). Consequently, mutant plants lacking RVE4 and RVE8 exhibit increased susceptibility to freezing conditions owing to the impaired activation of DREB1A and its downstream transcriptional targets (Kidokoro *et al.*, 2021). Under normal ambient temperatures, RVE4 and RVE8 are predominantly located in the cytoplasm. However, at lower temperatures, both proteins undergo modifications, resulting in shifted bands, and rapid relocation to the nucleus is observed when the temperature decreases below 10 °C. This translocation correlates with increased binding of RVE4 and RVE8 to the EEs in the promoter region of *DREB1A*. On the other hand, the authors also noted that the binding of LHY and particularly CCA1 to the same regions decreased during the transition from ambient to cold conditions, and that the degradation of CCA1 and LHY under cold-stress conditions is crucial for the full activation of *DREB1A*. Thus, they proposed a model in which CCA1/LHY binding to the EE motif of *DREB1A* represses its expression under normal growth conditions. Subsequently, as the temperature decreases and CCA1/LHY are degraded, coordinated shuttling of RVE4/RVE8 to the nucleus results in the direct activation of *DREB1A* through RVE binding to the same EE motif. Taking into account that previous investigations had proposed a role for CCA1/LHY as activators of the expression of *DREB* genes under cold conditions, the findings of Kidokoro *et al.* (2021) suggest that this

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positive regulation might be indirect. Still, the precise molecular mechanism involved in this regulation remains unclear.

More recently, the same group extended this model to encompass the role of the entire LNK family in temperature-stress responses. The authors discovered that all LNK proteins can physically interact with RVE4 and RVE8 and that under both normal and cold conditions, they are all expressed and located in the nucleus. Moreover, LNK3 and LNK4, but not LNK1 or LNK2, appear to undergo cold-specific phosphorylation. Surprisingly, under cold-stress conditions, LNK3 and LNK4 seem to play the most critical roles in activating cold-responsive genes, such as *DREB1A*, whereas LNK1 and LNK2 contribute to this response only when LNK3 and LNK4 are absent. Consequently, when plants lacking all four *LNK* genes were incubated at 4 °C for 1 d and then exposed to −10 °C for 8 h, they exhibited significantly greater sensitivity to freezing than wild-type plants. Conversely, plants overexpressing *LNK3* and *LNK4* displayed a higher degree of cold tolerance under the same conditions. As expected, RNA-seq analysis conducted after 3 h at 4 °C revealed that the quadruple *lnk1;2;3;4* mutant displayed defects in the induction of cold-responsive genes at the whole-transcriptome level. In fact, the deregulated expression profile of the multiple *lnk1;2;3;4* mutant substantially overlapped with the profile of differentially expressed genes in the quintuple *rve3;4;5;6;8* mutant (Kidokoro *et al.*, 2023). Taken together, these data suggest that LNK3 and LNK4 function as co-activators of *RVE4* and *RVE8* in the induction of cold-responsive genes under cold-stress conditions.

Based on these findings and the previously discussed results regarding heat stress, Kidokoro *et al.* (2023) proposed a dynamic model in which LNK and RVE co-regulate temperature responses in plants (Fig. 3). In this model, under normal growth conditions, RVE4, RVE6, and RVE8 activate the expression of clock-controlled genes in the evening, with LNK1 and LNK2 acting as co-activators. When the temperature decreases to cold-stress levels, RVE4 and RVE8 translocate to the nucleus, and their accumulation, along with the phosphorylation of LNK3 and LNK4, activates the expression of many cold-induced genes, such as *DREB1s*. Conversely, when the temperature rises, the accumulated RVE4 and RVE8 in the nucleus collaborate with LNK1 and LNK2 to induce the expression of heat-inducible genes such as *ERF53* and *ERF54*.

Conclusion

This expert review provides a comprehensive overview of the complex relationship between the circadian clock and temperature response in plants. It highlights the essential role of the circadian clock in regulating plant responses to temperature fluctuations and recent key findings into the role of the LNK and RVE families of transcriptional (co-)activators in temperature responses under both regular and stressful conditions. Further research on this topic is a promising avenue for developing heat-resistant crop varieties that could help mitigate the impact of rising temperatures on agriculture and food security worldwide.

Author contributions

MJdL and MJY: writing—review and editing.

Conflict of interest

No conflict of interest declared.

Funding

This work was supported by the Argentinean National Council of Sciences (CONICET) and the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (PICT ANPCYT).

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