

Plant circadian networks and responses to the environment

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1 **Plant circadian networks and responses to the environment**

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23 **Summary Text for the Table of Contents.**

24 Many environmental stresses are periodic and predictable, and plants have adapted to these by
25 temporally organising their stress-responses to maximise efficiency and efficacy. We review the current
26 evidence for temporal regulation of responses to the environment and the environmental impacts on
27 the plant circadian clock. Better understanding of the reciprocal interactions between the plant
28 circadian clock and environmental stresses may aid in the identification of mechanisms to improve plant
29 growth to increase food security.

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33

1 **Abstract**

2 There are regular, and therefore predictable environmental changes on Earth due to the rotation of the
3 planet on its axis and its orbit around the sun. Thus organisms have adapted their metabolism,
4 physiology and behaviour to minimise stresses caused by unfavourable conditions and maximise
5 efficiency of growth. Additionally, most organisms are able to anticipate these changes and accordingly
6 maximise metabolic efficiency and growth, because they have a complex biological time-keeping system
7 commonly referred to as the circadian clock. Multiple pathways in plants are organised in a temporal
8 manner through circadian clock-regulation of gene transcription and post-translational modifications.
9 What is becoming more apparent, is the bi-directional nature of interactions between the clock and
10 stress-response pathways. Until recently, the focus of many studies had been on the unidirectional,
11 hierarchical control of biological processes by the circadian clock, and impacts on the clock in response
12 to environmental stress had been largely ignored. Studies of interactions of the circadian clock with the
13 environment have primarily been to understand mechanisms of entrainment. We review the evidence
14 and implications of the reciprocal interactions between the clock and the environment.

15

16 **Introduction**

17 Plants are challenged with a wide range of biotic as well as abiotic stresses within the environment. Due
18 to their sessile nature, plants are unable to evade these stresses and are therefore equipped with
19 various mechanisms to cope and respond to these stresses. Although activation of stress responses is
20 crucial for survival, these processes draw on the same energy resources required for growth. Various
21 mechanisms have evolved to balance growth and stress responses (Coley *et al.* 1985; Simms and
22 Rausher 1987; Herms and Mattson 1992).

23 Due to the 24 hour light dark cycles generated by the rotation of the earth about its axis, certain abiotic
24 stresses such as light, heat and cold occur with a fairly predictable rhythm. Plants have evolved a
25 mechanism which allows them to align their internal metabolic and physiological processes with the
26 external rhythms of the environment. This mechanism is referred to as the circadian clock and plants
27 with a circadian clock that is in phase with the external environment have greater fitness than those
28 whose clocks are dysfunctional or do not resonate with the environment (Dodd *et al.* 2005). The
29 circadian clock is able to direct plants to restrict their maximal responses to a stimulus to times of day
30 when such strong responses were likely to be appropriate (a phenomenon referred to as circadian
31 gating) (Harmer 2009). In this way plants can regulate the magnitude of their responses to optimise the
32 allocation of energy to diverse processes required to ensure survival. The circadian clock allows plants to
33 coordinate their gene expression to ensure that physiological and biochemical processes occur at
34 optimal times of the day, (Dodd *et al.* 2005; McClung 2006).

35 The circadian clock has been well described in the model plant species *Arabidopsis thaliana*. It is
36 comprised of multiple interlocked transcriptional and translational regulatory feedback loops. The core
37 feedback loop consists of the morning expressed MYB domain transcription factors CIRCADIAN
38 ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) with overlapping functions. These
39 proteins act to repress the expression of the evening expressed *TIMING OF CAB EXPRESSION 1 (TOC1)*
40 also known as *PSEUDORESPONSE REGULATOR 1 (PRR1)* (Schaffer *et al.* 1998; Wang *et al.* 1998; Strayer *et*
41 *al.* 2000; Alabadi *et al.* 2001). The sequential expression of the TOC1 homologues PRR9, PRR7, PRR5 and

