Mathematical models of the plant circadian clock: impact of phase regulation by sugar on plant growth

Takayuki Ohara
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Preface

Organisms living on the Earth are exposed to environmental fluctuations such as daily light-dark cycles and annual changes of the day length due to Earth’s rotation and revolution. Biological apparatus that allows the organisms to anticipate the rhythmic environmental changes should be advantageous for their survival. Endogenous biological oscillator, called circadian clock, generates about 24-h (circadian) rhythmicity of biological processes even in constant environments where external timing cues are not available. The circadian clock is ubiquitous in organisms in diverse taxonomic groups and is crucial for the appropriate coordination of the timing of physiology and behavior in response to cyclic environmental change (Young and Kay, 2001). The synchrony of the circadian clock with the environmental cycles could be advantageous for survival and competition (Woelfle et al., 2004; Dodd et al., 2005). There are three major components that characterize circadian clock or circadian system; a core oscillator, input and output pathways (Harmer, 2009). A core oscillator generates the rhythm of clock gene expression necessary to keep track of time, input pathways regulate or reset the phase of the oscillator in response to external signals such as temperature and light (Johnson et al., 2003). Output pathways modulate a variety of processes such as leaf movement in plants (Yakir et al., 2007), locomotor activity in animals (Vitaterna et al., 1994), and eclosion in insects (Konopka and Benzer, 1971). Generation of the circadian rhythm stems from the transcriptional-translational feedback loop of clock genes, which is highly conserved in in eukaryotic clocks (Hardin, 2011; Hsu and Harmer, 2014; Papazyan et al., 2016).

The first scientific observation of the circadian rhythm was reported in 1729 using leaf movements in plants. The French astronomer de Mairan reported the rhythmic opening and closing of leaves of mimosa plants in constant darkness (McClung, 2006), suggesting that the rhythm is self-sustaining. Recent advances in molecular genetic studies using model plant species including *Arabidopsis thaliana* have unraveled that the circadian clock regulates many aspects of metabolic and physiological processes, e.g. hypocotyl elongation and stomatal opening (reviewed by Yakir et al., 2007). Expression of over 30% of genome is under the control of the clock in *A. thaliana* (Covington et al., 2008). Moreover, the integration of molecular-genetic studies and mathematical modeling has boosted the discovery of building blocks of the plant clock and their connections. In 2005, Locke et al. (2005) proposed the first mathematical model of the
A. thaliana clock based on the earliest hypothesis on the network structure that afternoon-expressed clock gene TIMING OF CAB EXPRESSION 1 (TOC1) activates dawn-peaked clock genes CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCHOTYL (LHY), which in turn repress TOC1 (Alabadi et al., 2001). New experimental findings continuously updated the mathematical description of the network (Bujdosó and Davis, 2013) and now we have fully complex models of plant circadian clock consisted of multiple interconnected feedback loops (Hsu and Harmer, 2014).

The circadian-phase regulation or entrainment by environmental inputs such as light underlies the synchronization of the clock and environments. In the plant circadian clock, the effect of light on the circadian phase has been intensively studied using so-called phase response curves (PRCs), which describes the phase shifts induced by stimulation (e.g. brief exposure to strong light) as a function of the phase of the stimulation (Johnson, 1992). Light stimulus generally advances the phase in the former half of the night and delays in the latter half (Covington et al., 2001; Ohara et al., 2015). The circadian clock appropriately entrained to external light-dark cycles can increase plant growth and competitive advantage (Dodd et al., 2005; Graf et al., 2010). In addition to signals from external environments, endogenous signals such as photosynthetic sugars also affect the A. thaliana circadian clock. The addition of sucrose to growth media restores the circadian rhythm in constant darkness, which is abolished without sucrose (Dalchau et al., 2011; Haydon et al., 2017), and also shortens the period of the rhythm in constant low light (Haydon et al., 2013). The phase of the clock is shifted by sugars; a PRC for sucrose pulses shows phase advance in the morning and delay in the afternoon and night (Haydon et al., 2013), which is clearly different from that for light pulses.

