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Understanding Circadian Regulation of Carbohydrate Metabolism in Arabidopsis Using Mathematical Models

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C_3 plants assimilate carbon by photosynthesis only during the day, but carbon resources are also required for growth and maintenance at night. To avoid carbon starvation, many plants store a part of photosynthetic carbon in starch during the day, and degrade it to supply sugars for growth at night. In Arabidopsis, starch accumulation in the day and degradation at night occur almost linearly, with the shape of this diel starch profile adaptively changing to allow continuous supply of sugar even in long-night conditions. The anticipation of dawn required to ensure linear consumption of starch to almost zero at dawn presumably requires the circadian clock. We review the links between carbon metabolism and the circadian clock, and mathematical models aimed at explaining the diel starch profile. These models can be considered in two classes, those that assume the level of available starch is sensed and the system ensures linearity of starch availability, and those in which sugar sensing is assumed, yielding linearity of starch availability as an emergent property of sucrose homeostasis. In the second class of model the feedback from starch metabolism to the circadian clock is considered to be essential for adaptive response to diverse photoperiods, consistent with recent empirical data demonstrating entrainment of the circadian clock by photosynthesis. Knowledge concerning the mechanisms regulating the dynamics of starch metabolism and sugar homeostasis in plants is required to develop new theories about the limitations of growth and biomass accumulation.

Keywords: Carbon starvation • Circadian clocks • Growth • Mathematical model • Photoperiod • Sucrose homeostasis.

Abbreviations: AGPase, ADP-glucose pyrophosphorylase; BOA, BROTHER OF LUX ARRHYTHMO; CCA1, CIRCADIAN CLOCK ASSOCIATED 1; CHE, CCA1 HIKING EXPEDITION; CO, CONSTANS; EE, evening element; ELF, EARLY FLOWERING; FKF1, flavin-binding kelch repeat F box1; GBSS, GRANULE BOUND STARCH SYNTHASE; GI, GIGANTEA; LHY, LATE ELONGATED HYPOCOTYLl; LUX, LUX ARRHYTHMO; MED16, Mediator16; pgm, phosphoglucomutase; PRR, PSEUDORESPONSE REGULATOR; REV, REVEILLE; SFR6, SENSITIVE TO FREEZING TOLERANCE 6; SIG5, SIGMA FACTOR5; ZTL, ZEITLUPE.

Introduction

Plants are sessile organisms on a rotating planet that use energy from the sun to fix carbon dioxide into sugars. In C_3 plants, the rate of carbon assimilation by photosynthesis usually exceeds the rate of carbon release by respiration and therefore in many species the assimilated carbon is partitioned between sucrose, which is used for immediate growth and maintenance, and starch, which accumulates in the leaf for later demand at night (Caspar et al. 1985, Geiger and Servaites 1994, Gibon et al. 2004). On most parts of the Earth, the period of oscillation of these cycles of carbon flux is 24 h due to the light and dark cycle and therefore it seems reasonable to assume that there is evolutionary advantage for linkage between carbon metabolism and an internal timing device. Here we describe theoretical and experimental approaches to understand how a plant might exert control over the timing of starch accumulation and consumption, and the possible role of the circadian clock in the regulation of carbon homeostasis.

The circadian clock provides an internal estimate of time that contributes to the regulation of carbon metabolism

Temporal analyses of transcripts and metabolites, and experiments in circadian mutants demonstrate a broad role for the circadian clock in regulating aspects of carbon metabolism (Haydon et al. 2013a). The core of the circadian oscillator comprises a network of transcriptional regulators (Hsu and Harmer 2014). These include proteins that can act as transcriptional repressors, encoded by the genes *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *PSEUDORESPONSE REGULATOR* (*PRR*) *1*, *3*, *5*, *7* and *9*, *CCA1 HIKING EXPEDITION* (*CHE*) and *LUX ARRHYTHMO* (*LUX*), and the transcriptional activators encode by *BROTHER OF LUX ARRHYTHMO* (*BOA*) and *REVEILLE* (*REV*) *4*, *6* and *8*. The transcriptional regulators participate in interlocking feedback loops of transcription and translation which, along with targeted protein degradation, generate rhythmic patterns of abundance of the transcriptional regulators. Phase-specific rhythmic expression of circadian clock genes and circadian clock-controlled output genes is conferred by specific regulatory promoter

