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Abstract

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The survival and reproduction of plants depend on their ability to cope with a wide range of daily and seasonal environmental fluctuations during their life cycle. Phytohormones are plant growth regulators that are involved in almost every aspect of growth and development as well as plant adaptation to myriad abiotic and biotic conditions. The circadian clock, an endogenous and cell-autonomous biological timekeeper that produces rhythmic outputs with close to 24-hour rhythms, provides an adaptive advantage by synchronizing plant physiological and metabolic processes to the external environment. The circadian clock regulates phytohormone biosynthesis and signaling pathways to generate daily rhythms in hormone activity that fine-tune a range of plant processes, enhancing adaptation to local conditions. This review explores our current understanding of the interplay between the circadian clock and hormone signaling pathways.

Keywords: Circadian clock, hormone, signaling, growth, immunity, adaptation

Introduction

As sessile organisms, plants spend their entire life cycle in the same place that they germinated. This, along with their poikilothermic nature, forces them to adapt to a variety of abiotic and biotic stresses that change both on short- and long-term time scales. Persistent challenges such as drought, shade, cold, and the changing seasons are dealt with in part by the impressive developmental and physiological plasticity of plants (de Jong and Leyser 2012). Hormone signaling pathways have long been known to play key roles in plant responses to such long-term environmental challenges. Daily environmental fluctuations also present plants with significant difficulties. For example, day/night cycles cause huge alterations not only in light levels but also in water availability; plants undergo profound daily changes in their metabolism to cope with fluctuations in these essential resources (Farre and Weise 2012; Muller et al. 2014). The circadian clock plays a central role in plant adaptations to daily and even seasonal changes in the environment. It is therefore perhaps not surprising that multiple connections between the clock and hormone pathways have recently been revealed. In this review, we will focus on studies demonstrating circadian modulation of hormone levels and physiological pathways controlled by hormones. We will also discuss evidence that hormone signaling may feed back to influence the circadian network.

Circadian clocks are found in most eukaryotes and some prokaryotes. They are cell-autonomous biological timekeepers that generate roughly 24-hour rhythms in many metabolic and physiological processes (Greenham and McClung 2015; Hsu and Harmer 2014). Daily rhythms can be diel, observed when there are regular rhythmic inputs such as daily light and dark cycles, or circadian, persisting in the absence of rhythmic environmental cues. It has been demonstrated in plants, bacteria, and mammals that circadian clocks that run with a period matched to that of external environmental cycles provide a competitive advantage (Dodd et al. 2005; Ouyang et al. 1998; Spoelstra et al. 2016), presumably by allowing organisms to correctly anticipate regular changes in the environment including alterations in temperature, light, and humidity. The circadian system can be generalized as consisting of input or entrainment pathways, the central clock or oscillator, and output pathways. Inputs such as light perceived by plant receptors entrain the central oscillator to generate precisely-phased rhythmic outputs, such as the release of volatiles timed to attract appropriate pollinators and enhanced resistance to cold at night (Greenham and McClung 2015; Yakir et al. 2007). The plant circadian clock generates daily and even seasonal rhythms in many physiological processes including stomatal opening, leaf movement, hypocotyl elongation, photosynthesis and carbon metabolism, resistance to abiotic and biotic stresses, and flowering time (Angelmann and Johnsson 1998; Farre 2012; Hsu and Harmer 2014; Muller et al. 2014; Song et al. 2015; Webb 1998; Yakir et al. 2007).

In addition to generating obvious daily rhythms, the circadian clock plays a more subtle role in the regulation of plant physiology. Many signaling pathways are modulated by the clock so that they are differentially active at different times of the day or night in a process known as “gating”. For example, plants treated with auxin (indole-3-acetic acid; IAA) at night are more responsive than plants treated with the same auxin concentration during the day (Covington and Harmer 2007; Went and Thimann 1937). Similar gating of responses to environmental cues such as light and temperature have also been reported (Adams and Carre 2011). It is thought that circadian gating may help plants distinguish between random fluctuations in the environment and longer-term alterations.

The plant circadian clock

The plant circadian clock is the most complex yet reported in any organism and consists of a highly interconnected network of transcription factors that regulate each other’s expression (Figure 1). Here we present a brief overview of our current understanding of the plant circadian clock. Readers are directed to recent excellent reviews for more details about the clock machinery (Hsu and Harmer 2014; McClung 2014). The closely-related, morning-expressed MYB-like transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) and the evening expressed TIMING OF CAB EXPRESSION 1 (TOC1/PRR1) reciprocally repress each other’s expression. The repression of CCA1 and LHY by TOC1 requires the *CCA1 HIKING EXPEDITION* (*CHE*) gene. The morning-phased CCA1 and LHY proteins repress expression of the “evening complex” (EC) components *LUX ARRHYTHMO* (*LUX*), *EARLY FLOWERING 3* (*ELF3*), and *ELF4*, while the afternoon-phased MYB-like transcription factor REVEILLE8 (RVE8) activates their expression. Another double-negative feedback loop exists between CCA1 and LHY and the day-phased transcription factors *PSEUDO RESPONSE REGULATOR9* (*PRR9*), *PRR7*, and *PRR5* (Adams et al. 2015; Fogelmark and Troein 2014). In addition to repressing *CCA1* and *LHY* expression, these PRRs repress expression of *RVE8* (Figure 1).