Whereas the effects of entrainment by environmental signals on plant fitness have been well studied (Dodd et al., 2005; Graf et al., 2010), the significance of sugar entrainment has been largely unknown. In addition, how the above-mentioned phase response to sugars is realized has been poorly understood at mechanistic levels. In the present thesis, I theoretically study the physiological importance and mechanisms of the circadian-phase regulation by sugar signals. Seki et al. (2017) have previously investigated the effect of sugar entrainment on carbon metabolism under environmental fluctuations using the integrated approach of physiological experiments and
mathematical modeling. They have proposed a phase oscillator model of the circadian clock that is regulated by the feedback from carbon metabolism. In Chapter 1, I briefly explained the study by Seki et al. (2017) because the proposed model plays a major role also in Chapter 2. In Chapter 2, in order to theoretically investigate the effect of sugar entrainment on plant growth (a good proxy for plant fitness; Younginger et al., 2017), I extended the phase oscillator model proposed by Seki et al. (2017) to a whole plant scale. In the new model, growth dynamics of sink tissues are formalized as a function of sucrose transported from source to sink through phloem (Seki et al., 2015; Satake et al., 2016). I compared growth patterns of the sugar-sensitive wild type and sugar-insensitive mutant that cannot adjust the circadian phase in response to sugar signals. The model predicted that the wild type grows faster than the sugar-insensitive mutant by stabilizing sucrose supply to sink tissues. These results highlight the importance of clock entrainment by endogenous signals for optimizing plant carbon metabolism and growth.

In Chapter 3, I theoretically investigate the conditions under which the circadian phase is advanced in the morning and delayed in the night in response to sugar signals by using the clock-gene regulatory network model with explicit formalization of sugar effects. I simulate PRCs for sucrose pulses using various combinations of a target clock gene for sugar signals and their property (activation or repression effect). The model suggests that sugar-induced repression of a morning-phased clock component that acts as a repressor is essential to realize optimal phase shifts for carbon homeostasis. The biological significance of this specific combination was discussed in terms of the efficient usage of carbon resources for growth.
References


Chapter 1
Adjustment of the Arabidopsis circadian oscillator by sugar signalling dictates the regulation of starch metabolism

This chapter is based on the study published at:
1.1 Introduction

In Arabidopsis thaliana, an important output of the circadian system is the timing of the diel turnover of starch (Smith and Stitt, 2007; Graf and Smith, 2011). A. thaliana stores a portion of the carbon fixed through sugars as insoluble starch, which accumulates during the day. At night, starch is broken down to provide sugars to sustain growth under dark. This process has several remarkable properties that are not easily explained within the context of known cellular signalling pathways. Starch abundance increases and decreases in an almost linear manner despite the exponential dynamics of cellular processes. Starch is consumed during night and reaches a minimum almost precisely at dawn regardless of changing photoperiod, due to regulation from the circadian oscillator (Lu et al., 2005; Graf et al., 2010). The system adjusts the rate of change of starch accumulation and loss in response to seasonal changes of photoperiod, such that A. thaliana in shorter photoperiods accumulate starch faster during the day and lose starch more slowly than those grown in longer photoperiods (Stitt et al., 1978; Chatterton and Silvius, 1981; Gibon et al., 2004; Lu et al., 2005; Graf et al., 2010). Lastly, early, or late onset of night, causes an immediate change in the rate of loss of starch abundance (Lu et al., 2005; Graf et al., 2010).