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sequences; for example, the evening element (EE) [(A)AAATAT CT], which is the binding site for CCA1 and LHY, regulates the phase of circadian clock oscillator genes and output genes (Covington et al. 2008). Gene expression is also regulated through alterations in RNA splicing (Hong et al. 2010, Sanchez et al. 2010). In addition to the regulation of gene expression, the abundance of the transcriptional regulator proteins is modulated by targeted protein degradation pathways. For example, the F-box protein ZEITLUPE (ZTL), which is bound to and stablized by GIGANTEA (GI) in a blue-light-dependent manner, is a major controller of ubiqutin targeting-mediated degradation of circadian clock proteins including PRR1 (also known as TOC1). Post-translational modifications such as phosphorylation and cyclic ADPR-mediated Ca^{2+} signaling also contribute to circadian timing (Dodd et al. 2007, Wang et al. 2010). The activity of the transcriptional regulators can be modulated by the control of subcellular location through complexing with proteins such as EARLY FLOWERING 3 (ELF3) and 4 (ELF4) (Herrero et al. 2012). Hypotheses concerning the architecture of the circadian system in Arabidopsis are developing (Hsu and Harmer 2014).

Circadian regulation of photosynthesis contributes to rhythmic carbon accumulation

There are diel oscillations of photosynthesis due to the light/dark cycle. Additionally, there are circadian oscillations in the rate of net carbon assimilation that persist in constant light with a period of 24 h (Dodd et al. 2005), which might be due to the modulation of the rate of chloroplastic gene expression by timing signals arising from the circadian oscillator in the nucleus. In Arabidopsis, the expression of the nuclear-encoded *SIGMA FACTOR5* (*SIG5*) is modulated by the circadian clock (Noordally et al. 2013). SIG5 confers promoter specificity to plastid-encoded RNA polymerases and is required for the circadian expression of transcripts from the *psbD* operon, which encodes the rapidly turned over D2 protein of the reaction center of PSII. Many nuclear- and plastid-encoded transcripts associated with photosynthesis are under circadian control; this, and presumably post-translational effects, result in the observed free-running oscillations in carbon assimilation.

Starch as a buffer against fluctuation in carbon supply

Growth and maintenance processes requiring carbon must continue despite the daily cycles in photosynthetic activity that arise from diurnal and circadian modulation, and shorter term fluctuations in light availability. For example, in Arabidopsis, like many other species, the maximum relative growth occurs in the day, but 30% of the growth activity in leaves occurs at night (Walter and Schurr 2005, Wiese et al. 2007). The sustained growth both in the light and in the dark is the result of careful management of carbon because even short periods of carbon starvation lead to an inhibition of growth (Smith and Stitt 2007, Stitt et al. 2007).

Buffering against daily fluctuations in the carbon supply is achieved through a diel turnover of starch. In the *sft1* mutant, which is unable to synthesize starch as a result of altered plastidic phosphoglucomutase function (Kofler et al. 2000), there is a surplus of carbohydrate in the afternoon, but little carbon available at night due to the failure to accumulate starch. This loss of the starch buffer in *sft1* decreases the relative growth rate at the end of the night, while increasing it during the day (Wiese et al. 2007). Reduced growth in roots and leaves has also been reported in the chloroplast phosphoglucomutase (*pgm*) starchless mutant (Caspar et al. 1985, Gibon et al. 2004). The strong positive correlation between the starch degradation rate or soluble sugar availability and relative growth rate occurs across a range of photoperiods (Gibon et al. 2009, Sulpice et al. 2014). In addition, there is a strong negative correlation between the starch content at the end of the night and the biomass of 20 Arabidopsis accessions in short-day conditions (Cross et al. 2006).

Starch accumulation in the light and degradation in the dark occur in a near-linear manner, with 5–10% remaining at the end of the night (Caspar et al. 1985, Gibon et al. 2004, Smith et al. 2004, Zeeman et al. 2010). The near-linear pattern of starch accumulation and degradation has been observed even when irradiances, daylength, CO_2 concentrations, and nutrient and water supply have been altered (Gibon et al. 2009, Tschoep et al. 2009, Hummel et al. 2010). Plants that are grown in shorter daylengths accumulate starch faster in the light and degrade it more slowly at night in an apparent anticipation of the requirement to endure long nights (Stitt et al. 1978, Chatterton and Silvius 1981, Lu et al. 2005, Graf et al. 2010). Early or late onset of night causes an immediate change in the starch degradation rate, demonstrating that the system is capable of dynamic adjustment (Lu et al. 2005, Graf et al. 2010). Flexible adjustments of the rates of starch synthesis and degradation have also been reported in conditions with lower light intensity and lower CO_2 concentration (Chatterton and Silvius 1981, Mullen and Koller 1988, Matt et al. 2001).