1 TOC1 repress the expression of *CCA1* and *LHY* (Matsushika *et al.* 2000; Strayer *et al.* 2000; Alabadi *et al.*
2 2001; Nakamichi *et al.* 2010; Huang *et al.* 2012). EARLY FLOWERING3 (ELF3), ELF4 and LUX ARRHYTHMO
3 (LUX) make up the evening complex (Schaffer *et al.* 1998; Wang *et al.* 1998; Strayer *et al.* 2000). The
4 members of the evening complex serve to relieve the repression of *CCA1* and *LHY* late at night by
5 repressing the expression of *PRR7* and *PRR9* allowing the cycle to start again (Hicks *et al.* 2001; Doyle *et*
6 *al.* 2002; Hazen *et al.* 2005; Onai and Ishiura, 2005; Nusinow *et al.* 2011; Huang *et al.* 2012; Pokhilko *et*
7 *al.* 2012 ; Dixon *et al.* 2011; Helfer *et al.* 2011).

8 The plant circadian clock has been implicated in the response to various stresses such as drought, cold,
9 salinity and pathogen attack. A large number of studies have shown that the circadian clock is able to
10 modulate various metabolic and physiological processes (reviewed by Seo and Mas 2015). It is also
11 becoming increasingly clear that external environmental factors are themselves able to feed into the
12 clock and influence certain clock parameters. The paradigm of the clock regulating certain pathways in a
13 unidirectional manner has already been queried and revised (Paz and Kay 2010). Both TOC1 (Huang *et*
14 *al.*, 2012; Gendron *et al.*, 2012) and *CCA1* (Nagel *et al.*, 2015; Kamioka *et al.*, 2016) are transcription
15 factors that have been demonstrated to bind to elements in the promoters of genes involved in diverse
16 physiological processes and thus directly influence the expression of genes in multiple pathways. The
17 expression of these two transcription factors themselves have been found to be influenced by a number
18 of environmental factors, other than zeitgebers. This review will discuss some current examples of
19 external factors that have been shown to impact on clock function as well as explore possible functional
20 consequences of clock plasticity on the various systems discussed.

21

22 **Abiotic Stresses**

23 Temperature

24 One of the key features of circadian rhythms is their temperature compensation and the genes that
25 make up of the morning loop of the circadian clock have been implicated in this (Salomé *et al.* 2010;
26 Johnson *et al.* 2003). Because most biological processes occur faster at higher temperatures than they
27 do at lower temperatures maintaining a 24 hour rhythm in fluctuating temperatures is a challenge. To
28 address this challenge plants have evolved a variety of mechanisms which allow them to do so (Gould *et*
29 *al.* 2006). However, it has also been reported that heat stress shortens the circadian period while cold
30 results in loss of rhythmicity (Bieniawska *et al.* 2008; Kusakina *et al.* 2014). This feature was observed to
31 be conserved over a variety of accessions of *A. thaliana*. Plants with robust temperature compensation
32 mechanisms seem to perform poorer in warmer environments (Kusakina *et al.* 2014). This is because
33 their dry mass was decreased as compared to those plants whose periods were more sensitive to
34 fluctuations in temperature. The flexibility in clock periodicity conferred by having reduced temperature
35 compensation may allow plants to uncouple certain physiological processes from clock control and
36 thereby shift them to a more optimal time. This may have the advantage of allowing plants to perform
37 certain metabolic and physiological activities at times of day when the damage brought on by heat
38 stress is less deleterious (Kusakina *et al.* 2014).