In addition to regulating expression of other oscillator components, the transcription factors that make up the plant clock regulate expression of thousands of output genes. Genome-wide studies carried out with RNA extracted from intact seedlings suggest that about 30% of expressed genes are clock regulated (Covington et al. 2008; Hsu and Harmer 2012; Michael et al. 2008b), although the fraction of the transcriptome that is clock regulated in some but not all cell types is likely considerably higher (Endo et al. 2014). Intriguingly, genes regulated by the hormones abscisic acid (ABA), brassinosteroids (BR), cytokinins (CK), ethylene (ET), gibberellins (GAs), IAA, jasmonates (JAs), and salicylic acid (SA) are more likely to be clock-regulated than expected by chance (Covington and Harmer 2007; Covington et al. 2008; Dodd et al. 2007; Mizuno and Yamashino 2008). Our recent analysis with a more complete list of cycling genes (Hsu and Harmer 2012) than used in previous studies reveals that between 35 and 46% of hormone related genes are also clock regulated in Arabidopsis, significantly more than the 29% expected by chance (Figure 2). Recent chromatin immunoprecipitation studies demonstrated that CCA1, TOC1, and the PRRs bind to the promoters of hundreds of genes (Huang et al. 2012; Liu et al. 2013; Liu et al. 2016; Nagel et al. 2015; Nakamichi et al. 2012). Interestingly, more of these putative direct targets of the circadian clock machinery are regulated by plant hormones than expected by chance (Figure 3). Additional genome-wide analyses suggest functional links between clock components and plant hormone pathways. For example, more than one-third of the likely direct targets of PRR7 also contain ABA-responsive elements in their upstream regions; the functional relevance of this finding is supported by the reduction of ABA-induced gene expression in plants overexpressing *PRR7* (Liu et al. 2013). Thus the circadian clock machinery has been implicated in direct control of genes involved in hormone signaling.

Daily rhythms in hormone levels

Clock regulation of hormone signaling occurs at additional levels as well. It has long been noted that levels of many phytohormones oscillate over 24-hour day/night cycles. For example, diel variations in ethylene levels have been demonstrated in bean, cotton, sorghum, Arabidopsis, rice, low-elevation longstalk starwort, red goosefoot, and *Kalanchoe daigremontiana* (Emery et al. 1994; Finlayson et al. 1998; Kapuya and Hall 1977; Lee et al. 1981; Lipe and Morgan 1973; Machackova et al. 1997; Morgan et al. 1990; Thain et al. 2004). In many species, ethylene production has been reported to persist in constant conditions and can thus be classified as circadian regulated (Finlayson et al. 1998; Jasoni et al. 2000; Morgan et al. 1990; Thain et al. 2004). However, in other species daily oscillations do not persist in constant conditions (Machackova et al. 1997) or have even been reported to have no pronounced daily rhythms in any condition tested (Emery et al. 1994).

Diel oscillations in the growth-related hormones IAA, GAs, CKs and BRs have been reported in multiple species. Diel changes in IAA levels have been observed in leaves of *Coffea arabica* and tobacco, with peak levels in the middle of the day (Janardhan et al. 1973; Novakova et al. 2005). Similar diel oscillations were reported in the tropical tree West Indian locust (Velho do amaral et al. 2012) and in red goosefoot (Krekule et al. 1985), but with peak IAA levels occurring at night. Circadian regulation of free IAA levels has been demonstrated in Arabidopsis and *Chenopodium rubrum* (Jouve et al. 1999; Pavlova and Krekule 1984), but with peak levels at the end of the subjective day and midday, respectively. Interestingly, the cycling patterns of expression of many IAA biosynthetic and signaling genes are highly conserved across poplar, rice, and Arabidopsis (Filichkin et al. 2011)

Levels of some but not all GAs have been reported to peak at the end of the day in spinach and sorghum (Foster and Morgan 1995; Lee et al. 1998; Talon et al. 1991), at the beginning of the day in pea (Stavang et al. 2005), and to show no significant daily variation in begonia (Myster et al. 1997). CK levels showed diel cycling in tobacco leaves, with peak levels at midday (Bancos et al. 2002; Novakova et al. 2005) while in pineapple levels were reported to peak near dawn in shoots but in the middle of the night in roots (Freschi et al. 2009). Finally, in Arabidopsis and tobacco, BR and CK levels were reported to show diel regulation with peak levels at midday (Bancos et al. 2002; Novakova et al. 2005).

Stress and defense-related hormones undergo diel oscillations as well. Levels of SA and JA are clock regulated in Arabidopsis, with peak accumulation in the middle of the subjective night and in the middle of subjective day, respectively (Goodspeed et al. 2012). Diurnal rhythms of JA have been reported in roots but not leaves of *Nicotiana attenuata* (Kim et al. 2011); however, in this plant JA levels peak at night. Similar variations in the timing of ABA oscillations have been reported. While ABA levels oscillate in poplar (Barta and Loreta 2006), field-grown pearl millet (Henson et al. 1982), Arabidopsis (Lee et al. 2006), and *Arbutus unedo* (Burschka et al. 1983) with peak levels around midday, ABA levels in soybean are circadian regulated with peak levels occurring at night (Lecoq et al. 1983). Finally, in tobacco leaves, ABA levels showed a complex pattern with two peaks during the day and a higher peak at the beginning of the dark phase (Novakova et al. 2005).

In summary, diel and circadian regulation of hormone levels is widespread in plants, but species- and tissue-specific variation is considerable. Thus there are undoubtedly many ways in which the circadian system interacts with hormone metabolic pathways. We will discuss a few below.

Circadian regulation of genes that control hormone levels

Genome-wide transcriptome studies have revealed that expression of many genes that encode hormone biosynthetic enzymes is clock regulated. For example, many genes that function in the synthesis of isoprenoids, precursors of the hormones ABA, BR, CK, and GA (Vranova et al. 2013), are clock controlled. In Arabidopsis, the circadian clock regulates at least 50% of the genes encoding key enzymes of the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways leading to isoprenoid synthesis (Figure 4). Key genes in the MEP pathway have been shown to be targets of the central clock proteins CCA1 and LHY (Pokhilko et al. 2015). Interestingly, the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is also clock regulated in mammals (Shapiro and Rodwell 1969).

Downstream of the MVA and MEP pathways, the carotenoid biosynthesis pathway supplies precursors for the biosynthesis of ABA. Circadian clock regulation of many of the genes encoding enzymes in this pathway (Figure 4) has been demonstrated in both Arabidopsis and maize (Covington et al. 2008; Khan et al. 2010). Diel regulation of genes involved in ABA synthesis has also been shown in species such as tomato (Thompson et al. 2000) and the perennial desert plant *Rhazya stricta* (Yates et al. 2014). Studies in plants deficient for the circadian clock components PRR5, 7, and 9 have shown that these pseudo-response regulator-like proteins negatively regulate the expression of both genes involved in the ABA biosynthetic pathway and ABA levels (Fukushima et al. 2009). This regulation may be direct, as PRR7 directly binds to the promoter region of *ABA DEFICIENT 1 (ABA1)*, which encodes a zeaxanthin epoxidase involved in ABA biosynthesis (Liu et al. 2013). Another mechanism controlling daily levels of active ABA may be via the diel regulation of AtBG1, a β-glucosidase that releases active hormone from glucose-conjugated, inactive ABA (Lee et al. 2006).