The circadian clock regulates diel starch turnover

In wild-type and circadian period mutants (*toc1-1* and *ztl-1*), carbon assimilation and biomass accumulation were reduced when the circadian period was not matched to the length of the total light and dark cycle (Dodd et al. 2005). It was hypothesized that this might be a consequence of the measured reduction in Chl observed when the circadian period and the diel cycle were mismatched (Dodd et al. 2005); however, later studies suggest that altered starch turnover might be the cause of the reduced growth. Graf et al. (2010) found that the timing of starch exhaustion was regulated by the circadian oscillator rather than the external light/dark cycle, such that starch was exhausted at the internal circadian dawn, irrespective of the actual dawn. For example, in 24 h light/dark cycles, *cca1-11 lhy-21*, loss-of-function circadian mutants that retain a circadian clock with a short period, degrade starch too quickly and starch

reserves are exhausted 3–4 h before the end of the night (Graf et al. 2010, Yazdanbakhsh et al. 2011). The role of the circadian system in starch degradation is exemplified by the finding that wild-type plants under 24 h environmental cycles and *cca1-11 lhy-21* plants under 17 h cycles had almost identical patterns of starch degradation in relation to the actual dawn (Graf et al. 2010). The involvement of the circadian clock in starch metabolism is also suggested by the very high starch levels in *gi* mutants (Eimert et al. 1995). The difference in biomass between matched and mismatched conditions was decreased by supplying sucrose in the media, suggesting that the lower growth rate in mismatched conditions is a consequence of sucrose starvation caused by early depletion of starch (Graf et al. 2010). Mugford et al. (2014) has recently speculated that acceleration of starch synthesis in short days could be regulated by flavin-binding kelch repeat F box1 (FKF1) and GI because mutants of these genes did not show correct adjustment of starch synthesis in response to the change of photoperiod. Mugford et al. (2014) also suggested that the adjustment of starch synthesis to a different photoperiod is dependent on post-translational modulation of the activity of the starch synthesis enzyme ADP-glucose pyrophosphorylase (AGPase).

Two theoretical mechanisms explaining diel starch turnover

The mechanism whereby starch breakdown is paced such that starch is exhausted at the dawn anticipated by the circadian clock is unknown (Graf et al. 2010, Graf and Smith 2011). Mathematical models have provided insight into the potential underlying mechanisms (Feugier and Satake 2013a, Scialdone et al. 2013).

The starch degradation process during the night can be approximated by the formula including the starch degradation rate at time t, denoted as $\beta(t)$, as follows;

$$\frac{dC}{dt} = -\beta(t)C(t) \quad (1)$$

The above equation expresses that the starch amount C decreases due to degradation with rate $\beta(t)$. $\beta(t)$ is likely to be an aggregate parameter of multiple processes such as cascades of regulations; transcription, translation, degradation and chemical modification of enzymes involved in starch metabolism. Starch-degrading enzymes cannot access all available starch because starch exists as large polymers and the degrading process only occurs at the surface of the granule. Thus, a more appropriate formulation would be to replace $C(t)$ in the right-hand side of Equation 1 as $C(t)^{\kappa}$ because $C(t)^{\kappa}$ is in proportion to the surface area of the spherical starch granule when $\kappa = 2/3$. Here, for simplicity, we use Equation 1 to approximate starch dynamics because the following arguments are unchanged even if we use the more realistic formulation.