39 The heat stress transcription factor (Hsf) family are known to function in responses to environmental
40 and heat stresses (reviewed in von Koskull-Doring *et al.* 2007). Recent studies have implicated the heat
41 inducible HEAT SHOCK FACTOR B2b (HsfB2b) as a member of the circadian clock input pathway in *A.*

1 *thaliana*. HsfB2b has been shown to exert transcriptional repression on the morning clock gene *PRR7* by
2 binding to a conserved heat shock element (HSE) site, present in the promoter of *PRR7* (Kolmos *et al.*
3 2014). Quantitative RT-PCR (qPCR) revealed a low abundance of the *PRR7* transcript in *hsfB2b-ox*
4 mutants together with a loss of *PRR7*-promoter-driven luciferase bioluminescence. Analysis of the
5 *hsfB2b-1* mutant revealed a subtle increase in *PRR7* transcript levels with a phase advance compared to
6 WT. Taken together this data supports the role of HsfB2b as a repressor of *PRR7* (Kolmos *et al.* 2014).

7 High temperatures have also been shown to be capable of reversibly inhibiting the repressive activity of
8 the evening complex on *PRR7* and *PRR9* thus increasing the transcription of these genes during the dark
9 phase (Mizuno *et al.* 2014). The elevated expression of *PRR7* and *PRR9* in response to exposure to higher
10 temperatures was not observed in mutants for the genes of the evening complex suggesting that the
11 evening complex was involved in the temperature responsiveness of these genes during the night period
12 (Mizuno *et al.* 2014).

13 Upon exposure to low temperatures plants respond by activating mechanisms that result in freezing
14 tolerance in a process known as cold acclimation (Thomashow, 1999; Smallwood and Bowles, 2002).
15 Low temperatures induce a suite of transcriptional activators known as C-REPEAT BINDING FACTORS 1-3
16 (CBF1-3) (Stockinger *et al.* 1997; Gilmour *et al.* 1998; Medina *et al.* 1999) which are also known as
17 DEHYDRATION RESPONSIVE ELEMENT BINDING1 proteins (DREB1a-1c; Lui *et al.* 1998). *CBFs/DREB1s*
18 recognise and bind DNA cis-acting regulatory elements known as C-repeat response elements (CRT) or
19 dehydration response elements (DRE) in the promoters of a range of cold-inducible genes (Baker *et al.*
20 1994; Yamaguchi-Shinozaki & Shinozaki, 1994; Stockinger *et al.* 1997). Nakamichi *et al.* 2012
21 demonstrated that *PRR5* associates with the *CBF/DREB1* genes *in vivo* and directly activates cold-stress
22 responses by repressing *CBF/DREB1* genes. It has been observed that constitutive expression of *CCA1*
23 results in freezing tolerance (Seo *et al.* 2012), while loss of *CCA1/LHY* impairs freezing tolerance (Fowler
24 *et al.* 2005; Dong *et al.* 2011; Espinoza *et al.* 2010). In addition, freezing tolerance was heightened in
25 *toc1*, *prp9*, *prp7*, *prp5* mutants and it was observed that *TOC1* associates with *CBF* promoters (Nakamichi
26 *et al.* 2009; Nakamichi *et al.* 2012; Lui *et al.* 2013; Kiely *et al.* 2013). Freezing tolerance and responses to
27 cold temperature are regulated by the direct association of *CCA1* and *TOC1/PRRs* to the promoter of
28 *CBFs/DREB1s* to gate their expression (Fowler *et al.* 2005; Dong *et al.* 2011).

29 In addition to clock regulation of responses to cold, low temperatures also affect clock function. Plants
30 subjected to cold treatment (4°C) for 14 days in light-dark conditions, showed reduced amplitude *TOC1*
31 oscillations, but maintained the amplitude of *LUX* expression (Bieniawska *et al.* 2008). These differential
32 effects on clock component genes are intriguing and support the idea that central clock genes have
33 distinct roles in other pathways, and may have been recruited as clock components secondarily
34 (Rosbash, 2009; McClung, 2013). Cold responses which feedback into the clock to alter its function could
35 possibly be mediated via *CBF1* binding to the *LUX* promoter (Chow *et al.* 2014). Chow *et al.* also
36 demonstrated that *LUX* contributes to freezing tolerance as *lux-1* and *lux-4* mutants did not survive cold
37 treatment.

