Tick Tock: Circadian Regulation of Plant Innate Immunity

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Abstract
Many living organisms on Earth have evolved the ability to integrate environmental and internal signals to determine time and thereafter adjust appropriately their metabolism, physiology, and behavior. The circadian clock is the endogenous timekeeper critical for multiple biological processes in many organisms. A growing body of evidence supports the importance of the circadian clock for plant health. Plants activate timed defense with various strategies to anticipate daily attacks of pathogens and pests and to modulate responses to specific invaders in a time-of-day-dependent manner (gating). Pathogen infection is also known to reciprocally modulate clock activity. Such a cross talk likely reflects the adaptive nature of plants to coordinate limited resources for growth, development, and defense. This review summarizes recent progress in circadian regulation of plant innate immunity with a focus on the molecular events linking the circadian clock and defense. More and better knowledge of clock-defense cross talk could help to improve disease resistance and productivity in economically important crops.
INTRODUCTION

Many living organisms on Earth have evolved the ability to measure time, using the endogenous circadian clock to anticipate day and night and seasonal changes and to subsequently coordinate their metabolism, physiology, and behavior with changes in the environment. A precisely tuned circadian clock is critical for the growth and development of many organisms, including photosynthetic bacteria, archaea, fungi, animals, and plants, and confers fitness and competitive advantages to the organisms under both benign and stressful conditions (44, 69). Circadian oscillation is also an integral component of the human immune system and influences human health (7, 120). Like animals, plants encounter various pathogens and pests with different lifestyles on a daily basis. Although plants lack adaptive immunity involving antibodies, plants use sophisticated mechanisms to anticipate and counter the incursions of pathogens and pests. Emerging evidence has established the circadian clock as integral to plant defense against pathogen and pest challenges. The circadian clock coordinates immune responses to pathogens and pests, and circadian dysfunction disrupts responses to invasion. Interestingly, pathogen challenge can reset the plant circadian clock. This reciprocal regulation of the circadian clock and plant innate immunity is likely important for the reallocation of limited resources to ensure proper growth and development of plants and their timely responses to biotic stresses. It could also offer a strategy for pathogens and pests to manipulate plant innate immunity. There are excellent reviews covering topics related to the circadian clock and plant innate immunity (22, 27, 44, 56, 107). This review examines recent progress in our understanding of the cross talk between the circadian clock and plant innate immunity. We specifically focus on the molecular events linking these two important biological processes.

PLANT INNATE IMMUNITY

Among various defense mechanisms employed by plants, some are preformed physical or chemical barriers and others are induced by the invaders. Plant surface structures, such as trichomes, cuticles, epidermal cells, and stomata, provide a frontline of physical barriers to prevent invasion by pathogens and pests. Preexisting secondary compounds can also be used to deter and repel the invaders. In addition, plants can actively recognize pathogens using at least two major surveillance systems and subsequently mount an induced defense. In the first system, plants use pattern recognition receptors to detect pathogen-associated molecular patterns (PAMPs), which are conserved molecules or structures present in groups of related microbes (12). Such recognition activates PAMP-triggered immunity (PTI), a basal level of defense, against nonadapted pathogens. A common counteracting strategy of pathogens is to suppress PTI using pathogen effectors. For instance, bacterial pathogens can produce and deliver effector proteins to the vicinity of or inside a plant cell via the type three secretion system (137). These effectors employ various biochemical mechanisms to compromise host innate immunity, causing effector-triggered susceptibility (ETS). In response, plants have evolved the second surveillance system, employing resistance (R) proteins to specifically recognize cognate pathogen effectors and subsequently activate effector-triggered immunity (ETI). ETI, also termed R gene–mediated resistance, is a much stronger form of defense. ETI is typically associated with hypersensitive response (HR), a process characterized by programmed cell death (42), and systemic acquired resistance (SAR), which leads to enhanced and long-lasting disease resistance at the whole-plant level (33). The activation of induced defense is often associated with increased defense signaling, activating pathways mediated by the small molecules, such as salicylic acid (SA), jasmonic acid (JA), and reactive oxygen species (ROS), reprogramming of genome-wide transcription and global metabolism, and further strengthening of the cell wall. Networks of defense genes act in concert to orchestrate PTI, ETI, and SAR to ward
The circadian clock regulates many biological processes. (a) Basic concepts of the circadian clock, period, phase, amplitude, and zeitgebers. Although it is endogenous, the circadian clock can be reset by external and internal time cues (zeitgebers). Examples of zeitgebers shown from left to right are light, temperature, metabolic status, and infection of pathogens, such as *Pseudomonas syringae*, *Hyaloperonospora arabidopsidis*, and *Botrytis cinerea*. Zeitgebers can affect clock period, phase, and amplitude (red dashed lines). (b) The circadian clock regulates many biological processes as outputs. Activation of some biological processes (red), such as increased signaling in defense, reactive oxygen species (ROS), and hormones and activation of cold response as well as the nutrient status of an organism, is known to feedback-regulate clock activity. It is worth mentioning that the position of each biological process on the circle does not imply the time of day when it occurs.

The circadian clock is an internal timekeeper that sustains itself under free-running conditions (in the absence of environmental time cues) and exhibits temperature compensation, meaning that the pace of the clock (period) remains constant over a range of ambient temperatures. The period of the circadian clock is approximately 24 h (Figure 1a), coinciding with the period of Earth’s rotation around its axis. Although the period of the clock does not vary seasonally, the phase and amplitude of some aspects of clock function may vary seasonally in response to changes in the duration of day and night. The circadian system can be rather simplistically organized into three interconnected parts: the input pathways, the central oscillator (clock), and the output pathways. Although it is endogenous, clock activity can be reset by the input pathways with zeitgebers (“time givers” in German), which are environmental and internal time cues. Light, temperature, and metabolic state in an organism are examples of zeitgebers that can influence rhythmic parameters, including clock period, amplitude, and phase (Figure 1a). The circadian clock has a profound influence on numerous biological processes in plants, some of which often display distinct and overt circadian rhythms (Figure 1b). Some clock output pathways feed back and serve as inputs to modulate clock activity (Figure 1b, red) (45, 48, 54, 73, 111, 155) and some cross talk with each other (51, 112, 150).
Figure 2
A simplified model of the transcription-translation feedback loops (TTFLs) of the Arabidopsis circadian clock. Details of the interactions among the TTFL genes illustrated in this cartoon can be found in the main text. Blue lines with an arrow indicate activation of target genes by a TTFL gene, and red lines with a circle indicate repression of target genes by a TTFL gene. The dashed line with an arrow indicates an indirect regulation of CCA1/LHY by the evening complex. The line underneath the TTFL network shows times of day. Each TTFL gene is roughly positioned according to its time-of-day expression. Color circles depict genes that functionally work together. It is worth mentioning that this cartoon illustrates only a few examples of TTFL gene interactions mentioned in this review but does not mean to provide a comprehensive view of the TTFLs of the circadian clock. Abbreviation: ZT, zeitgeber time.

