



Circadian coordination of cellular processes and abiotic stress responses

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Abstract

Diel changes in the environment are perceived by the circadian clock which transmits temporal information throughout the plant cell to synchronize daily and seasonal environmental signals with internal biological processes. Dynamic modulations of diverse levels of clock gene regulation within the plant cell are impacted by stress. Recent insights into circadian control of cellular processes such as alternative splicing, polyadenylation, and noncoding RNAs are discussed. We highlight studies on the circadian regulation of reactive oxygen species, calcium signaling, and gating of temperature stress responses. Finally, we briefly summarize recent work on the translation-specific rhythmicity of cell cycle genes and the control of subcellular localization and relocalization of oscillator components. Together, this mini-review highlights these cellular events in the context of clock gene regulation and stress responses in Arabidopsis.

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Introduction

For most multicellular organisms, integration of daily and seasonal environmental cues such as light and temperature is primarily controlled by the circadian clock [1]. In plants, synchrony between fluctuating external stimuli and internal cellular and physiological processes is critical for plant growth and fitness [2]. In Arabidopsis, the primary model for plant circadian studies, the transcriptional

feedback activation, and repression between multiple clock components that are expressed at different times throughout the 24 h period is important for sustaining robust circadian rhythms within the cell [3].

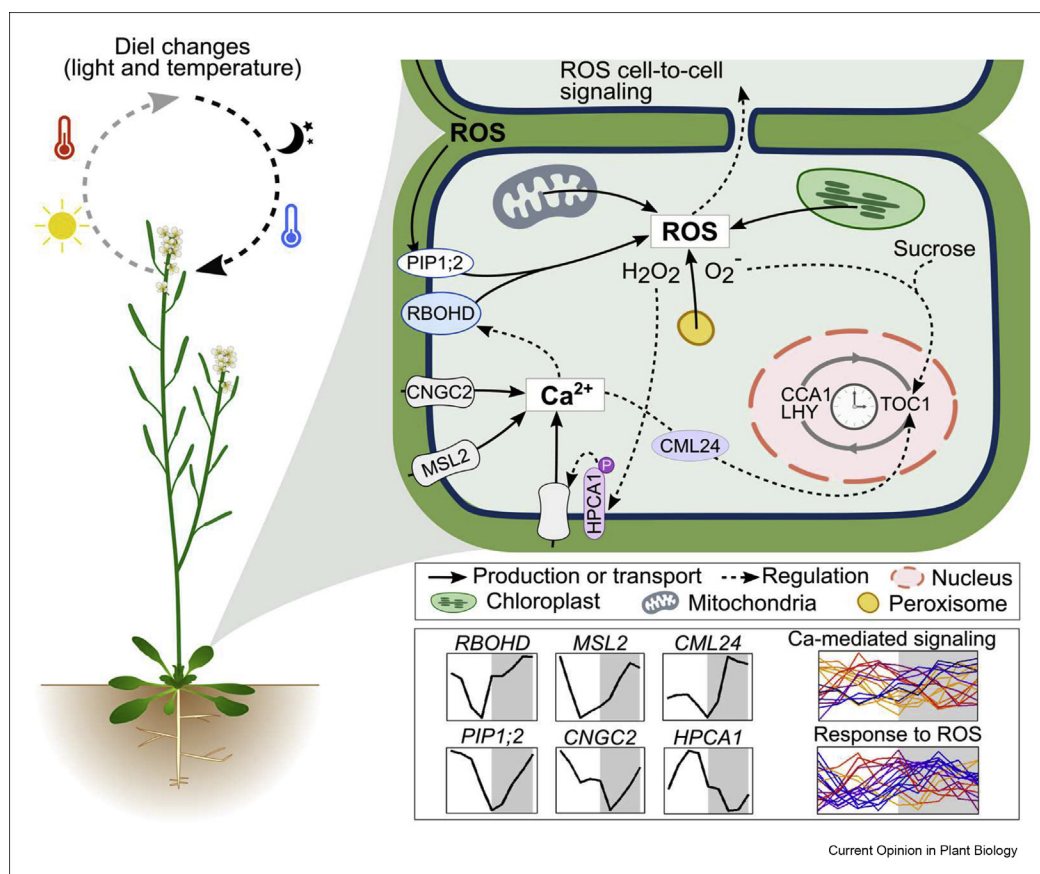
Briefly, the morning expressed clock genes, *CIRCADIAN CLOCK -ASSOCIATED 1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)*, participate in a feedback transcriptional repression between the afternoon expressed *PSEUDO-RESPONSE REGULATOR (PRR)* genes (*PRR5*, *PRR7*, and *PRR9*), the evening-expressed *PRR*, *TIMING OF CAB EXPRESSION1 (TOC1)*, and members of the evening complex (EC), *EARLY FLOWERING 3 (ELF3)*, *ELF4*, and *LUX ARRHYTHMO (LUX)* [4–10]. In addition, a dynamic transcriptional activation regulation involving the mid-day expressed *REVEILLE (RVE)* genes (*RVE4*, *RVE6*, and *RVE8*), the *PRRs*, and the *EC* contribute to the robust functioning of the oscillator [11–13]. Together with several other components that are not described here, accurately timed information is transmitted to key cellular events and communication within the plant cell [3,14–16]. For recent reviews with more comprehensive clock network descriptions in plants, please refer to studies reported by Markham and Greenham [17] and Paajanen et al.[18].