GAs are also major hormones generated from isoprenoid precursors. At least in diel conditions, many GA biosynthetic genes show daily rhythms in Arabidopsis, pea, potato, and maize (Carrera et al. 1999; Garcia-Martinez and Gil 2002; Hisamatsu et al. 2005; Khan et al. 2010). In Arabidopsis, expression levels of the clock-regulated gene AtGA20ox1 are increased in *toc1* mutants (Blazquez et al. 2002). Similarly, expression levels of several GA biosynthesic genes and levels of active GAs are increased in barley mutant for the clock gene *ELF3* (Boden et al. 2014), further implicating the circadian clock in regulation of GA biosynthesis. The clock may also be involved in the inactivation of active GAs: mRNA levels of all six Arabidopsis *GA2ox* genes, which encode enzymes that catabolize active GAs, exhibited diel rhythms, with GA2ox1 and possibly GA2ox2 also being circadian regulated (Zhao et al. 2007).

Less has been published on mechanisms underlying diel and circadian regulation of CK and BR levels, two other types of hormones generated from isoprenoid precursors. However, the expression of two BR-biosynthetic genes has been reported to be under circadian control in Arabidopsis (Bancos et al. 2002).

The most abundant auxin in plants is IAA. In land plants it is thought to be primarily derived from tryptophan via the action of the tryptophan aminotransferase (TAA)/flavin monooxygenase (YUCCCA) pathway, although tryptophan-independent biosynthetic pathways have also been proposed (Yue et al. 2014). In rice, at least one member of each paralogous set of genes from each of the six reactions in tryptophan biosynthetic pathway is under strong diel regulation (Dharmawardhana et al. 2013). A number of homologous genes are also circadian regulated in Arabidopsis (Figure 4). One mechanism by which the clock regulates free auxin levels is through the circadian-regulated MYB-like transcription factor *RVE1*. RVE1 directly promotes the expression of the auxin biosynthetic gene *YUCCA8* (*YUC8*) and thus increases free auxin production during the day (Rawat et al. 2009). Several transcripts encoding GH3 enzymes, which join auxin to amino acids to produce inactive conjugates, are also clock-regulated (Covington et al. 2008; Khan et al. 2010), suggesting an additional mechanism by which daily rhythms in free auxin levels may be generated.

Although ET emissions have long been recognized as clock controlled, mechanisms underlying this regulation are elusive. Under typical conditions, ACC synthase (ACS) is thought to be the rate-limiting step for ET synthesis, and in Arabidopsis transcript level of *ACS8* shows circadian rhythm of expression with a peak phase similar to that of ET production. However, plants mutant for *ACS8* do not exhibit altered ethylene rhythms (Thain et al. 2004), indicating other biosynthetic components are under clock control. Under some conditions ACC oxidase can be the rate-limiting step in ethylene synthesis (Rieu et al. 2005), and two genes encoding putative ACC oxidase enzymes are clock regulated with a phase similar to that of *ACS8* (Covington et al. 2008; Khan et al. 2010). It is therefore possible that these enzymes might act with ACS8 to generate rhythms in ethylene production.   Diel cycling of genes predicted to encode homologs of the ethylene receptors ERS1 (ETHYLENE RESPONSE SENSOR1) and EIN1 (ETHYLENE INSENSITIVE1) has been reported in Japanese cedar (Nose and Watanabe 2014), suggesting the clock may gate ethylene signaling in addition to regulating ethylene production.

As described above, circadian regulation of JA and SA levels has been reported in both Arabidopsis and other plants. In Arabidopsis, the clock protein CCA1 has been shown to bind to the promoter of the JA biosynthetic gene *LOX2* (Nagel et al. 2015). It has been proposed that similar regulation of the JA biosynthesis gene *LOX3* also occurs in *Nicotiana attenuata* (Kim et al. 2011). More is known about daily regulation of SA levels in Arabidopsis. *ICS1* (ISOCHORISMATE SYNTHASE1) encodes an enzyme essential for SA biosynthesis (Wildermuth et al. 2001) and its expression is clock-regulated. The clock protein CHE directly, and perhaps also indirectly, regulates *ICS1* expression and is required for daily rhythms in SA levels (Zheng et al. 2015). The clock may also regulate SA signaling via additional mechanisms: CCA1 has been implicated in the regulation of expression of the phosphate transporter gene *PHT4;1*, a negative regulator of plant defenses that acts genetically upstream of SA signaling (Wang et al. 2014).

In summary, the circadian clock has been implicated in control of most major plant hormones and therefore by extension most physiological events. Below, we discuss recent findings regarding joint clock and hormone regulation of two important processes, plant growth and plant defense.

The roles of hormones and the clock in growth regulation

Plant growth is a complex process controlled by many environmental and endogenous signals including major roles for the phytohormones IAA and GAs. Daily rhythms in stem and leaf growth are observed in multiple species and at least in dicots are generated by the circadian clock (Ruts et al. 2012). The mechanisms underlying these rhythms have been best studied in the Arabidopsis hypocotyl. Clock and environmental regulation of hypocotyl elongation is mediated in part via the transcription factors PHYTOCHROME INTERACTING FACTOR (PIF) 4 and 5 (Dowson-Day and Millar 1999; Nozue et al. 2007). Daily rhythms in *PIF4/5* expression and thus hypocotyl elongation are generated by the evening complex, ELF3, ELF4, and LUX (Nusinow et al. 2011). Additional regulation may be provided by PRR7 and PRR5, which also bind to the promoters of *PIF4* and *PIF5* (Franklin et al. 2011; Liu et al. 2013; Nakamichi et al. 2012).

A number of studies have linked PIF4 and 5 to the control of IAA and GA signaling (de Lucas et al. 2008; Koini et al. 2009; Kunihiro et al. 2011; Nozue et al. 2011) and biosynthesis (Filo et al. 2015; Franklin et al. 2011; Hornitschek et al. 2012). A simple model for regulation of daily growth patterns can be generated from the following results: PIF4 and PIF5 are required for expression of key GA biosynthetic enzymes (Filo et al. 2015) and plants deficient for GA production exhibit large reductions in rhythmic growth (Nozue et al. 2011). Expression of GA receptors is clock controlled and plant responsiveness to GA is accordingly gated by the clock (Arana et al. 2011). Together, these data suggest that daily hypocotyl growth rhythms are driven by the PIF-dependent rhythmic production of GA combined with circadian gating of GA perception.