Although the central oscillators of different organisms do not share conserved core molecular components, the architecture of eukaryotic circadian clocks is conserved and consists of networks of interlocked transcription-translation feedback loops (TTFLs) (7, 44, 56, 120). Indeed, the central oscillator of the model plant Arabidopsis contains many core clock genes that are expressed and act at different times of day to affect activities of other clock proteins and/or themselves (44, 56, 107). For instance, CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) are morning-phased Myb transcription factors that are involved in multiple TTFLs (Figure 2). They directly repress expression of other morning-phased genes, such as PSEUDO-RESPONSE REGULATOR 9 (PRR9), PRR7, and PRR5, the evening-phased genes, such as TIMING OF CAB EXPRESSION 1 (TOC1; also known as PRR1), and the evening complex (EC) genes Lux-ARRHYTHMO (LUX; also known as PHYTOCLOCK1), EARLY FLOWERING 3 (ELF3), and ELF4. CCA1 and LHY can also negatively regulate expression of each other and themselves. In addition, expression of CCA1 and LHY can be negatively regulated by multiple clock proteins, including TOC1, the TOC1 interactor CCA1 HIKING EXPEDITION (CHE), PRR9, PRR7, and PRR5. TOC1 is a negative regulator of LUX, ELF4, GIGANTEA (GI), and PRR5. The EC directly represses expression of PRR7, PRR9, TOC1, GI, and LUX (58). Because PRR9, PRR7, and TOC1 are direct repressors of CCA1 and LHY, the repression of the corresponding encoding genes by the EC relieves their repression of CCA1 and LHY, making the EC also an indirect activator of CCA1 and LHY. The CCA1/LHY relatives REVEILLE 8 (RVE8) and RVE4, together with the transcriptional coactivators NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED GENE 1 (LNK1) and LNK2, activate transcription of the evening-expressed clock genes ELF4, LUX, PRR5, and TOC1 (55, 111, 116, 151). Along with transcriptional-translational control of the circadian clock, post-translational modifications are also key to clock function (121, 128). For
example, alternative splicing was shown to be important for the function of several clock genes. In addition, the evening gene ZEITLUPE (ZTL) encodes an F-box protein in a complex that targets TOC1 and PRR5 for ubiquitination and subsequent degradation (5, 82). ZTL also interacts with GI to control the stability of GI and itself (68). Such complicated regulation of the circadian clock at multiple levels is critical to ensure the precision of the circadian clock.

REGULATION OF PLANT INNATE IMMUNITY BY THE CIRCADIAN CLOCK

The Circadian Clock Genes and Disease Resistance

The circadian clock integrates environmental signals with internal cues to enable proper growth, development, and response to stimuli in organisms (44, 56, 69). Light, one of the main clock zeitgebers, has long been known to affect plant responses to biotic stress (57, 64, 113). In the presence of pathogens, light conditions influence plant defense responses, including HR, SAR, and defense signaling. Some photosynthesis-related genes and photoreceptors were revealed as part of the plant immune system. In addition, constitutive defense and cell death phenotypes conferred by some mutations are light-dependent (13, 62, 83, 154).

Most previous defense assays were conducted under diurnal conditions, which would make it difficult to distinguish the effects caused by light from those caused by the circadian clock. To address this problem, Bhardwaj et al. (10) infiltrated Arabidopsis leaves with the bacterium Pseudomonas syringae at different times of day and subjected the infected plants to the free-running condition of continuous light. They found that plants showed a temporal variation over the day in their resistance to P. syringae with a peak resistance at dawn. This temporal resistance pattern was disrupted when a clock gene, CCA1 or ELF3, was misexpressed. More studies with additional clock mutants provide further support of the direct role of the circadian clock in disease resistance against various pathogens and pests (Table 1).

Several points regarding the role of the circadian clock in plant innate immunity are clear from Table 1. First, the defense role of a clock gene is not associated with a specific expression phase. Mutations in clock genes expressed in the morning (CCA1 and LHY) or evening (CHE, TIME FOR COFFEE (TIC), and ELF3) conferred enhanced disease susceptibility (eds) to P. syringae. Second, the change of clock activity caused by a clock mutation does not predict pathogen resistance of the mutant. Both short-period mutants (e.g., cca1 and lhy) and arrhythmic plants [e.g., plants overexpressing CCA1 (CCA1ox) or LHY (LHYox)] were more susceptible to P. syringae infection. Both the short-period mutant cca1 and the long-period mutant ztl showed eds to the oomycete pathogen Hyaloperonospora arabidopsidis (Hpa). Third, the synergistic or antagonistic relationship between two TTFL genes in clock regulation does not determine the defense phenotypes caused by each mutation. Mutations in mutual repressors CCA1 and CHE enhanced susceptibility to P. syringae (157). These observations suggest that although TTFL genes work together to maintain clock precision, some clock genes could affect the specific output to defense and perhaps also other biological processes. However, what defense mechanisms are deployed by individual clock genes and how these clock genes orchestrate the integration of temporal information with defense responses remain to be addressed. More discussion of how the circadian clock regulates plant defense (see below) could clarify some aspects of these important questions.

The Circadian Clock Regulates Stomata-Dependent and -Independent Defense

Although they are important for photosynthesis and water transpiration, the plant surface structures known as stomata also provide a portal for some pathogens to break into plant tissue and
Table 1  Circadian clock genes are direct regulators of plant defense