In this mini-review, we briefly discuss the most recent advances in Arabidopsis on how the clock coordinates molecular and cellular processes along with responses to external stress. Specifically, in the first half, we highlight recent work on clock gene regulation as it relates to alternative splicing (AS), polyadenylation, translation, noncoding RNAs, and the rhythmic coordination of second messengers such as reactive oxygen species (ROS) and calcium (Ca^{2+} , Figure 1), primarily in the context of temperature stress. In the second half of this review, we discuss circadian gating of temperature stress responses (Figure 2), clock regulation of cell cycle genes, and dynamic subcellular localization of oscillator components (Box 1, Box 2, and Figure 3).

Circadian regulation of gene expression in plant cells

The clock's precision in conferring robust circadian rhythms relies on the regulation of clock components at

Figure 1



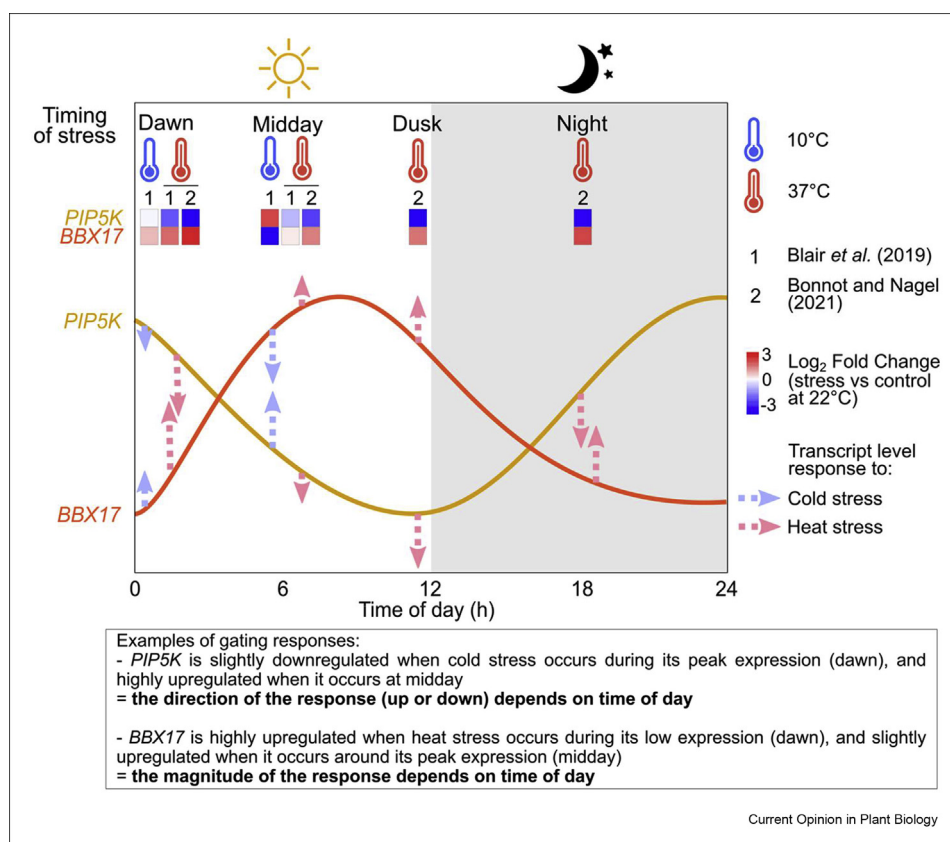
the transcriptional, post-transcriptional, translational, post-translational, and chromatin levels [19–24]. Although most of our understanding of clock gene expression is from knowledge gained at the transcriptional level, deeper insights into other important aspects of clock gene regulation such as post-transcription (AS and polyadenylation), translation, and noncoding RNAs (microRNAs [miRNA] and long noncoding RNAs [lncRNA]) are accumulating and discussed in the following paragraphs.