However, the full story is much more complex. PIF function is modulated by other hormone signaling pathways: a BR-dependent kinase phosphorylates PIF4 and promotes its degradation (Bernardo-Garcia et al. 2014) while the ability of multiple PIF proteins to bind to DNA is inhibited by their binding to DELLA proteins, negative regulators of GA signaling that are themselves degraded in response to GA (de Lucas et al. 2008; Feng et al. 2008). PIF4 transactivation activity is also inhibited upon binding to the clock protein ELF3 (Nieto et al. 2015), providing another layer of clock regulation on PIF function. PIFs have also been implicated in auxin signaling. PIF4 and 5 regulate auxin biosynthesis (Franklin et al. 2011; Hornitschek et al. 2012) and PIF4 and 5 modulate plant sensitivity to auxin (Nozue et al. 2011). Finally, auxin- (but not GA-) responsive genes are overrepresented among those misexpressed in *pif4 pif5* seedlings (Nozue et al. 2011), suggesting that PIFs may play a more important role in growth control via auxin signaling than the GA pathway.

PIF-independent clock control of plant growth has also been reported. Circadian rhythms in leaf growth persist in plants mutant for *PIF4* and *5* (Dornbusch et al. 2014). Since daily rhythms in floral stem elongation require IAA (Jouve et al. 1999) and plant growth and transcriptional responses to IAA are gated by the clock (Covington and Harmer 2007), clock regulation of auxin signaling may play a role in PIF-independent growth rhythms. Indeed, the clock-regulated transcription factor RVE1 promotes hypocotyl growth by increasing free auxin levels; this is independent of PIF4 and PIF5 function (Rawat et al. 2009). However, further complexity is suggested by a genome-wide transcriptome study implicating daily rhythms in ABA and BR signaling, in addition to rhythms in IAA and GA signaling, in daily growth rhythms (Michael et al. 2008a). Therefore diel and circadian regulation of plant growth likely involves a complex network of hormone signaling pathways that are modulated at many steps.

The roles of hormones and the clock in defense responses

Plants are subjected to various biotic stresses throughout their sedentary life cycle. In general, SA and JA are recognized as the major defense hormones with SA being essential for the immune response against biotrophic pathogens and JA helping defend against necrotrophic pathogens and herbivorous insects. The other phytohormones act as modulators of the plant immune signaling network (Pieterse et al. 2012). The roles of hormonal signaling pathways may vary depending on the plant and the type of the threat (Kunkel and Brooks 2002; Lund et al. 1998; Thomma et al. 2001).

Not surprisingly, defense responses are diel and circadian regulated. Susceptibility of plants to bacteria, oomycetes, fungi, and chewing insects has been shown to be clock regulated (Bhardwaj et al. 2011; Goodspeed et al. 2012; Hevia et al. 2015; Ingle et al. 2015; Wang et al. 2011). Even after harvest, the circadian clock regulates pest resistance and plant nutritional value (Goodspeed et al. 2013). Many hormone pathways and mechanisms have been implicated in clock modulation of plant defense, even to the same pathogen. For example, while plant defense responses to mechanical infiltration of the bacterial pathogen *Pseudomonas syringae* into leaves are maximal in the morning, defense responses are maximal in the evening when these bacteria are simply sprayed on plants (Korneli et al. 2014; Zhang et al. 2013). These distinct phases of peak resistance are likely due to circadian regulation of both stomatal aperture and downstream defense signaling pathways. Regulation of stomatal aperture is perturbed in plants mutant for the clock genes *CCA1* and *LHY* (Dodd et al. 2005; Shin et al. 2012; Zhang et al. 2013), which regulate expression of *GLYCINE-RICH RNA-BINDING PROTEIN7* (*GRP7*) (also known as *COLD AND CIRCADIAN REGULATED 2* [*CCR2*]) (Zhang et al. 2013), a protein shown to promote stomatal closure (Kim et al. 2008). Notably, GRP7 also promotes translation of FLS2, a receptor for bacterial flagellin

(Nicaise et al. 2013), demonstrating the complexity of clock regulation of defense signaling. The clock genes *PRR7*, *TIC*, *ELF3* and the clock output gene *PATHOGEN AND CIRCADIAN CLOCK CONTROLLED 1* (*PCC1*) have also been implicated in regulation of stomatal aperture (Kinoshita et al. 2011; Korneli et al. 2014; Liu et al. 2013; Mir et al. 2013).

Roles for JA in circadian-driven variation in non-stomatal dependent defense pathways have been demonstrated. Circadian-driven variation in susceptibility to the fungus *Botrytis cinerea* requires a functional JA signaling pathway (Ingle et al. 2015), as do daily rhythms in resistance to cabbage looper (Goodspeed et al. 2012). Expression of the JA receptor *CORONATINE INSENSITIVE1* (*COI1*) is clock regulated, as is expression of the transcription factor MYC2, a positive regulator of JA signaling. In addition, the clock-associated protein TIC interacts with MYC2 and is required for daily variation in JA-mediated defense responses (Shin et al. 2012). Studies demonstrating the functional importance of circadian regulation in SA-mediated defense pathways have not yet been published, and in fact one report suggests that CCA1 and LHY modulation of defense responses is largely SA-independent (Zhang et al. 2013).

Most studies to date have focused on the role of the plant circadian clock in daily rhythms of plant susceptibility to pathogens and pests. However, studies on *Botrytis cinerea* (Hevia et al. 2015) and chewing insects (Goodspeed et al. 2013) have shown that circadian regulation of pathogen and pest physiology helps determine the outcome of their interactions with plants. Future work on how circadian clocks act in both plants and pathogens to modulate defense responses is likely to be of great interest.