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>Expression phase</th>
<th>Clock phenotypes conferred by function loss</th>
<th>Defense-related phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CCA1</em> (partially redundant with <em>LHY</em>)</td>
<td>AT2G46830</td>
<td>Morning</td>
<td>Short period</td>
<td>A <em>cca1lhy</em> mutant shows enhanced disease susceptibility (eds) to <em>Pseudomonas syringae</em>, <em>Hyaloperonospora arabidopsidis</em>, and <em>Botrytis cinerea</em>, and <em>CCA1</em>ox plant is more susceptible to <em>P. syringae</em> and <em>Trichoplusia ni</em> but more resistant to <em>H. arabidopsidis</em></td>
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<td></td>
<td><em>CCA1</em> affects stomata-dependent and stomata-independent defense, reactive oxygen species (ROS) homeostasis and response, and defense gene expression. <em>CCA1</em> acts independently of salicylic acid (SA)</td>
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<td>Expression of <em>CCA1</em> is affected by flg22, RPP4, and infection of <em>P. syringae</em>, <em>H. arabidopsidis</em>, and <em>B. cinerea</em></td>
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<td></td>
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<td></td>
<td></td>
<td>10, 37, 38, 52, 60, 73, 99, 143, 155</td>
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<tr>
<td><em>LHY</em> (partially redundant with <em>CCA1</em>)</td>
<td>AT1G01060</td>
<td>Morning</td>
<td>Short period</td>
<td>A <em>cca1lhy</em> mutant shows eds to <em>P. syringae</em>, <em>H. arabidopsidis</em>, and <em>B. cinerea</em>, and an <em>LHY</em>ox plant is more susceptible to <em>P. syringae</em></td>
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<td></td>
<td><em>LHY</em> affects stomata-dependent and stomata-independent defense and ROS homeostasis and response. <em>LHY</em> acts independently of SA</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>SA increases the amplitude of <em>LHY</em> expression</td>
<td>60, 73, 155</td>
</tr>
<tr>
<td><em>TOC1</em> (<em>PRR1</em>)</td>
<td>AT5G61380</td>
<td>Evening</td>
<td>Short period</td>
<td>A toc1 mutant shows higher SA-induced resistance to <em>P. syringae</em> than Col-0</td>
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<td><em>TOC1</em> affects stomatal opening and closure, ROS response, and defense gene expression</td>
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<td>The amplitude of <em>TOC1</em> expression is increased by SA and suppressed by mutations disrupting <em>ICS1</em>, <em>NPR1</em>, or ROS signaling</td>
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<td></td>
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<td>The <em>TOC1</em> promoter is bound by NPR1 and TGA proteins</td>
<td>26, 59, 73, 158</td>
</tr>
<tr>
<td><em>TIC</em></td>
<td>AT3G22380</td>
<td>Evening</td>
<td>Short period</td>
<td>A <em>tic</em> mutant shows eds to spray-inoculated <em>P. syringae</em> and is compromised in stomata-dependent defense</td>
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<td></td>
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<td><em>TIC</em> is a negative regulator of jasmonic acid (JA) response and affects ROS response</td>
<td>70, 73, 124</td>
</tr>
<tr>
<td><em>ELF3</em></td>
<td>AT2G25930</td>
<td>Evening</td>
<td>Arrhythmic</td>
<td>An elf3 mutant shows eds to <em>P. syringae</em> and <em>B. cinerea</em></td>
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<td><em>ELF3</em> affects ROS response</td>
<td>10, 60, 73</td>
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(Continued)
Table 1  (Continued)

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>Expression phase</th>
<th>Clock phenotypes conferred by function loss</th>
<th>Defense-related phenotypes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELF4</td>
<td>AT2G40080</td>
<td>Evening</td>
<td>Arrhythmic</td>
<td>ELF4 affects ROS response</td>
<td>73</td>
</tr>
<tr>
<td>LUX</td>
<td>AT3G46640</td>
<td>Evening</td>
<td>Arrhythmic</td>
<td>A lux mutant is compromised in temporal expression of some defense phenotypes induced by flg22 or P. syringae infection and in phase-dependent resistance to T. ni LUX affects ROS response</td>
<td>38, 70, 73</td>
</tr>
<tr>
<td>ZTL</td>
<td>AT5G57360</td>
<td>Evening</td>
<td>Long period</td>
<td>A ztl mutant shows eds to H. arabidopsis</td>
<td>143</td>
</tr>
<tr>
<td>CHE</td>
<td>AT5G08330</td>
<td>Evening</td>
<td>No phase and period change</td>
<td>A che mutation disrupts the diurnal oscillation of SA and ICS1 transcription in a light/dark cycle and compromises P. syringae–caused systemic acquired resistance (SAR), SAR-induced SA accumulation, and expression of ICS1 and two ICS1 regulators CHE binds to the ICS1 promoter</td>
<td>157</td>
</tr>
<tr>
<td>GRP7</td>
<td>AT2G21660</td>
<td>Afternoon</td>
<td>No phase and period change</td>
<td>A grp7 mutant shows eds to P. syringae GRP7 affects stomata-dependent and stomata-independent defense GRP7 directly interacts with P. syringae effector HopU1 and transcripts of pathogen-associated molecular pattern (PAMP) receptors FLS2 and EFR</td>
<td>66, 106, 155</td>
</tr>
</tbody>
</table>

Studies show that some clock genes regulate plant defense via a stomata-dependent pathway. Correlating with the rhythmicity of stomata aperture, Arabidopsis plants spray-infected with P. syringae showed more resistance at night than in the morning. Plants misexpressing clock genes (e.g., CCA1, LHY, TOC1, LUX, and TIC) exhibited disrupted diurnal stomatal opening and closure (26, 70, 155). Some of these plants also lost the temporal variation in resistance to P. syringae (10, 70, 155). However, open stomata during the day are not just a passive portal for P. syringae to access the leaf interior. In fact, plants can actively close stomata to restrict pathogen entry through sensing the invader as nonself. This sensing can be achieved by plant receptors to recognize PAMP molecules of P. syringae, such as flg22 and elf16, which are conserved peptides from flagellin and elongation factor Tu, respectively (71, 159). Although the mechanisms by which TOC1, LUX, and TIC affect stomata-dependent defense remain to be elucidated, CCA1 and LHY were shown to impose time-of-day dependence on (gate) the plant response to P. syringae–induced stomatal closure (155). CCA1 and LHY likely act through a potential downstream gene, GRP7 (At2g21660; also known as COLD AND CIRCADIAN REGULATED 2), which encodes an RNA binding protein (34). GRP7 is likely a direct target of CCA1 and LHY based on the over-representation of two CCA1 binding motifs, evening element (EE) and CCA1 binding site (CBS),
in the GRP7 promoter. Expression of GRP7 is circadian regulated and dependent on CCA1 (41). Because loss of function in GRP7 does not affect clock activity, GRP7 is not considered as part of the clock TTFLs but rather as part of a slave oscillator (129). GRP7 was previously shown to regulate stomatal defense, possibly partly through its direct interaction with transcripts of PAMP receptors FLS2 and EFR (66, 155). The P. syringae effector HopU1 binds to the GPR7 protein, likely blocking this interaction and thereby interfering with host stomatal defense (34, 71, 106, 159). Thus, functional TTFL genes (CCA1 and LHY) and their output pathway target (GRP7) are required for the normal function of at least some PAMP receptors to keep P. syringae from disrupting stomatal defense. Additional clock genes have also been shown to affect stomatal opening and closure in a diurnal cycle (76, 125). Further investigation is needed to reveal whether clock genes could broadly gate P. syringae entry into the plant interior via stomata.

Spraying P. syringae onto the plant surface mimics the natural process of foliar bacterial infection and has been used to study stomata-dependent defense in plants. In contrast, pressure infiltration forces bacteria into plant tissue, largely bypassing stomatal restriction on initial bacterial invasion and providing an opportunity to study stomata-independent defense. Interestingly, plants have the opposite sensitivity to P. syringae when infected with these two methods at different times of day, showing more susceptibility to infiltrated bacteria in the evening and to sprayed bacteria in the morning (Figure 3) (10, 70, 155). These observations suggest that plants activate different defense mechanisms at different times of day to deal with pathogens with varying invasion tactics. The core clock gene CCA1 was shown to be required for plant resistance to P. syringae delivered by both methods, underscoring the importance of the circadian clock in plant defense throughout the day.