Alternative splicing and polyadenylation

AS of clock components is impacted by various abiotic stresses [25,26]. Several components of the splicing machines appear to alter the oscillator function through

the regulation of clock genes [25]. A recent high-resolution transcriptomic study determined the extent of circadian-controlled AS events and revealed that Splicing Factor 30 regulates a subset of clock-controlled splicing events, by increasing the baseline level of intron retention events [27]. Furthermore, the *Splicing Factor 30* transcript is induced by heat stress implicating a role for this splicing modulator in rhythmic splicing events and heat stress [21]. Intriguingly, the Romanowski et al., (2021) [27] study also revealed that the rhythmicity of some AS events is independent of rhythmic transcripts suggesting that for a subset of genes in Arabidopsis, clock regulation of AS may occur in the absence of circadian gene expression.

Figure 2



Representation of the circadian gating of temperature stress responses. Transcript abundance for two selected genes exhibiting circadian oscillations is represented: a member of the *Phosphatidylinositol 4-phosphate 5-kinase* (*PIP5K*; *AT1G01420*) family, peaking in the early morning; and *B-BOX DOMAIN PROTEIN 17* (*BBX17*; *AT1G49130*), peaking in the evening. Transcript profiles are simplified and based on data published in Bonnot and Nagel (2021) [21]. Heatmaps represent the temperature stress responses of the selected genes at different times of day and correspond to Log_2 Fold Change values identified in Blair *et al.* (2019) and Bonnot and Nagel (2021) [21,53]. Arrows summarize the transcript abundance level in response to temperature stress, the direction indicating an upregulation (above the line) or a downregulation (below the line), and the length representing the magnitude of the response.

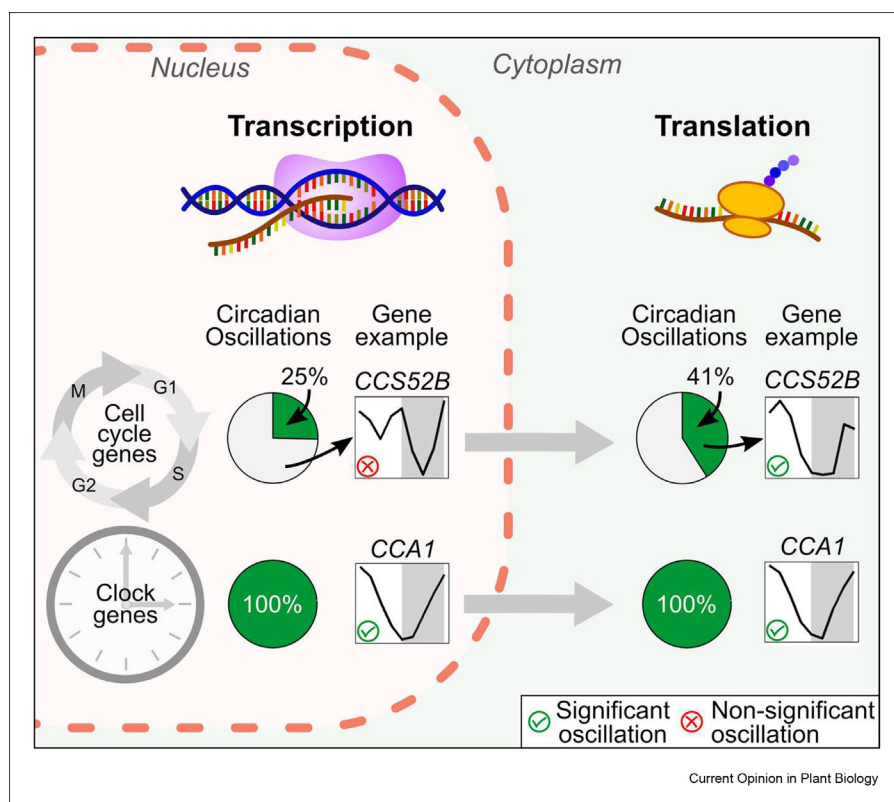
Other mechanisms such as alternative polyadenylation (APA) are circadian-controlled, and genes involved in APA machinery are rhythmic [28]. In fact, circadian-regulated alternatively spliced genes appear to also undergo rhythmic APA events, suggesting a similar clock-controlled pathway facilitating post-transcriptional regulation. It is generally regarded that rhythmic AS and APA may contribute to the robustness of clock-controlled molecular processes in response to environmental perturbations. However, future experiments on specific/individual rhythmic alternatively spliced and polyadenylated transcripts could provide functional insights.