The molecular mechanisms that coordinate the turnover of starch with the external photoperiod remain unknown. Mathematical modelling aimed at providing a theoretical framework to consider the processes that regulate the diel turnover of starch has generated two competing hypotheses (Scialdone and Howard, 2015; Webb and Satake, 2015). In the first, it is assumed that the abundance of starch is measured (Scialdone et al., 2013; Pokhilko et al., 2014; Scialdone and Howard, 2015), whereas in the other, it is assumed that sucrose is measured (Feugier and Satake, 2013, 2014; Webb and Satake, 2015; Larbat et al., 2016). Both hypotheses consider that the circadian oscillator is required for the correct regulation of diel starch dynamics but with major differences in the assumed role of the clock. In the starch sensing models, the circadian clock is a passive timer used to measure the time of day (Scialdone et al., 2013; Pokhilko et al., 2014; Scialdone and Howard, 2015). In the second, sucrose feedbacks to the circadian clock to dynamically regulate the phase of the circadian oscillator (Feugier and Satake, 2013, 2014; Webb and Satake, 2015). There is strong experimental evidence for the role of the circadian oscillator in the timing of turnover of starch, which is critical to both hypotheses (Gibon et al., 2004; Mugford et al., 2014). In addition, inhibition of starch
degradation rate in response to elevated sucrose levels, mediated by trehalose-6-phosphate (Tre6P) supports the hypothesis that sugar-sensing contributes to the regulation of starch turnover (Martins et al., 2013). However, because of the interrelationships between starch, sugars and the clock it has been difficult using experimental data alone to differentiate between the profoundly different assumptions of starch or sucrose sensing and the role of dynamical feedback from sucrose to the circadian clock.

A recent experimental finding that sugars can adjust the phase and period of the circadian oscillator of *A. thaliana* (Haydon et al., 2013) motivated Seki et al. (2017) to examine further the hypothesis that the diel turnover of starch arises from sucrose-sensing and feedback to the circadian oscillator (Feugier and Satake, 2013, 2014). They tested this hypothesis by developing a new phase oscillator model, which describes the adjustment of the circadian oscillator as a function of the phase at which the sucrose signal is received. Using the model, Seki et al. (2017) demonstrated how the circadian oscillator in *A. thaliana* shows a phase advance in the subjective morning and a phase delay at night in response to sugar signals (Haydon et al., 2013). They then predicted that the metabolic regulation of the circadian clock contributes to appropriate carbon use in changing photoperiods. Because my thesis is based on the model proposed by Seki et al. (2017), I here explain the structure of their model and results of model analyses.

1.2 Results

1.2.1 Linear starch dynamics is an emergent property that arises from sucrose homeostasis

To theoretically assess the diel turnover of starch in the light of empirical data demonstrating a feedback from sugar sensing to the circadian system (Haydon et al., 2013), Seki et al. (2017) combined a phase oscillator model of the dynamical adjustment of the circadian system with a description of carbon metabolism. In the model, the following assumptions about the dynamics for sucrose (*S*) and starch (*C*) are made. During the light period photoassimilates are produced at a constant rate *a* (Dodd et al., 2004; Gibon et al., 2004). A fraction, *γ*, of those photoassimilates is partitioned into starch for storage, and the remaining portion, 1 - *γ*, is transformed into sucrose for use in respiration or transportation at rate *H* (Fig. 1.1). To produce sucrose, starch is
degraded at a rate of $\beta(t)$ per unit surface area of the starch granule. The model was simplified by considering the constant rates for $\gamma$ and $H$ because oscillations of $\gamma$ and $H$ were less important to explain observed starch dynamics (Feugier and Satake, 2014). The length of a day was normalized as 1, and the fractions of light and dark periods in a day are given as $\tau_L$ and $\tau_D$, respectively ($\tau_L + \tau_D = 1$). Based on these assumptions, the dynamics of sucrose and starch are represented with the following equations:

\[
\frac{d}{dt}S(t) = \begin{cases} 
  a(1 - \gamma) + \beta(t)C(t)^{\kappa} - HS(t) & \text{under light} \\
  \beta(t)C(t)^{\kappa} - HS(t) & \text{under dark} 
\end{cases} \quad (1.1)
\]

\[
\frac{d}{dt}C(t) = \begin{cases} 
  a\gamma - \beta(t)C(t)^{\kappa} & \text{under light} \\
  -\beta(t)C(t)^{\kappa} & \text{under dark} 
\end{cases} \quad (1.2)
\]

Starch-degrading enzymes cannot access all available starch because starch exists as large polymers (granules), and the degradation process only occurs at the surface of each granule. Thus, Seki et al. (2017) assume that starch degradation occurs in proportion to the surface area of starch granule represented as $C(t)^{\kappa}$ in Eqs. (1.1) and (1.2). They mainly assumed $\kappa = 2/3$, which is widely applied for three-dimensional objects, and confirmed that the value of $\kappa$ has small effect on the model outcomes (see Seki et al. 2017).