38

39 Water

40 One of the best studied cases of the circadian clock being influenced by external cues is in response to
41 drought. One of the first stress-responses of plants to drought is the inhibition of leaf growth

1 (Baerenfeller *et al.* 2016; Dubois *et al.* 2017). Plant growth is rhythmic, controlled by the circadian clock,
2 with day-time and night-time growth phases (Poiré *et al.* 2010; Dornbusch *et al.* 2014). When the effects
3 of drought on growth dynamics were examined over the course of the day, it was found that plants
4 restricted the day-time growth only (Dubois *et al.* 2017). *A. thaliana* mutants deficient in *cca1* or *lhy*
5 behaved similarly to wild-type, but those lacking *toc1* had reduced leaf growth at night too, and thus
6 displayed a greater reduction in leaf area than wild-type (Dubois *et al.* 2017). Loss-of-function *toc1*
7 mutants had better survival rates under drought conditions than wild-type plants (Legnaioli *et al.* 2009),
8 possibly due to having a much more conservative response to drought-stress (Dubois *et al.* 2017). Plants
9 that constitutively express *TOC1* (*TOC1-ox*) showed increased susceptibility and impaired responses to
10 drought stress (Legnaioli *et al.* 2009). Many water-stress responsive genes in both *A. thaliana* and
11 soybean (*Glycine max*) show diurnal and circadian expression patterns (Covington and Harmer 2007;
12 Covington *et al.* 2008; Marcolino-Gomes *et al.* 2014), and many are *TOC1* and *PRR5* targets (Huang *et al.*
13 2012; Nakamichi *et al.* 2016). As *TOC1* and the *PRRs* mainly act as transcriptional repressors, and
14 perhaps restrict drought-stress responses at times when water-stresses are likely to be lowest, at night.
15 The *toc1*-null mutants lack the repressive function and display a more active drought-stress response.

16 *TOC1* binds to promoter of the gene which encodes a putative abscisic acid (ABA) receptor: *ABSCISIC*
17 *ACID RECEPTOR/CHELATASE H SUBUNIT/GENOMES UNCOUPLED 5* (*ABAR/CHLH/GUN5*), and negatively
18 regulates its expression (Legnaioli *et al.* 2009). ABA is a plant hormone involved in a number of plant
19 growth and development pathways, including seed germination/dormancy, seedling growth and water-
20 stress responses (Finkelstein & Somerville, 1990; Hirayama & Shinozaki, 2007). Legnaioli *et al.* (2009)
21 observed that the *TOC1-ox* plants were less responsive to ABA-induced stomatal closure. This reduced
22 sensitivity to ABA contributes to increased drought susceptibility seen in the *TOC1-ox* plants. It was also
23 observed that when wild-type plants were treated with ABA during the day *TOC1* expression was
24 induced, but not when treated at night (Legnaioli *et al.* 2009). This may serve to curtail the duration of
25 ABA signalling effect to deal with a transient water stress, and allow plants to continue growth at night.
26 Thus it seems that in the case of drought the reciprocal regulation between ABA and *TOC1* serves to
27 induce an acute response whereby the early on plants switch on mechanisms that prevent water loss,
28 however due to the cost to photosynthesis they also switch on mechanisms which subsequently lead to
29 the relief of the mechanisms involved in maintaining water. The balance of the process may be
30 controlled by the relative amounts of ABA to which the plant is exposed (Legnaioli *et al.* 2009).