Although stomata-dependent defense is critical to prevent the initial invasion of foliar bacteria like some P. syringae strains that proliferate aggressively in the apoplast of plant cells, the virulence of many other pathogens of different lifestyles is less dependent on the limitations imposed by stomata (6, 75). For instance, epiphytic bacteria can complete their life cycles on the leaf surface without entering the plant interior (6, 75). Some oomycete and fungal pathogens do not use stomata as the main passage to host internal tissue. Instead, they produce hyphae to directly penetrate into or between host epidermal cells to interfere with host immunity, obtain nutrients and water, and eventually kill host cells (18, 90, 146). Thus, like stomata-dependent defense, stomata-independent defense is a major mechanism for plant resistance to pathogens. How the circadian clock regulates stomata-independent defense against a broad spectrum of pathogens with different lifestyles is not well understood. Stomata-independent defense overlaps with induced defense, the regulation of which by the circadian clock is discussed further in the next section.

The Circadian Clock Regulates Induced Defense

Besides restricting pathogens with physical barriers like stomata, plants can actively recognize and subsequently mount induced defense against the invaders. Accumulating evidence indicates the importance of the circadian clock in activating induced defense. This section focuses on circadian regulation of defense gene expression, different layers of defense responses, and key defense signaling pathways.

Circadian regulation of defense gene expression. The recognition of molecular control of plant defense by the circadian clock was first made via observations that expression of some defense genes oscillated in a circadian manner. This individual gene-based notion was quickly validated by large-scale studies. A genetic screen with the enhancer trapping experiment revealed that 36% of Arabidopsis genes were circadian regulated (92). Time-series transcriptomics studies suggest
Timed defense in *Arabidopsis*. Microbial pathogens demonstrate timed virulence whereas insects have timed feeding behavior (outer black text). In response to attacks from pathogens and pests, plants mount a defense to anticipate the time when the invasion of a certain pathogen or pest is most likely to occur and to gate immune responses to specific invaders in a time-of-day-dependent manner. Many of the anticipatory responses oscillate with relatively low amplitude and peak at a certain time of day. Examples of such responses (from inside to outside) are production and/or signaling of salicylic acid (SA) (red), jasmonic acid (JA) (blue), reactive oxygen species (ROS) (purple), and plant growth hormone, expression of genes involved in RPP4-mediated defense and phenylpropanoid biosynthesis, and stomata opening/closure (green). The growth hormones include auxin (IAA), cytokinins (CKs), brassinosteroids (BRs), ethylene (ET), and abscisic acid (ABA). The position of each label represents the phase of an event, which is the peak activity during a day. Abbreviations: Hpa, *Hyaloperonospora arabidopsidis*; ZT, Zeitgeber time.

that one-third of *Arabidopsis* genes cycle under certain circadian conditions (21, 30, 49), whereas as much as 89% of *Arabidopsis* transcripts oscillate under one or more environmental cycling conditions (e.g., light-dark or warm-cool) (93). Together, these studies unequivocally support the overarching transcriptional control of *Arabidopsis* genes by the circadian clock, which is consistent with the fact that many core clock genes encode transcription factors. Indeed, such widespread transcriptional control by the circadian clock is a common theme in other organisms, including mouse, *Drosophila melanogaster*, *Neurospora crassa*, and cyanobacteria (86, 96, 109, 148). Similarly, biotic stress conditions induce global transcriptional reprogramming (133). It is possible that many gene transcripts change because of modifications in the chromatin structure under cycling and/or defense conditions. It is critical to distinguish which gene transcripts are passively affected by
Table 2  Gene Ontology (GO) analysis of target genes of circadian clock proteins

<table>
<thead>
<tr>
<th>GO analysis</th>
<th>CCA1</th>
<th>TOC1</th>
<th>PRR5</th>
<th>PRR7</th>
<th>PRR9</th>
<th>All combined&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Whole genome (all genes)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Whole genome (protein coding genes)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of target genes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,318</td>
<td>772</td>
<td>1,021</td>
<td>1,096</td>
<td>132</td>
<td>4,121</td>
<td>33,602</td>
<td>27,416</td>
</tr>
<tr>
<td>Response to biotic and abiotic stimuli&lt;sup&gt;d&lt;/sup&gt;</td>
<td>358 (15.4%)</td>
<td>164 (21.2%)</td>
<td>192 (18.8%)</td>
<td>278 (25.4%)</td>
<td>41 (31.1%)</td>
<td>720 (17.5%)</td>
<td>3,174 (9.4%)</td>
<td>3,174 (11.6%)</td>
</tr>
<tr>
<td>Developmental processes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>254 (11.0%)</td>
<td>98 (12.7%)</td>
<td>174 (17.0%)</td>
<td>179 (16.3%)</td>
<td>23 (17.4%)</td>
<td>499 (12.1%)</td>
<td>2,989 (8.9%)</td>
<td>2,989 (10.9%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The target genes were identified from ChIPseq experiments with the indicated clock proteins (top row) (59, 76, 77, 99, 100).
<sup>b</sup>The number of genes targeted by all five clock proteins.
<sup>c</sup>Represents similar GO analysis with all Arabidopsis genes or with protein coding genes only and was included as controls.
<sup>d</sup>Top numbers are the count of genes in each GO category. Percentages were calculated as follows: the count of genes in each GO category/total number of gene targets of a clock protein × 100.

these conditions and which ones operate at the intersection of the circadian clock and defense to regulate these processes.

To support a role of the circadian clock in defense control, bioinformatics analyses predict that some defense genes are potential targets of the core clock component CCA1, based on the enrichment of the two CCA1 binding motifs, the EE and CBS motifs, in the promoters of these genes (143, 155). To further identify direct gene targets for the circadian clock, chromatin immunoprecipitation coupled with deep DNA sequencing (ChIPseq) experiments were conducted with several core clock proteins, including CCA1, TOC1, PRR5, PRR7, and PRR9 (59, 76, 77, 99, 100). Target genes of these clock proteins were enriched with functions related to biotic and abiotic stress as well as to development-related processes (Table 2). Thus, multiple core clock components are likely linked directly to stress outputs. It is worth noting that these ChIPseq experiments were performed with nonchallenged samples and thus may miss additional clock targets that respond only under stress conditions. Further experiments are needed to reveal the biological relevance of circadian control of defense gene expression under both basal and biotic stress conditions.