Roles for hormones in regulation of the circadian clock

In animals, it has long been recognized that circadian rhythms are generated in diverse organs but that rhythms at the organismal level are coordinated via a ‘master clock’ in the brain that entrains ‘slave oscillators’ in peripheral organs. This coordination is achieved by multiple mechanisms including daily rhythms in body temperature, metabolism, and hormone levels (Mohawk et al. 2012). Although some work suggests at most only weak coupling between clocks in individual plant cells (Fukuda et al. 2007; Shimizu et al. 2015; Thain et al. 2000; Wenden et al. 2012), other studies suggest there may be significant interactions between clocks in different plant tissues (Endo et al. 2014; James et al. 2008; Takahashi et al. 2015). An obvious question is therefore whether hormone pathways can entrain the plant clock and help coordinate rhythms between far-flung organs such as roots and shoots.

A number of studies have found that application of exogenous hormones can affect clock function in plants. Although treatment with ET, SA, auxins, or GAs have been reported to have little or no effect on clock pace (Covington and Harmer 2007; Hanano et al. 2006), more significant changes in phase and/or period were reported after treatment with ABA, CKs, or BR (Hanano et al. 2006; Salome et al. 2006; Zheng et al. 2006). SA treatment did not affect clock phase or period (Hanano et al. 2006; Zhang et al. 2013; Zhou et al. 2015) but has been suggested to reinforce circadian robustness (Zhou et al. 2015). However, flg22, a peptide activator of basal defense pathways, has been reported to shorten clock pace (Zhang et al. 2013) via an unknown mechanism. Consistent with the ability of exogenous hormones to alter clock pace, mutation of genes that act in hormone signaling pathways can affect free-running period (Hanano et al. 2006; Salome et al. 2006; Zheng et al. 2006).

However, there are inconsistencies in the literature that complicate interpretation of these findings. For example, ABA treatment has been variously reported to cause either modest or significant period shortening (Lee et al. 2016; Liu et al. 2013) or period lengthening (Hanano et al. 2006). Such discrepancies may be due to differences in hormone concentration or formulation and the clock reporter gene examined: different concentrations of a CK can evoke either period shortening or period lengthening (Salome et al. 2006), and the periodicity of different circadian reporter genes may be oppositely affected by treatment with the same hormone (Hanano et al. 2006). Moreover, many of the above studies used high concentrations of hormones over prolonged periods of times, calling into question the physiological relevance of the observed effects on clock function. Finally, the authors of one study concluded that the effects of mutation of hormone-related genes on clock function were likely due to an unknown mode of action independent of hormone signaling (Salome et al. 2006). Thus our current understanding of the role of plant hormones in control of clock function remains incomplete.

In addition to changes in hormone levels, daily cycles in plant metabolites have been suggested to coordinate clock function in disparate organs (Haydon et al. 2013; James et al. 2008). In support of this hypothesis, acute treatment of plants with sucrose during the day was shown to cause phase advances while acute treatment at night caused phase delays (Haydon et al. 2013). Such time-of-day-dependent effects of sucrose on circadian phase are consistent with it acting as an endogenous regulator of the circadian system, making fixed carbon a strong candidate as a global coordinator of clock function. Similar studies on the acute effects of hormones on clock phase will be of great interest and may help reveal whether they normally coordinate clock function in disparate organs, as for example between the shoot apex and roots (Takahashi et al. 2015).

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Figure 1: Brief overview of the plant circadian clock. Three different size ovals represent levels of the indicated proteins. Arrows and perpendicular bars indicate activation and repression, respectively. For simplicity, the morning-expressed MYB-like transcription factor LATE ELONGATED HYPOCOTYL (LHY), which functions semi-redundantly with its homolog CCA1, is not shown. For more details about the clock machinery, see recent reviews (Adams et al. 2015; Hsu and Harmer 2014; McClung 2014).

Figure 2: The percentages of clock-regulated genes (Hsu and Harmer 2012) that are also regulated by individual phytohormones (Blanco et al. 2009; Nemhauser et al. 2006; Schenk et al. 2000) are plotted. Asterisks indicate statistically significant circadian enrichment over the 29% circadian regulation expected by chance (Fisher's exact test; $p < 0.05$).

Figure 3: The percentages of CCA1, TOC1, PRR9, PRR7, and PRR5 target genes (as identified by chromatin immunoprecipitation (Huang et al. 2012; Liu et al. 2013; Liu et al. 2016; Nagel et al. 2015; Nakamichi et al. 2012) that are regulated by individual phytohormones (Blanco et al. 2009; Nemhauser et al. 2006; Schenk et al. 2000). Asterisks indicate statistically significant enrichment of phytohormone-regulated genes among all identified clock protein target genes (Fisher's exact test; $p < 0.05$).

Figure 4: Circadian regulation of many phytohormone biosynthesis enzymes. (a) The times of peak expression of clock-regulated phytohormone biosynthesis genes. Light red, early subjective day; dark red, late subjective day; light blue, early subjective night; dark blue, late subjective night. (b) Overview of the major phytohormone biosynthesis pathways and the enzymes involved. Black, metabolites; blue, enzymes; red, enzymes with clock regulated gene expression. MVA-mevalonate, MEP- methylerythritol phosphate, ABA-abscisic acid, BR-brassinosteroids, CK-Cytokinins, ET-ethylene, GA-Gibberellins, IAA-indole-3-acetic acid, JA-Jasmonates, and SA-salicylic acid. More details are found at http://biocyc.org/ARA/NEW-IMAGE?object=Plant-Hormone-Biosynthesis and within the following references (Dempsey et al. 2011; Gupta and Chakrabarty 2013; Mano and Nemoto 2012; Ruiz-Sola and Rodriguez-Concepcion 2012; Vranova et al. 2013; Wang et al. 2002; Wasternack and Hause 2013; Xu et al. 2013; Zhao and Li 2012).