To determine the shape of $\beta(t)$, we first solve Equation 1 by assuming that the derivative of $C(t)$ by t equals to a constant [i.e. $dC(t)/dt = k$], and obtain $C(t)$ as the following linear decreasing function of time t:

$$C(t) = C_{dusk} - k(t - t_L)\, k(t^* + t_L - t) \quad (2)$$

where C_{dusk} is the starch amount at dusk, t_L represents the length of the day, and $t^* = C_{dusk}/k$ represents the length of time needed to deplete starch completely [i.e. $C(t^*) = 0$; see **Fig. 1a**]. Note that time is counted from dawn (i.e. $t = 0$ at dawn). Using $\beta(t)C(t) = k$ and Equation 2, we can determine the function for starch degradation as a hyperbolic function of time t (Feugier and Satake 2013b):

$$\beta(t) = \frac{1}{t^* + t_L - t} \quad (3)$$

As a result, Equation 1 becomes:

$$\frac{dC}{dt} = -\frac{1}{t^* + t_L - t}C(t) = -k = -\frac{C_{dusk}}{t^*} \quad (4)$$

In addition to a linear starch profile across time, Equation 4 insures that whatever the amount of starch at dusk, starch will always decrease to 0 exactly at the end of the night if t^* corresponds to the length of night, and hence a rate constant k, which is consistent with the slope of starch profile at night, decreases as the photoperiod decreases (i.e. the length of night increases). In reality, t^* would be slightly longer than the actual length of night because starch is almost but not entirely exhausted at dawn. Given the starch dynamics during the day as $dC/dt = p - \beta(t)C(t)$, we can determine the shape of $\beta(t)$ during the day as $\beta(t) = \frac{(pt_L/C_{dusk})-1}{t}$, using the same logic, where p indicates the increase rate of starch amount by photosynthesis. Because $\beta(t)$ cannot be negative, starch degradation during the day would not happen [i.e. $\beta(t) = 0$] when $p \leq C_{dusk}/t_L$.

Although Equation 4 gives the theoretical requirements that should be satisfied to explain the linearity and appropriate adjustment of slope in various environmental conditions, the underlying biological mechanisms to realize the dynamics in Equation 4 are unknown. The model proposed by Scialdone et al. (2013) assumed that plants dynamically measure the two quantities, the amount of starch remaining (C) and the time to dawn ($t^* + t_L - t$), and divide these as formalized by Equation 4, which allows plants to compute the appropriate slope given in Equation 4. In their model, measurements of starch amount and the time to expected dawn are performed by hypothetical molecules, S and T, respectively, and specific chemical kinetics between these two molecules and starch were assumed to realize a constant rate for starch decline during the night. Scialdone et al. (2013) analyzed mutants that lack a protein involved in starch degradation, and found that phosphoglucan water dikinase (PWD) might be a node at which information about expected time to dawn and starch content is integrated to set an appropriate rate of starch degradation, because the *pwd* mutant did not have appropriate adjustment of starch degradation in response to an unexpectedly early night.