Translation

Clock control of mRNA ribosome loading using ribosome profiling along with a new study using Translating Ribosome Affinity Purification revealed that a significant proportion of the transcriptome in *Arabidopsis* is under circadian control [21,29]. Genes representing many

plant processes that are rhythmic at the transcriptome level are also rhythmic at the translome level. A subset of eukaryotic translation initiation factors shows circadian rhythmicity, similar to observations in fungal and mammalian clock studies [21,30,31]. Strikingly, the observations by Romanowski *et al.* [27] showing the discordance between transcriptional (mRNA abundance) and post-transcriptional rhythmicity are also evident at the level of translation [21,32]. A notable subset of rhythmically translated mRNAs does not show significant cycling at the transcriptional level across multiple circadian data sets, suggesting that the mechanism of translation may somehow facilitate or reinforce translation-specific oscillations. Notably, translation-specific rhythmicity was observed for cell cycle genes (example and discussion provided below in Box 1). Future investigation into the extent to which translation-specific rhythms and environmental stress are coordinated could shed light on the underlying mechanisms.

Figure 3



(Box 1). Specific rhythmicity of cell cycle genes at the translational level. Cell cycle genes correspond to the list of annotated core cell-cycle regulators provided in Yang et al. (2017) [21,65] and that were expressed in Bonnot and Nagel (2021), which represents 122 genes in total. Clock genes correspond to 10 clock genes (*CCA1*, *LHY*, *PRR9*, *PRR7*, *PRR5*, *GI*, *ELF3*, *ELF4*, *LUX*, and *TOC1*) described in Bonnot and Nagel (2021) [21]. Green areas and indicated percentages in pie charts correspond to proportions of genes exhibiting circadian oscillations (rhythmic transcript abundance). This was calculated using the lists of Arabidopsis circadian genes identified at the transcriptome and translome levels in Bonnot and Nagel (2021) [21]. Data correspond to a 24 h time course performed in free-running conditions (constant light and temperature). Transcripts for all clock genes are significantly oscillating at the transcriptome and translome levels (green areas). Transcripts from the core cell cycle regulators, 31 (25%) and 50 (41%) are significantly oscillating at the transcriptome and translome levels, respectively. Transcript profiles of *CELL CYCLE SWITCH 52* (*CCS52B*; *AT5G13840*), significantly cycling only at the level of translation and *CCA1* (*AT2G46830*), are represented as examples of cell cycle regulators and clock genes, respectively. Black lines represent the mean for $n = 3$ replicates, and gray areas represent the subjective night. *CCA1*, CIRCARDIAN CLOCK-ASSOCIATED 1; *ELF4*, EARLY FLOWERING 4; *ELF3*, EARLY FLOWERING 3; *GI*, GIGANTEA; *LHY*, LATE ELONGATED HYPOCOTYL; *LUX*, LUX ARRHYTHMO; *PRR9*, PSEUDO-RESPONSE REGULATOR 9; *PRR7*, PSEUDO-RESPONSE REGULATOR 7; *PRR5*, PSEUDO-RESPONSE REGULATOR 5.

Improved large-scale quantitative proteomics methods are now making it possible to perform protein-level time-course studies to investigate the circadian-controlled proteome. A subset of quantified proteins (3–9%) exhibits diel and circadian rhythms in Arabidopsis [22,33]. Interestingly, these studies also corroborate the prevalence of rhythmic proteins originating from nonrhythmic transcripts, suggesting that this discordance may include regulation at the post-transcriptional, translational, and/or post-translational levels [33,34]. Advances in quantitative proteomics will help to shed light on the underlying regulatory mechanisms [35]. Moreover, comparing multiple studies highlights that genes with circadian rhythms across all three levels (transcriptome, translome, and proteome) of gene regulation are involved in clock-regulated processes such as photosynthesis, response to abiotic stimulus, and metabolism-related processes [21]. Emerging evidence also indicates that

changes in subcellular protein localization and cytoplasmic to nuclear relocalization in response to stress for clock genes might also be important for circadian coordination of environmental stimuli (discussed in more detail in Box 2).