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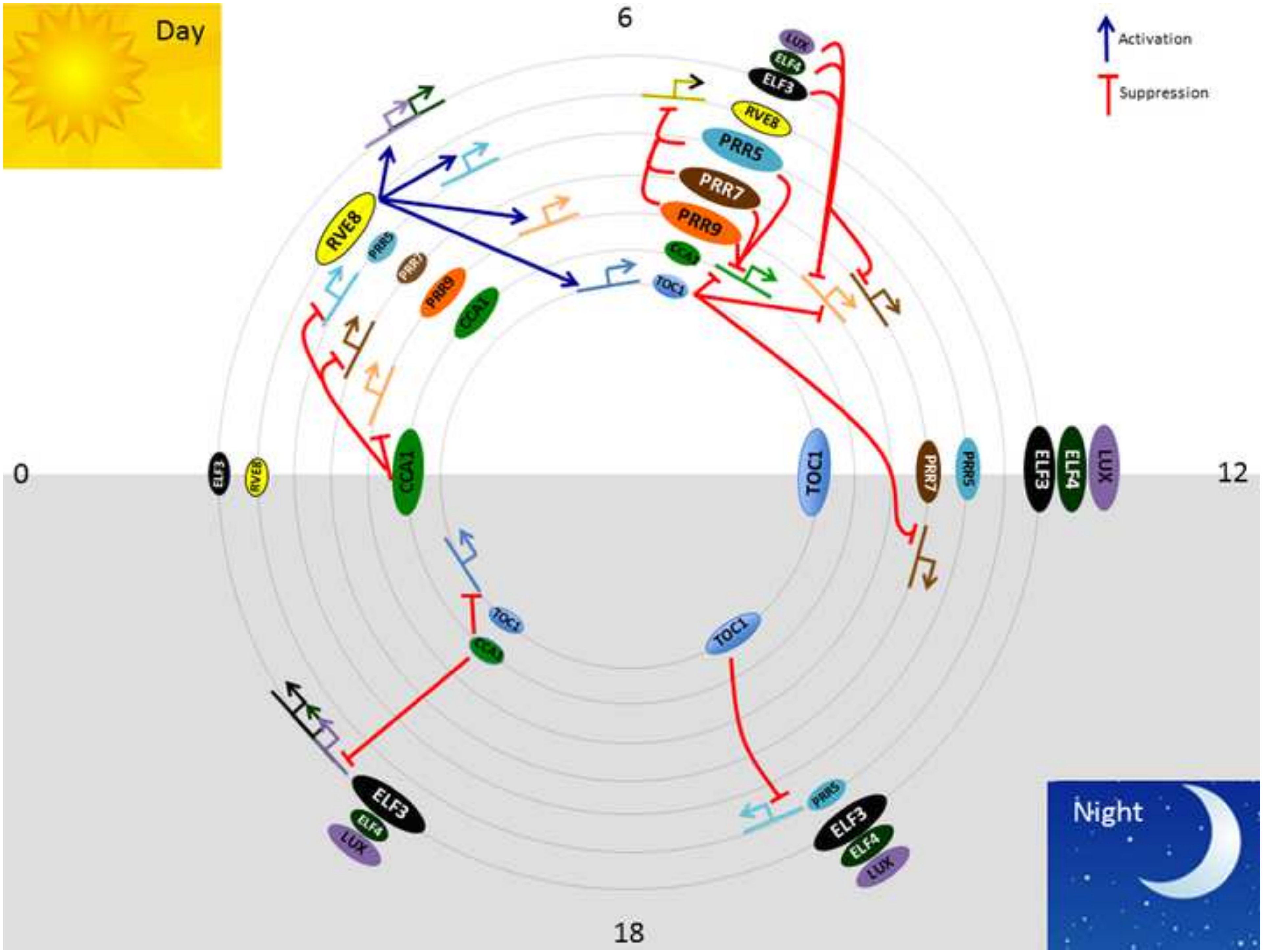
Key Message

The circadian clock regulates phytohormone biosynthesis and signaling pathways to generate daily rhythms in hormone activity that fine-tune a range of plant processes, enhancing adaptation to local conditions.

Author Contribution Statements

HSA and SLH both contributed to the writing of this manuscript.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PRR7
PRR9
CCA1
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PRR7
PRR5
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ELF4
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LUX
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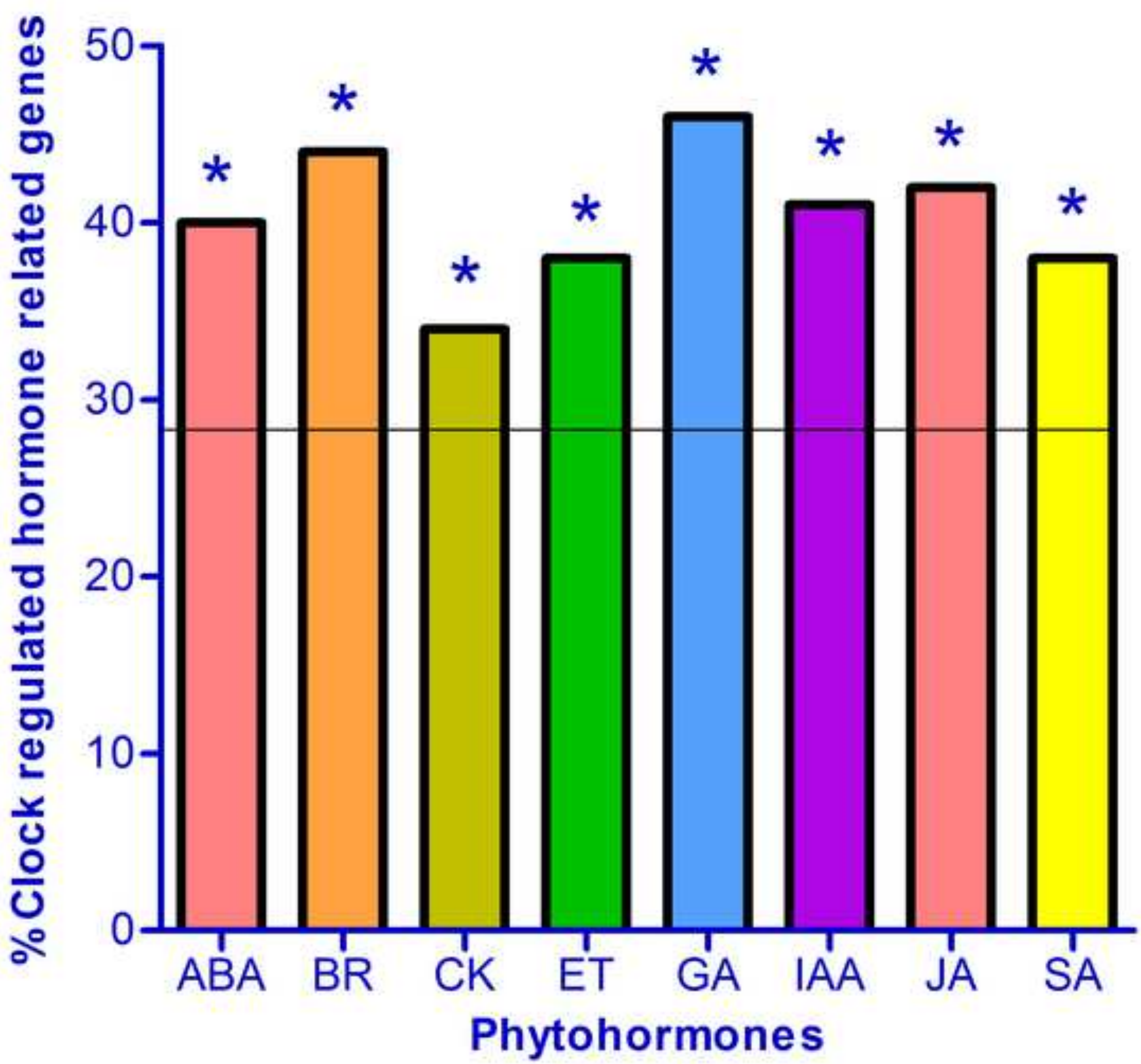
Night