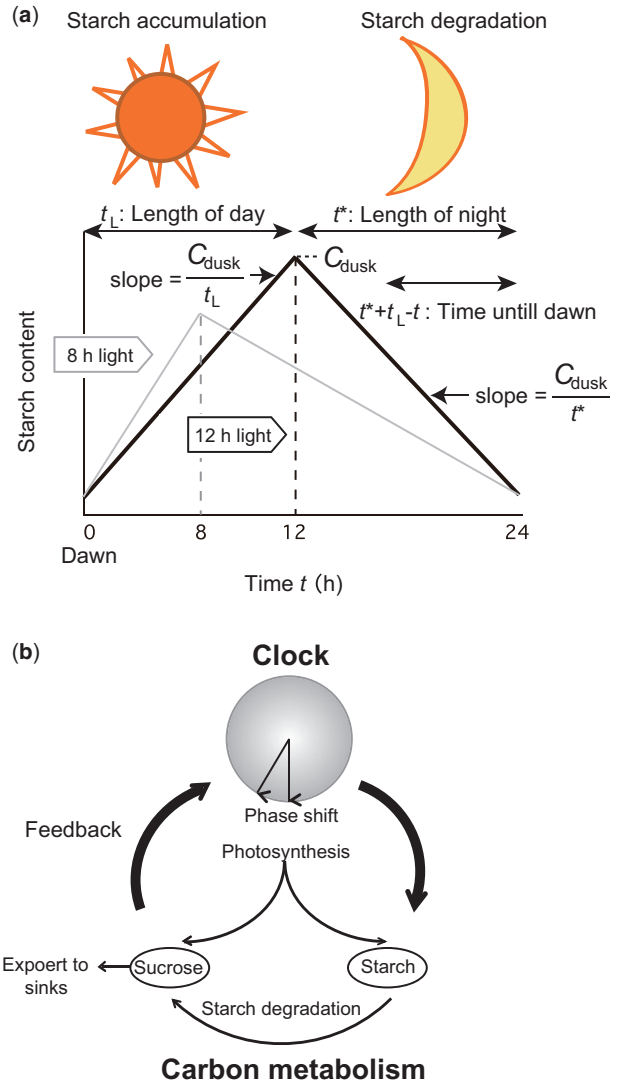

Fig. 1 (a) Typical starch profile in 12 h/12 h (black) and 8 h/16 h (gray) day-night cycles in plant leaves. C_{dusk} represents the amount of starch at dusk. When linearity with time is satisfied, the amount of starch should increase with the slope of C_{dusk}/t_L, while it should decrease with the slope of $-C_{dusk}/t^*$. (b) Flow chart of the model proposed by Feugier and Satake (2013a). The circadian clock regulates the processes in carbon metabolism such as starch degradation, which changes the diel sucrose profile. The signal of carbon starvation then feed backs to the clock by changing the phase of oscillation.

In contrast, the model proposed by Feugier and Satake (2013a) is based on the idea in which linearity in starch degradation is an emergent property that results from minimization of carbon starvation. Plants would minimize the carbon starvation risk because even a short period of carbon starvation leads to an inhibition of growth (Smith and Stitt 2007, Stitt et al. 2007). Feugier and Satake (2013a) hypothesized that the starch degradation rate [$\beta(t)$ in Equation 1] is regulated by the circadian clock, and the phase of the circadian clock changes in response to the severity of sucrose starvation (**Fig. 1b**). They showed that a phase shift of the circadian clock in response to sucrose status was sufficient to explain the three properties of starch turnover, linearity of starch profiles with time both in the day and in the night, almost complete depletion of starch by the end of the night, and adjustment of starch accumulation in response to photoperiod changes. The level of starvation threshold assumed in the model does not influence the conclusion. Feugier and Satake (2013b) also found that the ideal shape of $\beta(t)$ that avoids carbon starvation and realizes sucrose homeostasis was exactly the same as the hyperbolic function given in Equation 3. This result indicates that linearity of starch profiles can be a consequence of sucrose homeostasis.

Critical differences exist between the models by Scialdone et al. (2013) and Feugier and Satake (2013a). Scialdone et al. (2013) assume that the level of available starch is sensed and the system ensures linearity of starch availability, and this assumption has also been included in models that incorporate the detailed regulatory dynamics between major clock genes (Seaton et al. 2014) and diel regulation of carbon metabolism in the cytosol and chloroplast (Pokhilko et al. 2014). On the other hand, the model of Feugier and Satake (2013a) assumed that no signaling networks were present that measure starch levels and instead included the feedback term representing the signaling networks associated with sugar sensing. The model of Feugier and Satake (2013a) yielded linearity of starch availability as an emergent property of the system. Although Equation 1 could be interpreted to indicate that plants implicitly sense starch levels because starch degradation occurs in proportion to the total amount of starch that cannot be accessed, we can avoid such an interpretation by considering the degradation process occurring only at the surface of the starch granule [replacement of $C(t)$ in the right-hand side of Equation 1 as $C(t)^{\kappa}$ where $\kappa = 2/3$]. The models also differed in the way in which the circadian clock was incorporated. Because Scialdone et al. (2013) assumed starch sensing, the feedback from starch metabolism to the circadian clock was not essential, while Feugier and Satake (2013a) considered the feedback from starch metabolism to the circadian clock to be a key factor to adjust the starch degradation profile in diverse environmental conditions.

Regulation of Arabidopsis circadian clocks by sugars

The inclusion of feedback from sugars to the circadian clock in the model by Feugier and Satake (2013a) is consistent with recent empirical data. Soluble sugars such as sucrose, glucose and fructose have at least four effects on the circadian clock of Arabidopsis; sustaining circadian oscillations in constant darkness (Dalchau et al. 2011, Haydon et al 2013b), reducing circadian period (Knight et al. 2008, Haydon et al. 2013b), altering the phase of the oscillator (Haydon et al. 2013b) and abolishing circadian oscillations in the concentration of free Ca^{2+} ions in the cytosol ($[Ca^{2+}]_{cyt}$; Johnson et al. 1995) (**Fig. 2**). Circadian period is insensitive to sugar in plants carrying loss-of-function mutations in *CCA1* and *PRR7* (Haydon et al. 2013b) and also *SENSITIVE TO FREEZING TOLERANCE 6* (*SFR6*) (Knight et al 2008). SFR6 encodes Mediator16 (MED16), a subunit of the