Of note, owing to space constraints, regulation at the level of post-translation is not reviewed here. However, recent work on aspects of protein turnover and modification of clock components, such as ubiquitination, phosphorylation, sumoylation, and glycosylation, is reviewed by Yan et al., (2021) [23].

Noncoding RNAs

Lesser understood molecular layers are also temporally regulated such as miRNAs and lncRNAs. For example, the expression of the natural variant of *miR397*, *miR397b*, is dampened by *CCA1* binding to its promoter [36]. In turn,

miR397b targets Casein kinase II subunit beta-3 for degradation, which prevents phosphorylation of CCA1 and results in altered circadian rhythm (longer period) [36]. Furthermore, PRR9 has been shown to directly bind and repress *miR164* expression, resulting in control of senescence via accumulation of the aging regulator, *ORESARA 1* [37]. With the availability of new circadian transcriptome data sets, *miR167D*, *miR414*, *miR408*, and *miR157c* transcripts appear to oscillate at the transcriptome level [21]. Interestingly, three of these (*miR167*, *miR408*, and *miR157*) show increased accumulation in response to abiotic stresses [37]. These connections present an intriguing opportunity to investigate miRNAs in the context of the clock and stress responses.

LncRNAs also play a role in the plant's response to stress conditions, and their expression may also be circadian-regulated [38,39]. For example, an Arabidopsis lncRNA array identified clock-regulated lncRNA (*FLORE*) as a natural antisense transcript to *CYCLING DOF FACTOR 5* [40]. Both *CYCLING DOF FACTOR 5* and *FLORE* cycle in various photoperiods with antiphase expression but have opposite effects on flowering. Although lncRNAs are generally considered to lack protein-coding potential, they may be translated [41]. For example, the *FLORE* transcript is translated and also cycles at the translational level [21,40]. In response to heat stress, *FLORE* transcripts show increased accumulation at both the transcriptome and translational levels [21]. Although genome-wide circadian time-course experiments to determine the extent of clock control of the various classes of noncoding RNAs are not available, transcriptomic analysis from multiple studies revealed that a small subset of lncRNAs is rhythmic, although this is likely an underestimate owing to the experimental design [21,27,28]. Future dissection of these levels of clock gene regulation described previously in conjunction with additional layers of biological complexity such as signaling and metabolite pathways will undoubtedly allow for more accurate assessments of the functional implication of clock-controlled responses to stress.

Circadian coordination of reactive oxygen species and Ca²⁺ signaling and stress responses

Diurnal cycles and the clock have been shown to be regulators of the ROS homeostasis within the cell [42,43]. As a normal product of cell metabolism, ROS are produced in various cell compartments including chloroplasts, mitochondria, and peroxisomes, and are important signaling molecules at low or moderate concentrations to aid in the activation of diverse cellular responses (Figure 1) [44]. In response to high light stress, the systemic response of Arabidopsis requires the action of membrane-localized proteins RESPIRATORY

BURST OXIDASE HOMOLOG D and PLASMA MEMBRANE INTRINSIC PROTEIN to amplify the ROS signal and both of which show rhythmic expression under circadian conditions (Figure 1) [45].

Daily rhythms of Ca²⁺ in the cytoplasm are regulated by the clock and light signaling [46]. ROS and Ca²⁺ signals are intimately connected, and Ca²⁺ channels such as CYCLIC NUCLEOTIDE-GATED CHANNEL2 and Mechanosensitive channel of Small conductance-Like2 were recently shown as required to mediate the rapid ROS systemic signal [45]. In addition, in guard cells, the leucine-rich-repeat receptor kinase Hydrogen-Peroxide-induced Ca²⁺ increases (HPCA1), an essential protein for stomatal closure during extreme stress, is activated by hydrogen peroxide (H₂O₂) and triggers Ca²⁺ influx [47]. Although direct connections between the clock and these signaling components have not been investigated, oscillations observed at the transcriptome level in constant conditions suggest that the clock may impart an important regulatory role in this signaling cascade (Figure 1).