% Clock regulated hormone related genes
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10
20
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40
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ABA
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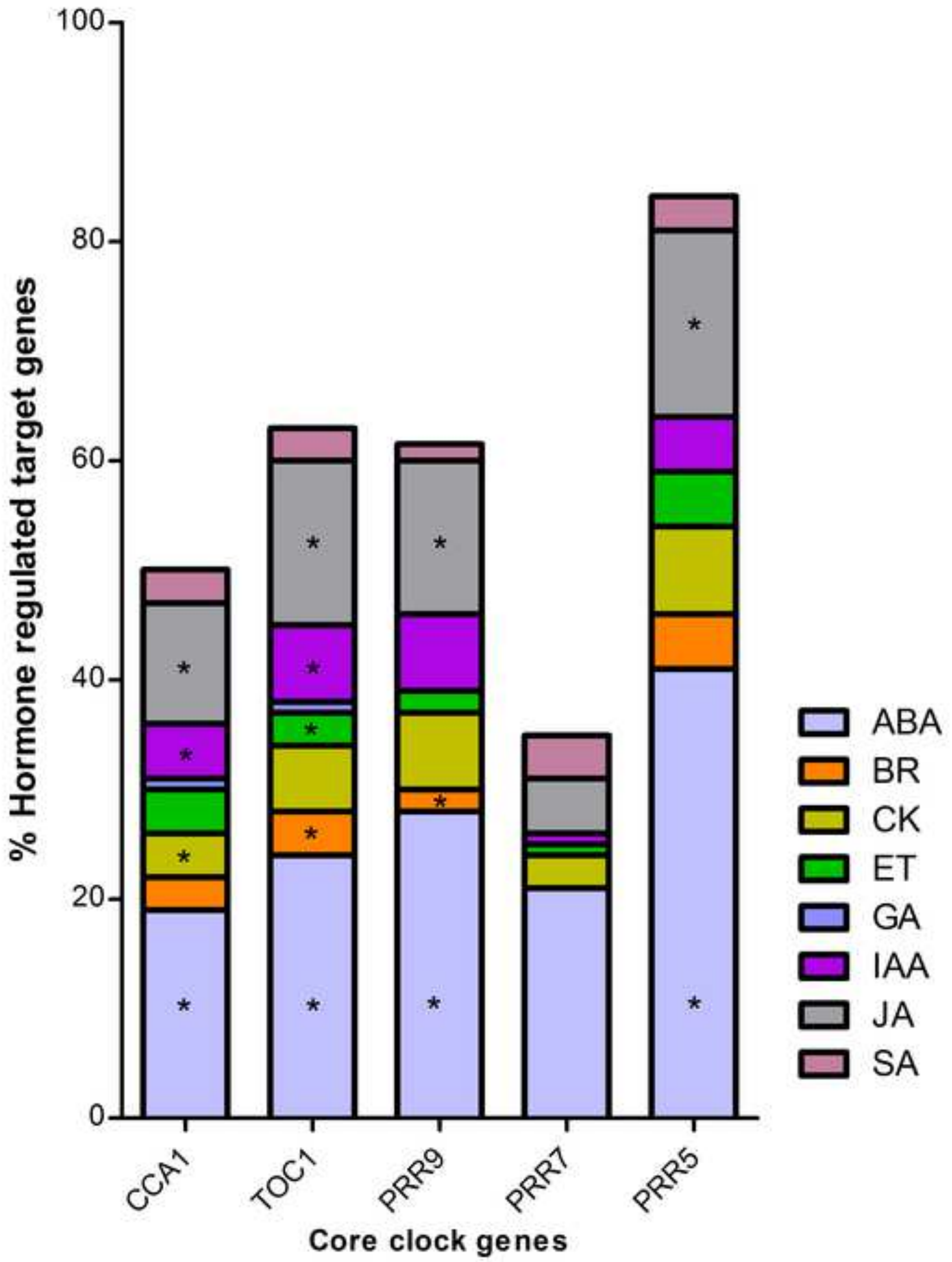
Phytohormones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Core clock genes
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GA
IAA
JA
SA
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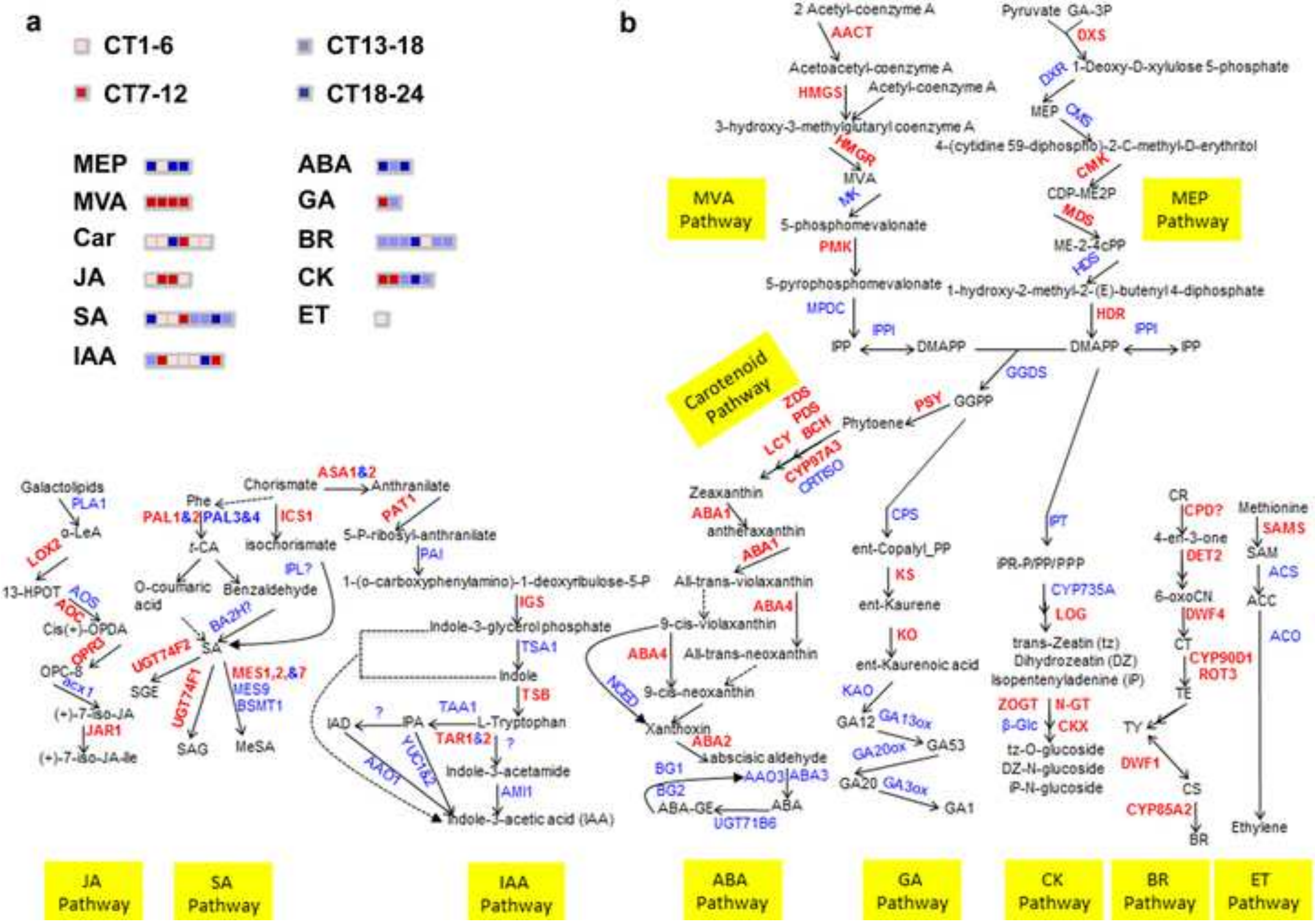
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CT1-6
CT13-18
CT7-12
CT18-24
MEP
MVA
Car
JA
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IAA
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ET
b
2 Acetyl-coenzyme A
AACT
Acetoacetyl-coenzyme A
HMGS
Acetyl-coenzyme A
3-hydroxy-3-methylglutaryl coenzyme A
HMGR
MVA
MK
5-phosphomevalonate
PMK
5-pyrophosphomevalonate
MPDC
IPP
IPPI
DMAPP
Pyruvate GA-3P
DXS
DXR
1-Deoxy-D-xylulose 5-phosphate
MEP
CMS
4-(cytidine 59-diphospho)-2-C-methyl-D-erythritol
CMK
CDP-ME2P
MDS
ME-2,4cPP
HDS
1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate
HDR
DMAPP
IPPI
IPP
MVA Pathway
MEP Pathway
Carotenoid Pathway
GGDS
GGPP
PSY
Phytoene
ZDS
PDS
BCH
LCY
CYP97A3
CRTISO
Zeaxanthin
ABA1
antheraxanthin
ABA1