31 The mechanism by which *TOC1* integrates drought signalling and the circadian clock is dependent on the
32 R2R3-type MYB transcription factor, *MYB96* which is induced by high levels of ABA and is itself under the
33 control of the circadian clock (Lee *et al.* 2016). Chromatin immunoprecipitation (ChIP) assays conducted
34 on transgenic plants revealed that *MYB96* may regulate clock activity by binding to the conserved R2R3-
35 type MYB-binding sequence motif in the *TOC1* promoter. *MYB96* deficient seedlings showed a reduction
36 in the amplitude of *TOC1* expression particularly at the time of peak expression. Treatment of wild type
37 plants with ABA resulted in an advanced phase in *CCA1* expression, and luciferase assays using a
38 *pCCA1:LUC* construct revealed an advanced phase and shortened period (Liu *et al.* 2013; Lee *et al.* 2016).
39 Higher amplitude and delayed phase of *CCA1* expression was observed in *myb96-1* mutants which may
40 suggest that by modulating *TOC1* expression, *MYB96* indirectly regulates *CCA1* expression. Analysis of
41 the *MYB96* promoter revealed multiple *CCA1*-binding sites (CBS: AAAATCT) as well as evening elements
42 (EE: AAATATCT) and ChIP assays confirmed binding of *CCA1* to the *MYB96* promoter at dawn but not at
43 dusk. In the *cca1-2* mutants (Seo *et al.* 2012) the peak phase of *MYB96* expression was advanced with a

1 decrease in amplitude but a slight increase in expression was observed around dawn. This further
2 supports the idea that TOC1 may indirectly strengthen MYB96 expression by its repressive action on
3 *CCA1* to coordinate circadian rhythms with the environment. Taken together the MYB96 transcription
4 factor possibly acts as a key molecular component which mediates the bidirectional regulation between
5 ABA and the circadian clock possibly via the clock component, TOC1.

6 The effect of drought stress on the clock has been more broadly examined in *A. thaliana* and *G. max*
7 (Marcolino-Gomes *et al.* 2014; Dubois *et al.* 2017). Under the water stress conditions used by Dubois *et al.*
8 *al.* (2017), only very minor changes in the expression of *TOC1*, *LHY* and *CCA1* were observed, but the
9 nadirs of expression were lower for both *TOC1* and *LHY*. Circadian clock gene expression was also
10 altered by moderate drought stress in soybean, with the many genes showing increased amplitude or
11 advanced phase of expression (Marcolino-Gomes *et al.* 2014). Under severe drought stress, the
12 amplitude and levels of expression of most genes was significantly reduced, with the *GmTOC1*, *GmPRR7*
13 and *GmLUX* orthologues having advanced phases of expression. This may be indicative of the plant
14 adapting to the long term water-stress by extending the drought-response gene repression-phase by
15 TOC1 while still saving resources to ensure survival.

16

17 **Biotic stresses**

18 Plants are able to anticipate and respond more strongly at particular times of the day to a variety of
19 pathogens and to herbivores (Wang *et al.* 2011; Bhardwaj *et al.* 2011; Goodspeed *et al.* 2012; Zhang *et al.*
20 *al.* 2013; Ingle *et al.* 2015). Zhou *et al.* (2015) proposed that the temporal regulation of defences were
21 reinforced by the cellular redox rhythm, and enabled plants to effectively defend themselves during the
22 day and grow during the night. Plant responses to pathogen attack depend on the recognition of
23 pathogen associated molecular patterns (PAMPs) which are epitopes generally displayed by plant
24 pathogens (Jones and Dangl, 2006).

25

26 There is a large-scale transcriptional response to PAMP detection (Zipfel *et al.* 2004) that up-regulates
27 plant defences. In two sets of experiments examining the transcriptional response of *A. thaliana* to
28 infection with the necrotrophic fungal pathogen *Botrytis cinerea*, it was noted that circadian clock gene
29 expression was repressed, and their amplitude was reduced (Windram *et al.* 2012; Ingle *et al.* 2015).
30 Treatment of *A. thaliana* with a PAMP of bacterial origin shortened the circadian period (Zhang *et al.*
31 2013). Knocking out key salicylic acid-responsive gene *NON-EXPRESSOR OF PATHOGENESIS-RELATED*
32 *GENE 1 (NPR1)* in *A. thaliana* resulted in reduced *TOC1* expression (Zhou *et al.* 2015). They proposed
33 that circadian clock gene expression is regulated by the plant defence protein NPR1's activity in
34 response to cellular redox status. This reciprocal regulation allow plants to temporarily divert resources
35 from growth to defence, but minimise the costs of the diversion by allowing growth at particular times
36 of day.