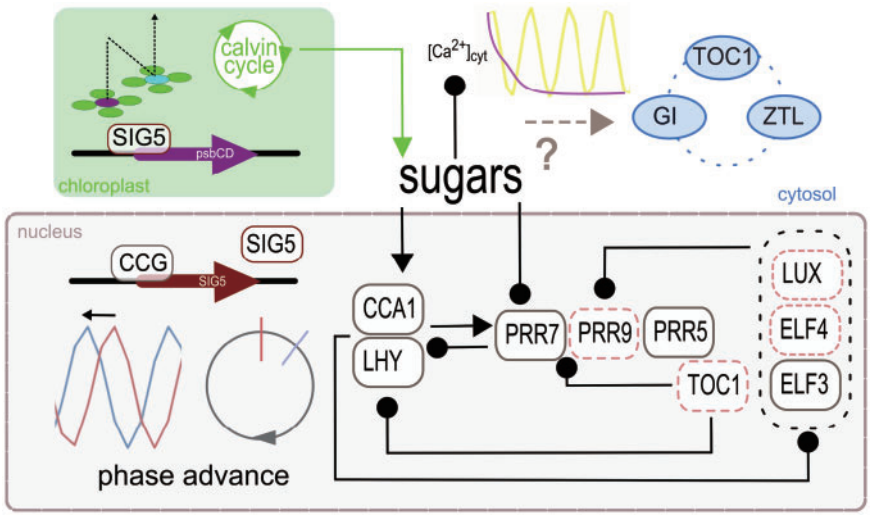

Fig. 2 Photosynthetic sugar production adjusts the phase of the circadian oscillator. The model depicts potential events in the chloroplasts, cytosol and nucleus of an idealized plant cell. In the chloroplast, the nuclear-encoded SIG5 (brown) activates expression from the *psbCD* operon of the chloroplast genome which encodes D2, a component of the reaction center of PSII (purple). Energy harvested by the photosystems is used to generate sugars through the Calvin cycle (green). During the day, sugars accumulate in the cytosol. Cytosolic sugars can affect circadian rhythms through a pathway involving *GI*. It is not known if sugars affect the protein–protein interactions between GI, ZTL and TOC1 (blue) in the cytosol. Sugars might also regulate the light-dependent transcription of *GI*. Also in the cytosol circadian oscillations of $[Ca^{2+}]_{cyt}$ (yellow) are abolished by high exogenous sugars (pink). Sugars activate *CCA1* expression and suppress *PRR7* expression, affecting the transcriptional translational feedback in the circadian oscillator in the nucleus. Activatory arms are indicated by arrows and repression by circles. Only transcriptional relationships are shown. The oscillator model is modified from Hsu and Harmer (2014). The circadian clock components that have evening elements (EEs) in the promoter are indicated by red dashed lines. The EE is a binding site for the activatory circadian clock transcription factor RVE8, which is omitted for clarity. The EE is also enriched in genes regulated by SFR6/MED16, which is involved in the response of the circadian clock to sugars through an unknown pathway. Sugar-mediated regulation of *CCA1/PRR7* causes a phase advance in the circadian oscillator indicated by the shift from the red to blue waveform, and an advance from the red to blue position on the phase circle diagram. This will affect the timing of expression of circadian clock genes that are outputs of the circadian clock (CCG) involved in regulating a range of activities, including the expression of circadian-regulated genes such as SIG5.

Mediator complex that physically links transcriptional regulators to the recruitment and regulation of RNA polymerase II activity (Hemsley et al. 2014) and is involved in the regulation of expression of genes containing the circadian-regulated EE in the promoter (Knight et al. 2008).