In the cytoplasm, circadian regulation of Ca²⁺ is also influenced by changes in environmental stress signals, and this can impact photosynthesis among other processes [48]. Furthermore, dark-induced fluctuations of Ca²⁺ in both the chloroplast and stroma are modulated by the clock [49]. Recent data show that superoxide (O₂⁻) can act as a metabolic signal to regulate the clock gene *TOC1* in the evening [50]. In addition, the Arabidopsis Ca²⁺ sensor CALMODULIN-LIKE24 appears to alter the circadian period through a Ca²⁺-dependent pathway and genetic interaction with *TOC1* [51].

Clock control of ROS and Ca²⁺ fluctuations may help to facilitate the integration of extracellular signals with internal cellular processes in response to stress. Both the response to ROS and Ca²⁺ mediated signaling-related genes are expressed at multiple times of day in Arabidopsis in free-running conditions, suggesting a coordinated and specific action controlled by the clock throughout the day (Figure 1). *HPCA1* and *CALMODULIN-LIKE24* are both upregulated (increased transcript abundance) in response to heat stress, whereas *PLASMA MEMBRANE INTRINSIC PROTEIN 1;2* and *RESPIRATORY BURST OXIDASE HOMOLOG D* are downregulated [21]. Interestingly, the magnitude of change in transcript abundance in response to heat stress is controlled by time of day [21]. Temporal control of cellular responses, such that the same stress stimuli occurring or perceived at two different times of day, results in different molecular response intensities, is known as circadian gating and described in the context of temperature stress responses below (Figure 2) [18].

Circadian gating of temperature stress-responsive genes

The first mechanistic study on circadian gating involved clock control of the cold stress response pathway. These early studies demonstrated that the response to cold for major players in the cold tolerance pathway, the *C-REPEAT/DRE BINDING FACTOR*, is gated by the clock [52]. A later large-scale study indicated that a large portion of the cold-responsive transcriptome is gated [53]. In fact, only ~10% of the cold-responsive genes were significant in both the early morning and afternoon, highlighting the importance of time of day in the molecular response to cold [53].

The clock also modulates the magnitude and the occurrence of changes in gene expression in response to heat stress depending on time of day [21,53,54]. Under moderate and extreme heat stresses, ~30% and ~50% of the Arabidopsis-responsive transcriptomes were significant at a specific time point, respectively [53,54]. Interestingly, circadian gating in response to heat stress is also seen at the level of translation. Approximately one-third of the Arabidopsis circadian transcriptome that is responding to heat stress is doing so in a time-of-day-dependent context [21]. In addition, differences in the magnitude of the response to heat stress between the transcriptome and transcriptome with similar levels of transcript accumulation under normal conditions were notable. Some of these differences may be explained by regulation at the post-transcriptional level, sequestration to stress granules, decrease in translation efficiency owing to the presence of upstream Open Reading Frames or preference to translate certain heat stress response genes such as Heat Shock Proteins [21]. Whether just a few or all layers of gene regulation are controlled by the circadian gating mechanism requires further investigation.

At individual gene levels, time-of-day-specific responses to heat stress include both general and specific stress-responsive genes. For example, the *Heat Shock Protein17.4* shows a greater induction in response to morning-applied heat stress. Similarly, the plant AT-rich protein and zinc-binding protein 2 (PLATZ2) has a more pronounced heat stress response in the afternoon [53]. The involvement of *RVE8* in plant thermotolerance is also gated by the clock with a specific regulation in the middle of day [55]. To illustrate the gating response to temperature stress, additional examples of genes are represented in Figure 2. It is also worth noting that even though we are unable to review in detail due to space limitations, in addition to circadian gating of cold and heat stress, the clock has also been shown to gate the transcript abundance in response to drought stress, in hormone responses, and of shade-induced genes [56–60].

We can assume that the clock controls the expression of genes involved in environmental stress responses so that these genes peak at the time corresponding to the maximum peak of stress in natural conditions. Studies on gating responses raise important questions. Can genes peaking at the same time as a given stress signal but not responding to this stress be potential regulators of the responses to that stimulus? Can high gene responsiveness (i.e. high magnitude of response) when transcript levels are low have a more significant impact than low responsiveness during the peak of transcript abundance? Answering these questions will help to understand how the clock modulates the cellular responses when environmental and molecular signals are changing during the day. Recent analyses in *Brassica rapa* showed differential timing of expression and drought responses among retained paralogs [61]. These new findings bring interesting elements to our mechanistic understanding of environmental adaptation and clock regulation. With global climate changes, environmental cues become less reliable, and circadian rhythms are likely to be influenced. Understanding how circadian rhythms are conserved and have evolved between plant accessions and species can improve our knowledge of complex traits such as water use efficiency [62].