37

38 **Starch and sucrose**

39 Photosynthesis is tightly regulated by the circadian clock (Millar and Kay, 1991; Dodd *et al.* 2005). The
40 circadian clock has been proposed to play a role in the breakdown of starch, which is a product of
41 photosynthesis (Graf *et al.* 2010). The ability of plants to adjust the rate of starch breakdown to match
42 the anticipated length of the night is dependent on a functional clock. Mutants lacking *cca1 lhy* are

1 unable to adjust the rate of starch breakdown in response to an extended night (Graf *et al.* 2010). As this
2 phenotype was not observed in the *toc1* loss of function mutant which also displays short-period
3 circadian rhythms, this suggests that it was not due to the clock 'running faster' in short-period mutants,
4 but rather that CCA1/LHY may be the link between starch metabolism and the clock (Graf *et al.* 2010).

5 It has also been shown that sucrose is capable of influencing clock parameters (Dalchau *et al.* 2011).
6 Application of exogenous sucrose has been shown to shorten the circadian period of the *pCCA1:LUC*
7 reporter construct in low light (Haydon *et al.* 2013). Experiments with the *pCCA1:LUC* reporter system
8 also showed that pulses of exogenous sucrose early in the morning were also shown to be capable of
9 inducing phase advances in the expression of *CCA1* while performing the same treatment at night was
10 shown to be capable of inducing phase delays (Haydon *et al.* 2013). It was however noted that
11 exogenous sucrose was most efficacious when applied in the subjective morning suggesting that
12 sensitivity to this stimulus was gated (Haydon *et al.* 2013). The same study also observed that PRR7 is
13 required for the entrainment of the clock by sucrose since the *prp7* loss of function mutant was
14 insensitive to phase changes induced by exogenous sucrose. The addition of 3 % (w/v) sucrose to
15 growth medium has been shown to induce rhythmicity in plants grown in constant darkness (Dalchau *et al.*
16 2011). This rhythm was dependent on functional GI as *gi* loss-of-function mutants were unable to
17 maintain rhythmicity in constant darkness in sucrose-containing media. These *gi* mutants were however
18 able to induce clock gene expression in response to exogenous sucrose (Dalchau *et al.* 2011).

19 That sucrose feeds back to alter the expression of *CCA1* which is normally expressed at dawn, has been
20 suggested to act as a 'metabolic dawn' signal (Haydon *et al.* 2013). It has recently been proposed that
21 sucrose feeds into the clock by causing an increase in the transcription of the Phytochrome Interacting
22 Factor (PIF) proteins (Shor *et al.* 2017). This study showed that there is an enrichment in PIF protein
23 occupancy of the *CCA1* promoter regions in response to exogenous sucrose and that this enrichment
24 correlated with an increase in the transcription of *CCA1*. The mechanism of phase setting of the clock by
25 sucrose in the morning may therefore involve the induction of PIFs which in turn cause the activation of
26 *CCA1*. This signalling may be the link back to the clock to modulate starch breakdown. The clock is
27 necessary to regulate starch reserves during the night, and the clock is set to dawn when levels of
28 sucrose are elevated signalling that starch breakdown is no longer required. In light of the fact that *CCA1*
29 plays a key role in regulating both the rate of starch breakdown as well as phase setting of the clock by
30 sucrose it is reasonable to speculate that *CCA1* may be the integrator of metabolic signals in a similar
31 fashion to the way *TOC1* plays that role in drought signalling.