**Circadian regulation of PAMP-triggered immunity and effector-triggered immunity.** The best-characterized PTI in plants is induced when the PAMP receptor FLS2 recognizes the elicitor flg22. This recognition leads to the internalization and degradation of FLS2, activation of a MAPK cascade and WRKY transcription factors, and global transcriptional reprogramming (12, 114). As a result, plants mount a basal defense, associated with a series of physiological changes happening within minutes to days after the recognition. Several studies suggest a role of the circadian clock in regulating PTI. An in silico analysis of microarray data revealed that several key genes involved in flg22-FLS2 PTI, including FLS2, MKK4/6, MPK3 and MPK6, and WRKY22, exhibit circadian-regulated expression (10). However, it is not yet known whether any flg22-FLS2 PTI genes are under the direct transcriptional control by a core clock protein(s). Korneli and colleagues showed that flg22 treatment caused temporal induction of ROS production and defense marker gene expression, depending on the time of day of flg22 treatment; this temporal pattern of induction was disrupted by the lux mutation (70). In addition, the two core clock proteins CCA1 and LHY are potential PTI regulators because their downstream target gene GRP7 was
shown to affect PTI, at least through regulating two PAMP receptors, FLS2 and EFR (106). It is unknown, however, whether misexpression of CCA1 and/or LHY could affect PTI. If so, how CCA1, LHY, LUX, and other core clock components regulate PTI would be an interesting topic for further investigation.

In addition to PTI, the circadian clock also controls ETI during biotic stress. The resistance protein RPP4 belongs to a large protein family with two conserved domains: a nucleotide binding site (NBS) and a leucine-rich repeat (LRR) (87). Although its cognate avirulence effector(s) have not been identified, RPP4 specifically recognizes and activates strong defense against the avirulent oomycete Hpa isolates Emoy2 and Emwa1 (18). Hpa strains cause downy mildew in Arabidopsis, sporulating mainly at night and disseminating spores at dawn (Figure 3). An elegant study by Wang and colleagues showed that the circadian clock controls RPP4-mediated Hpa resistance (143). RPP4 and some RPP4-dependent genes are enriched with CCA1 binding motifs in their promoters. Expression of these genes displayed a circadian pattern with a peak overlapping that of CCA1 at dawn, and this pattern was disrupted by a cca1 mutation. Although wild-type plants showed more resistance to Hpa Emwa1 when infected at dawn than at dusk, the cca1 mutant lost this temporally regulated resistance and showed more susceptibility than wild-type plants in the morning. However, CCA1ox was more resistant to the strain. These data led to the conclusion that plants can use the core clock gene CCA1 to anticipate Hpa infection in the morning when Hpa spore dissemination is the highest. Challenging of a cca1lhy double mutant with a different avirulent Hpa isolate, Emoy2, supports this role of CCA1 and further implicates a similar role of the close CCA1 homolog LHY in RPP4-mediated resistance (155). In addition, the cca1lhy mutant was more susceptible to the avirulent P. syringae strain expressing avrRpt2, which is recognized by another NBS-LRR protein, RPS2 (155). Thus, CCA1 (and perhaps also LHY) may act through multiple R gene–mediated pathways to regulate ETI. Consistent with this idea, some other NBS-LRR genes also have CCA1 binding motifs in their promoters and thus are potential transcriptional targets of CCA1 (C. Zhang & H. Lu, unpublished results). Further studies are necessary to establish direct CCA1 regulation of its target genes, such as RPP4, RPP4-dependent genes, and other R genes, and the biological relevance of such a regulation in ETI. It would also be interesting to determine whether other core clock genes are involved in ETI and, if so, to elucidate how they affect specific R gene–mediated disease resistance.

Circadian regulation of hormone signaling. Although SA and JA have long been recognized as defense hormones, the role of the six major plant growth hormones, auxin, cytokinins, ethylene, gibberellic acids, abscisic acid, and brassinosteroids, in plant defense regulation has only recently been demonstrated, highlighting the importance of balancing growth and defense during the plant life cycle (112, 126). Circadian control of plant growth hormones has been manifested in daily rhythmic hormone accumulation and diel expression of genes related to hormone biosynthesis, signaling, and response (Figure 3). Some of these hormone genes are directly controlled by core clock proteins (2). The circadian clock also gates plant responses to some growth hormones. Whether the circadian clock regulates defense through controlling the rhythmicity of growth hormones is unknown. Recent studies, however, indicate that the defense role of the circadian clock is at least partly coordinated through the regulation of the two defense hormones SA and JA.

Circadian regulation of salicylic acid and systemic acquired resistance. SA is a small phenolic molecule that plays an important role in signaling different layers of plant defense, from stomata-dependent defense to multiple induced defense responses (47, 89, 117, 138). SA is synthesized through several pathways (23, 79); the major one is catalyzed by ISOCORISMATE SYNTHASE 1 (ICSI) (103, 104, 145). ICSI is localized to the chloroplast (130), the primary site of SA biosynthesis.
SA and possibly some SA precursors can be further transported by EDS5 to the cytoplasm for action and/or further modifications (102, 122, 152). Additional genes, such as ACCELERATED CELL DEATH 6 (ACD6) and EDS1 (31, 80), also affect SA accumulation, but their functions have not been completely understood. SA perception is mediated by the SA receptors NPR1, NPR3, and NPR4 (149, 153). NPR1 exists as an oligomer in the cytoplasm under an unchallenged state. Higher SA levels change redox status and stimulate the release of the NPR1 monomer from the oligomer for nuclear translocation (97, 131). The cytoplasm-nucleus shuttling of NPR1 is important for SA signaling, SA cross talk to other signaling pathways, and NPR1 stability (33, 51).

Circadian regulation of the SA pathway is supported by several studies (Figure 3). Goodspeed and colleagues showed that basal SA levels oscillate daily, with a peak at night (38). Consistent with this, expression of major genes affecting SA levels, including ICS1, EDS1, EDS5, and ACD6, also shows circadian oscillations (10, 95, 157). Although the NPR1 transcript level remains constant, NPR1 monomer accumulates rhythmically, with a peak at night (141, 158). Direct circadian control of SA biosynthesis was further demonstrated by Zheng and colleagues (157); the clock protein CHE binds to the promoter of ICS1 and affects the basal oscillation of ICS1 transcript and SA. CHE is also required for the induction of two transcription factors known to directly regulate ICS1 expression, SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 and CALMODULIN BINDING PROTEIN 60g (142, 156, 157). Thus, these studies strongly establish that SA production and signaling under non-pathogen-challenged conditions are outputs of the circadian clock.