In constant dark, in the absence of exogenous sucrose in the growth medium, rhythms of *CCA1*, *TOC1* and *GI* promoter activity are very low amplitude or undetectable. The addition of 3% sucrose to the medium restores robust rhythmic activity, albeit at a lower amplitude than occurs in constant light (Dalchau et al. 2011). To identify potential mechanisms by which sucrose in the medium might regulate the circadian system, Dalchau et al. (2011) took advantage of the mathematical descriptions of the Arabidopsis circadian oscillator that had been developed using experimental data obtained in the presence of 3% sucrose (Locke et al. 2006). Dalchau et al. (2011) determined that singular or multiple alterations in the kinetic parameters of the hypothetical component *Y* could simulate the dynamics of circadian clock gene expression in the presence and absence of exogenous sucrose (Dalchau et al. 2011). *Y* was a hypothetical gene forming a loop of the circadian oscillator in feedback with *TOC1* (Locke et al. 2006). At least some of the simulated functions of *Y* in the network were considered to have been fulfilled by GI (Locke et al. 2006). In *gi* loss-of-function plants, exogenous sucrose was unable to restore circadian oscillations in constant dark, confirming the mathematical predictions (Dalchau et al. 2011). *Y* has been removed from newer models of the Arabidopsis circadian oscillator as more data concerning *GI* and also the evening complex of proteins (ELF3, ELF4 and LUX) (Herrero et al. 2012) have been incorporated (Pokhilko et al. 2012).

GI is involved only in long-term metabolic sensing by the circadian oscillator because *gi-11* mutants retain a transient induction of clock gene expression in response to sucrose application but are unable to maintain circadian oscillations in constant dark even in the presence of sugars (Dalchau et al. 2011). This *GI*-dependent sugar sensing pathway occurs through a different pathway from that by which sugars regulate circadian period because the *gi-2* mutation is without effect on the regulation of circadian period by sugars (Dalchau et al. 2011, Haydon et al. 2013b).

To determine if the circadian oscillator is sensitive to the daily oscillation of soluble sugars, Haydon et al. (2013b) inhibited photosynthesis using CO_2-free air, DCMU, an inhibitor of PSII, or low light intensity (10 $\mu mol^{-2}\ s^{-1}$), all of which dampened the daily oscillation of internal soluble sugar concentration, and increased the period of the circadian clock (Haydon et al. 2013b). Addition of exogenous sucrose in these conditions reduced the circadian period, whereas modifiers of oxidative stress or chloroplastic–nuclear retrograde signaling pathways

were without effect, leading to the conclusion that photosynthesis regulates the clock through sugar production (Haydon et al. 2013b).

Circadian oscillators are adjusted in phase by the light and dark cycle in a process called entrainment to ensure that the internal timing of biological events is appropriate for the local time. Phase adjustment also permits the oscillator to track dawn in those latitudes where the daylength varies with the season. Entrainment occurs due to light-mediated regulation of the circadian clock, through the cryptochromes and phytochromes and also by photosynthetically derived sugars adjusting the phase of the circadian oscillator (Haydon et al. 2013b). Sugars advance the phase of the circadian oscillator in the morning and are without effect, or delay circadian phase, during the night (Haydon et al 2013b). Sugar is not merely a proxy for light in the entrainment of the circadian oscillator because exogenous application of sucrose or light set the circadian system to different phases (Dalchau et al. 2011, Haydon et al. 2013b). The sensory pathway(s) by which sugars adjust the phase of the circadian system are not known, but it appears that these pathways terminate at the promoter of *PRR7* and/or *CCA1*. Sugars stimulate *CCA1* promoter activity and repress the activity of the *PRR7* promoter, whereas starvation activates *PRR7* and suppresses *CCA1* (Haydon et al. 2013b).

It could be argued that the effects of sugars on the circadian clock are related to relief of energetic restrictions affecting oscillator function. We consider it unlikely that the circadian oscillator is weakly buffered against energy supply since this would mean that oscillator speed would be dependent on the metabolic status of the plant, and therefore too variable to be of functional use in anticipating the day/night cycle. The insensitivity of single gene mutants in *PRR7*, *CCA1* and *GI* to sugars under specific conditions is also suggestive of regulatory rather than metabolic effects of sugar on circadian oscillator function. In constant dark, *CCA1* induction by cold was similar both with and without exogenous sucrose (Dalchau et al. 2011), and inhibition of photosynthesis by DCMU treatment results in a persistent induction of rhythmic *PRR7:LUC* activity which would not be possible if energy was limiting gene expression or clock function (Haydon et al. 2013b).

Metabolic dawn and its role for sucrose homeostasis

It has been proposed that the regulation of the PRR7–CCA1 feedback loop by photosynthetically derived sugars represents a metabolic dawn (Haydon et al. 2013b). In the metabolic dawn hypothesis, there is first entrainment of the Arabidopsis circadian oscillator to the physical dawn by light signaling, which adjusts the phase of the oscillator to 0 and activates the expression of both *CCA1* and *PRR7*. As the sun continues to rise, photon flux density increases, activating photosynthesis, resulting in the production of sugars; once these accumulate past a threshold, *PRR7* is suppressed, relieving repression on *CCA1*, and the circadian oscillator is phase advanced by 2–4 h.