32

33 **Mineral status**

34 Various minerals that act as both macro- and micronutrients have been shown to affect clock function.
35 Short term, i.e. up to 28 hours of Magnesium (Mg) deprivation was shown to result in the reduced
36 repression of *CCA1*, *LHY* as well as *PRR9* during the light period (Hermans *et al.* 2010a). Interestingly,
37 Mg-deprivation activated ABA-signalling (Hermans *et al.*, 2010a), previously demonstrated to be closely
38 tied to *TOC1* (Legnaioli *et al.* 2009) and recently, to be subject to clock regulation (Lee *et al.* 2016). Long
39 term, i.e. a week of Mg-deprivation resulted in reduced amplitude of circadian transcripts especially in
40 the leaves (Hermans *et al.* 2010b). Copper has also been shown to affect the circadian clock, with an
41 overabundance altering the amplitude and phase of circadian clock genes *CCA1* and *LHY* (Andrés-Colás

1 et al. 2010). Plants that constitutively expressed the copper transporter protein COPT1 grown in
2 medium supplemented with high copper levels had increased endogenous copper levels. Under these
3 conditions, circadian rhythms were dampened and photoperiodic flowering times were altered (Andrés-
4 Colás et al. 2010).

5 In three independent studies it was shown that iron (Fe)-deprivation leads to a lengthening of the
6 circadian period (Chen *et al.* 2013; Hong *et al.* 2013; Salome *et al.* 2013). The mechanisms by which this
7 is achieved are not yet fully understood but preliminary work has uncovered some potential players.
8 Hong *et al.* (2013) observed that the *cca1 lhy* double mutant nor the *ztl* loss-of-function mutant, *ztl-4*,
9 did not display Fe-deprivation period lengthening, unlike the long period *prr7 prr9* double mutant which
10 had an even longer period in response to iron deprivation. This suggested that CCA1/LHY and ZTL are
11 involved in the period lengthening in response to low Iron levels. However, the role of CCA1 and LHY in
12 the Fe-response could not be established in the study by Salome *et al.* (2013). This could be due to the
13 differences in growth media composition between the studies. Importantly the two studies utilised
14 different percentages of sucrose and as highlighted earlier sucrose itself is capable of impacting the
15 function of the central oscillator. In the study by Salome et al. (2013) it was also noted that the clocks of
16 etiolated seedlings, or light-grown seedlings treated with a plastid translation inhibitor had reduced
17 sensitivity to Fe-status, indicating the centrality of chloroplasts in Fe-sensing. Their results suggested that
18 a chloroplast-derived protein may be responsible for sensing the Fe-status of the plants. A role was
19 proposed for HEMERA (HMR) a plastid protein responsible for translocation of the phytochrome
20 proteins to the nucleus. The clock of *hmr* mutants showed reduced sensitivity to iron status compared
21 to plants with functional HMR. The requirement of chloroplast translation was also supported by the
22 Chen *et al.* (2013) study which also found that plants treated with inhibitors of plastid translation
23 showed an increased circadian period but they did not further increase their period in response to Fe-
24 deprivation. Taken together it seems plausible to suggest that Fe-status might be sensed by the plastid
25 derived protein HMR which then acts in concert with ZTL to regulate CCA1 levels.

26

27 **Conclusion**

28 The plant circadian clock co-ordinates the temporal execution of various metabolic and physiological
29 processes. It is capable of receiving temporally informative signals, or zeitgebers, from the external
30 environment which affect rhythm parameters allowing for the entrainment of the clock. It is becoming
31 increasingly apparent that other environmental signals are capable of affecting circadian clock functions.
32 Environmental signals affect clock parameters in a myriad of ways. However it seems that the various
33 ways in which the circadian clock is regulated by environmental signals have beneficial effects on plant
34 fitness. It seems likely that clock plasticity to various environmental signals evolved independently for
35 the various pathways as an additional regulatory mechanism. This would explain why the various
36 environmental inputs have varying effects on clock parameters and why they act via different targets.
37 The reciprocal connections between the clock and plant responses to the environment allow plants to
38 exquisitely fine tune their metabolism to enhance survival, and adds to the complexities of future
39 research to better understand plant growth regulation.

40

41 **Conflict of interest statement**

1 The authors declare no conflicts of interest.

2

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