Besides affecting basal SA oscillation in the absence of pathogens, a che mutation also compromises SAR induction in response to P. syringae infection, suggesting a role for CHE in SAR regulation (157). Interestingly, P. syringae infection is known to induce in the local infected tissue the acute synthesis of bulk SA and expression of SA key regulatory genes, both of which could increase more than tenfold higher than the basal levels within 24 h post infection without maintaining the circadian pattern (46). The che mutant is not known to affect local resistance to P. syringae and acute local defense responses, including SA synthesis. Mutations in the two core clock genes CCA1 and LHY conferred lower local defense to P. syringae but did not affect bulk SA biosynthesis based on a genetic study (155). Thus, CCA1 and LHY may act independently of SA in defense regulation. It is possible that the circadian clock gates the immune response under biotic stress conditions rather than through its regulation of diel synthesis of endogenous SA, using the TTFL gene CHE. This idea is supported by the work of Zhou et al. (158) showing that the TTFL genes TOC1, CCAI, and PRR7 are upregulated by NPR1 under stress conditions, and they potentially function to gate immune responses at a specific time of day. Nevertheless, because SA plays a key role in plant innate immunity, it remains an interesting topic to determine which, if any, clock genes exert a major impact on SA synthesis and/or signaling under biotic stress conditions.

Circadian regulation of jasmonic acid–mediated defense. Jasmonic acids (JAs) are a collection of lipid-derived molecules that play key roles in plant defense. Cross talk between JA and SA signaling is important for plants to balance their defense against different pathogens and pests (14, 112). In general, higher JA levels inhibit SA accumulation and signaling and favor resistance against most insect herbivores and necrotrophic microorganisms, whereas higher SA levels inhibit JA accumulation and signaling and promote resistance against most biotrophic pathogens.

Biotic challenges and wounding induce rapid JA biosynthesis, with jasmonoyl-L-isoleucine (JA-Ile) being the most active form of JA. In the absence of JA-Ile, JA repressors, JASMONATE ZIM-DOMAIN (JAZ) proteins, bind to the transcription factor MYC2 to inhibit JA signaling (16, 134). Upon JA-Ile binding to the receptor complex, consisting of the JA receptor CORONATINE INSENSITIVE1 (COI1; an F-box protein) and JAZ coreceptors, JAZ proteins are targeted for
ubiquitination and degradation, releasing repression of MYC2 and leading to the activation of JA signaling (16, 25, 123, 134).

The JA pathway is circadian regulated, as demonstrated in several studies. The JA level oscillates during a day with a peak at midday (39) (Figure 3). Expression of some key JA biosynthetic genes is circadian regulated (20), including those directly targeted by CCA1 (99). Expression of some core JA signaling genes, e.g., COI1, MYC2, and the JAZ genes, also showed circadian cycling that is dependent on the clock protein TIC (124). TIC interacts with the MYC2 protein and inhibits MYC2 accumulation. Consistent with the central role of TIC in JA regulation, a tic mutant was hypersensitive to JA treatment in a MYC2-dependent manner, suggesting that TIC gates JA signaling as a negative regulator. This tic mutant was also more susceptible to P. syringae infection, possibly due to increased JA signaling that suppresses SA signaling.

As opposed to P. syringae defense, JAs activate plant defense against insect feeding (14). The midday peak accumulation of JA is consistent with plants’ anticipation of midday feeding insects, such as the cabbage looper (Trichoplusia ni) (Figure 3). Chewing of T. ni increased but did not disrupt the rhythmic timing of JA accumulation in Arabidopsis. JA mutants and plants whose clocks are not in phase with T. ni feeding behavior suffered more tissue loss in the presence of the insect. Arabidopsis resistance against the necrotrophic pathogen Botrytis cinerea is also time-of-day dependent (Figure 3) and requires several core clock genes and intact JA signaling (52, 60). In addition, Arabidopsis activates a series of different defense responses chronologically over the time course of B. cinerea infection (147). Together, these findings support the importance of the circadian clock and the JA pathway in plant defense against herbivores and necrotrophic fungal pathogens. They also highlight that plants activate timed defenses to anticipate attacks from pathogens and pests before the attacks occur and to gate immune responses against specific invaders in a time-of-day-dependent manner. However, whether core clock genes other than TIC activate plant defense through a direct control of the rhythms of JA accumulation and/or JA signaling remains to be determined.

Circadian regulation of reactive oxygen species. ROS are small molecules produced as by-products during photosynthesis and respiration (35). ROS regulate cell and organ growth and cellular communication, and thus they are important for plant development. ROS production can also be induced rapidly and/or abundantly during biotic and abiotic stresses, as described by the term oxidative bursts (127). ROS can act as signaling molecules to activate defense and strengthen the cell wall through cross-linking. At high concentrations, ROS are toxic to both host and pathogen cells, causing lipid peroxidation, protein modification, nucleotide and cell membrane damage, and eventually cell death. The multifunctional role of ROS makes it particularly important to balance ROS homeostasis in plant cells.

The circadian clock has been implicated in regulating ROS production, scavenging, and signaling. The basal level of H2O2, a major type of ROS, showed a circadian pattern with a peak at 7 h after dawn (ZT7, where ZT stands for zeitgeber time and indicates the time after lights on) (Figure 3). The peak of H2O2 accumulation is preceded by the expression of many photosynthetic genes at ZT4 (49), consistent with ROS being by-products of photosynthesis. In addition, the activity of the ROS-scavenging enzyme catalase, the oxidation and reduction cycle of peroxiredoxin proteins, and the levels of the metabolic redox coenzymes NADP+ and NADPH showed specific time-of-day peaks (29, 73, 158) (Figure 3). The circadian clock also controls the expression of ROS-related genes (73). Of 517 ROS-related Arabidopsis genes, including genes involved in ROS producing, scavenging, and signaling, 39% show rhythmic expression under circadian conditions. The cycling expression of these ROS genes is largely dependent on CCA1, ELF3, LUX, and TOC1. Even some of the noncycling ROS genes are regulated by these core clock components.
In particular, CCA1 binds to some ROS gene promoters that are enriched with EE and CBS motifs. Mutations in CCA1 and LHY led to higher ROS accumulation, reduced catalase activity, and altered response to some ROS-inducing reagents (73). Like CCA1 and LHY, several other TTFL components, including ELF3, ELF4, LUX, TIC, PRR5, PRR7, PPR9, and GI, were also shown to gate responses to ROS stress (72, 73). Although these studies unequivocally establish a role of the TTFL clock in ROS control under nonchallenged conditions, a recent study showed that the redox cycle of the highly conserved peroxiredoxin proteins persists even in the absence of functional TTFL genes in all three domains of life: bacteria, archaea, and eukaryota (29). Thus, sensing and responding to oxidative cycles could be intrinsic metabolic processes independent of clock TTFLs. Redox status in the unstressed cellular environment depends on the intimate coordination between the metabolic clock and the TTFL clock.