All-trans-violaxanthin
ABA4
9-cis-violaxanthin
ABA4
All-trans-neoxanthin
NCED
9-cis-neoxanthin
Xanthoxin
ABA2
abscisic aldehyde
AAO3
ABA3
BG1
BG2
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ABA
UGT71B6
CPS
ent-Copalyl_PP
KS
ent-Kaurene
KO
ent-Kaurenoic acid
KAO
GA12
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GA20ox
GA53
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GA3ox
GA1
IPT
iPR-P/PP/PPP
CYP735A
LOG
trans-Zeatin (tz)
Dihydrozeatin (DZ)
Isopentenyladenine (iP)
ZOGT
N-GT
β-Glc
CKX
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DZ-N-glucoside
iP-N-glucoside
CR
CPD?
4-en-3-one
DET2
6-oxoCN
DWF4
CT
CYP90D1
ROT3
TE
TY
DWF1
CS
CYP85A2
BR
Methionine
SAMS
SAM
ACS
ACC
ACO
Ethylene
Galactolipids
PLA1
α-LeA
LOX2
13-HPOT
AOS
AOC
Cis(+)-OPDA
OPR3
OPC-8
acx1
(+)-7-iso-JA
JAR1
(+)-7-iso-JA-Ile
Chorismate
ASA1&2
Anthranilate
Phe
PAL1&2
PAL3&4
ICS1
t-CA
isochorismate
O-coumaric acid
Benzaldehyde
IPL?
BA2H?
SA
UGT74F2
SGE
UGT74F1
SAG
MES1,2,&7
MES9
BSMT1
MeSA
PAT1
5-P-ribosyl-anthranilate
PAI
1-(o-carboxyphenylamino)-1-deoxyribulose-5-P
IGS
Indole-3-glycerol phosphate
TSA1
Indole
TSB
L-Tryptophan
TAA1
TAR1&2
?
IPA
?
IAD
AAO1
YUC1&2
?
Indole-3-acetamide
AMI1
Indole-3-acetic acid (IAA)
JA Pathway
SA Pathway
IAA Pathway
ABA Pathway
GA Pathway
CK Pathway
BR Pathway
ET Pathway