Conclusions and perspectives

In this review, we briefly discuss recent progress on clock coordination of selected cellular processes. Many aspects of the clock control of other important processes are yet to be resolved. With advances in proteomics methodology, the dynamic nature of clock-regulated protein complexes should be investigated in a time of day and response to stress context. An understanding of circadian-regulated small RNAs on clock-controlled processes at the whole plant level and in specific tissues and cell types in response to stress is needed. In addition, as the epicenter of photosynthesis is the chloroplast, understanding the clock coordination in individual plastids using single plastid sequencing is necessary for a broader understanding of the relationship between clock and organellar function and how this might contribute to plant fitness. Using CRISPR technologies to modify circadian-associated *cis*-regulatory elements in promoters of transcription factors that are tightly regulated by the clock and important for stress tolerance and growth will aid efforts for creating stress-tolerant plants. Finally, taking advantage of clock-controlled transcription factor networks and machine learning analyses will allow us to propose beneficial outcomes for plant response to single and combinatorial stresses depending on the time of day.

Box 1: Clock control of cell division

Circadian regulation of cell cycle genes is known in eukaryotes as several proteins are rhythmic [63]. In developing *Arabidopsis* leaves, TOC1 modulates the mitotic cycle by repressing the expression of *CELL DIVISION CYCLE 6*, an important regulator of the G1-to-S phase transition [64]. *CELL DIVISION CYCLE6* and other cell cycle genes such as cyclins and *CYCLIN-DEPENDENT KINASES* inhibitors show transcript oscillations in constant conditions suggesting they are clock-regulated [21,27]. Interestingly, a recent study identified several transcripts that are rhythmic at the level of translation but not transcription. Among these, an overrepresentation of circadian-regulated cell cycle genes suggests that clock control of cell division may be modulated at the translational level (Figure 3) [21]. During mitosis, nuclear sequestration of mRNAs has been shown for cell cycle genes, these mRNAs are then redistributed to the cytoplasm for translation at prometaphase [65]. One of these genes, *CELL CYCLE SWITCH52* exhibited significant circadian oscillations at the translational but not at the transcriptional level (Figure 3). Mechanisms of nuclear retention for some mRNAs at specific times of day might explain the specific rhythmicity of the transcriptome. Together these observations raise an intriguing possibility for either a parallel or shared existence of the rhythmic control of the clock and the cell cycle machinery. A mechanistic understanding of the connections between these two types of oscillators is needed to better understand how plants coordinate developmental processes and specifically in response to environmental stresses.

It is worth mentioning that coordination of the circadian rhythms via local and long-distance signaling has been recently demonstrated and reviewed elsewhere [14,15]. However, insights into how this communication is altered in response to abiotic stresses depending on time of day may provide insights into how the oscillator facilitates growth and development in response to environmental changes. Future work using single-cell isolation and cell-type-specific promoters coupled with RNA sequencing with clock mutants under various abiotic stress conditions will provide additional insights.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Box 2: Circadian control of subcellular localization and movement

For clock components that are transcription factors, subcellular localization can be altered in response to abiotic stress. For example, CCA1 and LHY proteins are no longer detected in the nucleus during cold stress likely owing to cold-mediated protein degradation [66]. It is possible that phosphorylation or sumoylation of CCA1 may contribute to this observed degradation [67,68]. This modified localization state is critical for clock coordination of *Dehydration-Responsive Element Binding* transcription factors in response to cold [66]. However, in response to other stresses, CCA1 nuclear localization is maintained suggesting a cold-specific mechanism for CCA1 protein regulation [66]. Similarly, when exposed to either heat or cold stress, the subcellular localization of RVE4 and RVE8 changes from cytoplasmic during ambient conditions to nuclear [66]. Evening clock components are also generally nuclear-localized with TOC1, specifically, localizing in nuclear speckles [69]. ELF3, a component of the EC, is active and nuclear-localized under ambient temperature. However, after exposure to warm temperatures, the inactive ELF3 protein exhibits a speckled localization pattern [70]. In addition, a recent report shows a reduction of ELF3 protein localization to foci, a subnuclear structure, under warm temperature [71]. These observations suggest that subcellular localization of clock proteins dynamically responds to stress resulting in altered molecular function.

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