Although its role in regulating ROS homeostasis and signaling under unchallenged conditions is relatively well understood, how the circadian clock affects oxidative bursts during plant-pathogen interactions is less clear. Whereas whether the metabolic clock could affect oxidative bursts remains to be determined, the TTFL clock likely has such a role on the basis of prevailing transcriptional control of diel expression of ROS-related genes by TTFL components in non-stressed plants. However, how TTFL proteins affect the expression of ROS-related genes under pathogen challenge is still an open question. Some cycling ROS genes, e.g., AtrbohD and PEROXIDASE (Figure 3), are known to be part of defense networks (1, 135, 136). Whether TTFL components directly control these ROS-related defense genes remains to be determined. ROS distribution at the subcellular level shows a distinct spatial, temporal, and quantitative pattern during PTI, ETI, and ETS, possibly due to differential activation of different ROS production and/or scavenging enzymes (46). It is unknown whether the circadian clock affects such a ROS distribution pattern under stress conditions. Further research is needed to address these questions and gain insights into circadian regulation of ROS during plant-pathogen interactions.

**RECIPIROCAL REGULATION OF THE CIRCADIAN CLOCK BY PATHOGEN INFECTION**

The circadian clock is an endogenous timekeeper that can be reset by zeitgebers such as light, temperature, nutrient status, and hormone signaling (45, 48, 54, 111, 119). A growing body of evidence indicates that pathogen infection can also reciprocally regulate clock activity in plants (Figure 1a). For instance, infection with both virulent and avirulent P. syringae strains can shorten the plant circadian period as measured by luciferase activity driven by the CCA1 or GRP7 promoter (CCA1:LUC or GRP7:LUC) (155). Rhythmic expression of CCA1 can also be disrupted by infection with the oomycete pathogen Hpa and is dependent on the R gene RPP4 (143). In addition, infection by the necrotrophic pathogen B. cinerea dampens oscillations of a number of core clock genes, including CCA1, although neither the expression phase nor the period of these genes is altered (147). Thus, a change in clock activity appears to be a common host response to pathogen infections. It is worth mentioning that all these infections are associated with programmed cell death in the infected tissue, which may account for the altered clock activity. However, whether an acute pathogen attack without causing host cell death could perturb clock activity remains to be determined. Pathogen-induced host clock change could be a host mechanism to balance costly disease resistance with primary metabolism, thereby maximizing host growth and development of plants. It could also offer a strategy on the part of the pathogens to manipulate the host immune system to acquire resources.

How does pathogen challenge affect the host circadian clock? There is a paucity of data to directly link pathogen manipulation of the central oscillator. Instead, studies suggest that pathogens
could use PAMPs, effectors, and other molecules to at least indirectly hijack the plant circadian clock. For instance, \textit{P. syringae} could use its PAMP molecules, such as flg22, to shorten cycling of \textit{CCA1:LUC} or use the effector HopU1 to target GRP7 and thus affect CCA1-mediated clock outputs (34, 155).

Pathogen-induced host clock change could also be caused by the manipulation of hormone signaling. For instance, \textit{P. syringae} effectors could activate the production and/or signaling of auxin and abscisic acid in \textit{Arabidopsis} (15, 24, 101). Although they are clock regulated, these two growth hormones also reciprocally regulate clock activity and thus could be targeted by pathogens to influence the plant clock and defense. Besides growth hormones, the two defense hormones SA and JA are also frequent targets of pathogens for manipulation. \textit{P. syringae} strains employ several mechanisms to manipulate host defense signaling mediated by JA, using the toxic chemical coronatine to mimic JA or effectors to interfere with JA signaling (8, 36, 63, 65). Although JA is not known to affect clock activity, these studies support the hypothesis that pathogen infection affects at least some clock outputs. As with JA, SA production and signaling can be modulated by various pathogens (132). SA may reciprocally regulate the circadian clock, depending on plant developmental stage, because this role of SA was shown only with soil-grown 3-week-old plants but not with plate-grown 10-day-old seedlings (48, 155, 158). Because SA induces redox changes in plants and the master immune regulator NPR1 and its interacting TGA transcription factors are also important redox sensors in \textit{Arabidopsis} (51, 97, 131), the role of SA in clock regulation could stem from cross talk between SA and redox state. Indeed, Zhou et al. (158) showed that NPR1 exerts redox-sensitive transcriptional regulation of several TTFL genes, including \textit{LHY}, \textit{PRR7}, and \textit{TOC1}, in the absence of pathogen challenge. In particular, NPR1 and some TGA proteins bind directly to the \textit{TOC1} promoter to regulate its expression. In response to acute perturbation in the redox status triggered by SA, the upregulation of both morning (\textit{LHY} and \textit{PRR7}) and evening (\textit{TOC1}) clock genes by NPR1 reinforces rather than disrupts circadian rhythmicity. The reinforced clock is critical in gating of immune responses in a time-of-day-specific manner to minimize interference with other cellular processes, for example, confining a robust immune response in the morning could minimize costs on growth at night. Thus, reciprocal regulation of the circadian clock and defense could confer enhanced fitness to plants.

\textbf{PATHOGEN CLOCK}

One fascinating idea for host-pathogen coevolution would be that the coordination of circadian clocks from pathogens, pests, and their hosts could shape the outcome of interactions at the organismal level. Although much is known about host circadian clocks, especially in the model plant \textit{Arabidopsis}, studies on pathogen and pest clocks lag behind.

It is not known whether \textit{P. syringae} has a circadian clock, although its genome contains several conserved clock genes found in other organisms. Many fungal and oomycete pathogens exhibit time-of-day-dependent hyphae development, sporulation, and spore dissemination, suggesting the involvement of the circadian clock (11, 74, 110, 118). Indeed, some clock genes identified in fungal species were shown to be important for the virulence and development of fungi. For instance, a mutation in the \textit{bcfrq1} gene of \textit{B. cinerea}, a homolog of the core clock gene \textit{frq} in \textit{N. crassa}, adversely affected the reproduction and virulence of \textit{B. cinerea} (52). The \textit{N. crassa white collar-1} (\textit{wc-1}) ortholog in \textit{Magnaporthe oryzae}, the causal agent for rice blight disease, regulates blue-light-specific asexual development, conidia release, and light-dependent disease suppression (67, 74). The fungal causal agent of gray leaf spot disease in maize, \textit{Cercospora zeae-maydis}, also has a \textit{wc-1} homolog, \textit{crp1}, which regulates stomatal tropism, appressoria formation, conidiation, and pathogenicity of the fungus (67). In addition, insects can use their circadian clocks to keep pace
with oviposition, feeding, hatching, and locomotor activities (17, 19, 88, 108, 115). Interestingly, a recent study showed that it is the circadian clock of *B. cinerea*, but not that of its host *Arabidopsis*, that dictates the outcome of *B. cinerea*–*Arabidopsis* interactions (52). However, the paucity of data on pathogen and pest circadian clocks makes it difficult to assess whether the coordination of circadian clocks between plants and their invaders generally determines the outcomes of interactions at the organismal level. This clearly represents fertile ground for further study.