The purpose of metabolic dawn might be to deal with the competing demands of light as a signal that entrains the circadian oscillator, vs. light as the energy source for photosynthesis. While the timing of the light signals can be anticipated, the intensity of light is more variable, being a function of cloud cover, season and shading from other vegetation. Dependent on the intensity of the incident solar irradiation, dawn might precede significant photosynthetic sugar production by hours. Alternatively, photosynthesis might become very active immediately after dawn if the incident solar irradiation is high. By incorporating sugar signals into entrainment, the circadian oscillator might adjust the phase of the circadian oscillator to one which is appropriate to the photosynthetic capacity of the cell. The adjustment of the circadian phase by sugar signals has the potential to act as the feedback from starch metabolism required to adjust the phase of starch degradation predicted by Feugier and Satake (2013a), but this has yet to be tested. One possibility is to search for a role of metabolic entrainment in the optimization of carbon use by analysis of optimal phase responses of a phase oscillator model, as has previously been done for light (Hasegawa and Arita 2014). *PRR7* might also have wider roles in sugar signaling because *prr7-11* mutants are insensitive to sugars for the pathway regulating Chl production, a widely used assay for general sugar signaling (Moore et al. 2003, Haydon et al. 2013b). There might also be a role for sugars as diffusible signals that couple circadian oscillators either locally (Wenden et al. 2012, Fukuda et al. 2012) or between organs (James et al. 2008).

Conclusions and future prospects

Intense focus of research on Arabidopsis has resulted in the discovery that the correct timing of starch metabolism by the circadian oscillator is required for optimization of growth. The role for the clock in ensuring that starch is degraded to almost zero in anticipation of dawn is consistent with the finding that the Arabidopsis circadian clock is particularly important for the correct timing of nocturnal events (Dodd et al. 2014). Diel oscillations of starch also occur in plants undergoing C_4 and CAM forms of photosynthesis (Weise et al. 2011) and it will be interesting to determine if the principles discovered in Arabidopsis apply more widely. It is noteworthy that starch accumulation ceases in mature C_4 maize mesophyll cells, perhaps further suggesting that diel starch cycles are required for growth. The formulation of concepts derived from empirical studies in to mathematical models will provide a framework in which to understand the rules which govern carbon metabolism across species.

As the daylength changes through the seasons, plants have to manage not only starch turnover correctly through the diel cycle but also major alterations in resource allocation from supporting vegetative growth to the development of reproductive material occurring during the developmental switch to flowering which can occur in response to photoperiodic alterations (Ortiz-Marchena et al. 2014). The circadian-regulated transcriptional regulator CONSTANS

(CO) participates in altering the diel dynamics of transitory accumulation of starch to mobilize sugars in preparation for the transition to flowering, which is also regulated by CO (Ortiz-Marchena et al. 2014). It has been proposed that the accumulation of starch is in part regulated by GRANULE BOUND STARCH SYNTHASE (GBSS) activity, and the expression of *GBSS* expression is regulated by CCA1 and CO binding to the *GBSS* promoter (Ortiz-Marchena et al. 2014). The activity of CO is an output of the circadian clock and light signaling, and therefore these data show that understanding the circadian regulation of diel metabolism will require models that incorporate light-mediated regulation of circadian dynamics (Dalchau et al. 2010). The burst of sugars released by altered starch accumulation might contribute to the induction of flowering through trehalose-6-phosphate-mediated signaling (Wahl et al. 2013). These changes in resource allocation that occur in response to photoperiodic changes have yet to be considered in the mathematical models of transitory starch dynamics.

We have summarized new findings demonstrating the temporal organization of carbon metabolism, which in turn feeds back on to the circadian clock to regulate daily metabolism and growth and also long-term developmental changes, which themselves are sensitive to daylength and energy status. The heavily interlocked nature of the system, with a high degree of non-linear feedback, results in emergent properties that are simple but non-intuitive. Mathematical modeling and analyses will be powerful tools to provide insight that is difficult to gain only from empirical studies. This will be particularly important as international consortia seek efficient strategies to increase yield and alter sugar composition for biofuel production.

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The authors have no conflicts of interest to declare.

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