**TIMED DEFENSE AND PLANT FITNESS**

Plant growth, development, and responses to environmental stimuli are energy-costly processes that can be in conflict through competition for limited resources or via other forms of interference, such as the generation of an intermediate in one pathway that interferes with a second process. Resolution of such potential conflicts can entail spatial segregation of conflicting processes into different cells or subcellular compartments or, alternatively, can entail a temporal separation. Thus, a properly tuned circadian clock has a profound influence on the fitness of *Arabidopsis* and natural populations related to *Arabidopsis* during development and response to abiotic stresses (26, 28, 40, 41, 94, 105). This role of the circadian clock has not been well understood in plants under biotic stresses.

Because plants encounter different pathogens and pests at different times of day, it is conceivable that plants have evolved the ability to time defense responses to anticipate when the invasion of a certain pathogen or pest most likely occurs and to gate immune responses to individual invaders at a specific time of day. The amplitude of such anticipated defense responses is usually much smaller than that induced by biotic challenges. Such low and rhythmic responses likely reflect the rhythmic regulation of chromatin accessibility to some transcription factors. In the presence of pathogens and pests, the open chromatin structure permits the access of defense-related transcription factors and thus primes plants to mount much stronger defense responses. The circadian clock could gate the priming response at the peak of the basal oscillation as shown in *T. ni*–induced JA accumulation (38). This priming mechanism by the circadian clock appears to be widespread for other clock outputs and in other organisms (4, 50, 91). Alternatively, induced defense could be acute and independent of circadian oscillation as shown in *P. syringae*–induced SA accumulation (46). Whether such acute defense responses are also directly regulated by the circadian clock remains to be addressed. Defense activation could also reinforce the circadian clock, leading to more restricted immune responses at a specific time of day and subsequently minimizing costly effects associated with defense (158). Thus, timed defense likely reflects the adaptive nature of plants to their pathogens and pests.

The importance of such timed defense with the right strategy has already been demonstrated in *Arabidopsis* because clock dysfunction results in compromised resistance to broad-spectrum pathogens and pests (Table 1). Activating defense at the wrong time is detrimental (158), and constitutive defense activation could be at the cost of shortening the plant life cycle and/or reducing biomass (3, 78, 140). In natural populations related to *Arabidopsis*, hybrid plants show growth rigor when their stress genes are expressed in a timed manner, whereas necrosis occurs when these genes are constitutively expressed (95). Together, these observations indicate that the circadian clock-timed defense confers a fitness advantage in *Arabidopsis* plants.

Our understanding of the mechanisms of plant timekeeping and defense is largely derived from studies of *Arabidopsis*. It is broadly assumed that the *Arabidopsis* model applies to plants in general, but the degree to which this *Arabidopsis*-centric view of the circadian clock and defense can be extrapolated to other plants has not been tested in detail. As in *Arabidopsis*, widespread circadian regulation of gene expression is found in most plants, including important crop species
such as poplar, soybean, rice, and maize (53, 61, 81, 84). Orthologs of *Arabidopsis* clock genes are identified in other species, but few studies have established the importance of those orthologs to clock function or defense regulation (9, 85, 128). For instance, *Arabidopsis* orthologs of GI, each of the PRRs, and the evening complex genes ELF3, ELF4, and LUX/NOX are known to serve as important determinants of flowering time in a number of crops, including beet, soybean, pea, rice, barley, wheat, sorghum, and maize (9, 128). Only GI, ELF3, ELF4, and LUX have been established to function in the circadian clocks in some plants, and the functions of the PRR genes seem to be restricted to flowering time determination. None of these crop clock ortholog genes has yet been shown to play a role in regulating plant defense. Although the molecular mechanisms of the clock genes remain to be determined in non-*Arabidopsis* plants, at least two studies suggest that the circadian clock of some crop plants enhances plant fitness and performance (43, 98).

In particular, the circadian clock has played a significant role in tomato domestication because tomato has been selected for lagging phase and lengthened period, presumably as an adaptation to the longer and seasonally variable photoperiods encountered in the northern latitudes of Europe and North America (98). Additional studies in more crop species and functional characterization of *Arabidopsis* clock ortholog genes in crop plants are necessary to establish and substantiate a general role of the circadian clock in enhancing fitness in crop plants, especially under biotic stress conditions.

**CONCLUDING REMARKS**

The circadian clock is clearly an integral part of the plant innate immune system and is critical for multiple layers of defense responses and broad-spectrum resistance against various pathogens and pests. Timed defense to anticipate attacks from pathogens and pests and to gate immune responses to individual invasions at a specific time of day could be an adaptation of plants gained through coevolution with their pathogens and pests. We are still at the beginning of understanding the role the circadian clock plays in defense regulation. Much work remains to be done to elucidate the underlying molecular mechanisms in clock-defense cross talk, including but not limited to high-resolution time-series experiments with biotic challenges, systems biology to model large-scale data sets related to circadian and defense conditions, and identification and detailed characterization of biochemical functions of some genes regulating the clock-defense cross talk. Knowledge obtained from such studies and the uncovering of genes acting at the converging point of regulating the circadian clock and defense could be applied to improve disease resistance and productivity in economically important crop plants.

**SUMMARY POINTS**

1. The circadian clock regulates plant defense against a broad spectrum of pathogens and pests.
2. The circadian clock affects both preformed and induced defense. This review focuses on circadian regulation of stomatal defense, PTI, ETI, defense gene expression, and defense signaling pathways mediated by SA, JA, and ROS.
3. Pathogen infection can reciprocally affect clock activity of the host through mechanisms that are not yet well understood.
4. Some pathogens and pests have their own circadian clocks, which could be important for their interactions with the hosts.
5. Timed defense means that plants incorporate external and internal time cues to anticipate likely attacks from invaders at different times of day and ready defense responses for rapid deployment to individual invaders at a specific time of day.

6. Successful manipulation of timed defense could be an important strategy to improve disease resistance and productivity of economically important crop plants.

FUTURE ISSUES

1. The circadian clock affects multiple aspects of defense responses under challenged or nonchallenged conditions, but most mechanistic details remain incompletely elucidated.

2. The extent to which the plant innate immune response feeds back to modulate circadian clock function remains unclear, and the pathways by which this feedback is exerted need to be established.

3. The circadian clocks of pathogens and pests affect their virulence, as has been established in B. cinerea and T. ni, but the mechanistic understanding of this regulation remains limited.

4. It will be important to understand the temporal coordination between pathogens and pests and their hosts, using their circadian clocks, and how such coordination contributes to the outcome of the interactions at the organismal level.

5. The circadian clock increases fitness by temporally partitioning potentially conflicting biological processes to distinct times of day. In considering biotic challenge, this is usually simplified as a conflict between growth and defense, but this needs to be refined to identify more precisely the conflicts between biological processes and defense responses.

DISCLOSURE STATEMENT

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Errata
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