By combining Eqs. (1.1) and (1.2), Seki et al. (2017) derived the following equation:

\[
\frac{d}{dt}S(t) = \begin{cases} 
  a - \frac{d}{dt}C(t) - HS(t) & \text{under light} \\
  -\frac{d}{dt}C(t) - HS(t) & \text{under dark} 
\end{cases} \quad (1.3)
\]

Based on Eq. (1.3), the following equation can be generated:

\[
\frac{d}{dt}S(t) = 0 \iff \frac{d}{dt}C(t) = \begin{cases} 
  a - HS(t) & \text{under light} \\
  -HS(t) & \text{under dark} 
\end{cases} \quad (1.4)
\]

The left-hand side of Eq. (1.4) shows that the sucrose level does not change over time and, thus, is kept constant. Parameters $a$ and $H$ on the right-hand side of Eq. (1.4) are constant; therefore, if the sucrose level does not change, then the starch amount changes constantly with a linear increase during the day and linear decrease at night.

These results show that when the level of sucrose is constant, the starch profile is always linear and vice versa. Furthermore, this relationship suggests that the linearity of starch turnover is an emergent property of sucrose homeostasis. The emergent nature of the starch profile in the model represents a major difference from models in which starch sensing is assumed and specific chemical kinetics are assumed to realize the
linear starch profiles (Scialdone et al., 2013). The finding that linear starch kinetics can arise as an emergent property of homeostasis of sugars is consistent with the empirical data that demonstrate that rather stable sucrose levels are found through the day although starch levels change drastically through the diel cycle (Gibon et al., 2004; Sulpice et al., 2014). The idealized relationship between starch and sucrose presented here is realized even under fluctuating environments due to a feedback mechanism from sucrose to the circadian clock to minimize sucrose changes, as explained later.

1.2.2 The role of phase adjustment of the circadian oscillator in starch metabolism

It was previously demonstrated that the nearly linear dynamic of starch accumulation and loss is caused by a non-linear starch degradation rate. Seki et al. (2017) determined the starch degradation rate $\beta(t)$ required to achieve sucrose homeostasis in a given photoperiod, and showed that it has a peak at dawn regardless of the magnitude of $\kappa$ in Eqs. (1.1) and (1.2) (Fig. 1.2A), and it is independent from the amount of starch at dusk. This result is clearly different from the previous models assuming that the plants sense the starch amount at dusk and regulates starch degradation accordingly (Scialdone et al., 2013; Pokhilko et al., 2014). It was assumed that this diel fluctuation of starch degradation is generated under the influence of circadian clock because there is an increasing evidence that the activity of rate-limiting enzymes shows clear diel oscillation due to the regulation by the circadian clock in animals (Doi et al., 2006; Neufeld-Cohen et al., 2016), although detailed mechanisms underlying oscillating activity of starch degradation enzymes still remain unknown in plants. $\beta(t)$ is positive in the light when photoperiod is long (Fig. 1.2A), suggesting that starch degradation could occur in the light. Recent experimental studies have supported this theoretical finding that the starch degradation occurs during the light (Horrier et al., 2016; Fernandez et al., 2017), although the mechanism remains not elucidated (Smith et al., 2005). The discontinuous feature of $\beta(t)$ at dusk (Fig. 1.2A) implies that the starch degradation rate is regulated in a day mode and a night mode, possibly as a consequence of dual regulation by light signalling in addition to the circadian oscillator (Dalchau et al., 2010).

The theoretically determined starch degradation rate captures an important feature of the data: the starch accumulation rate increased, and the rate of the loss of starch decreased as the photoperiod shortened (Fig. 1.2B), while the sucrose level is less variable even
under different photoperiod conditions (Fig. 1.2C). In short photoperiods (4, 6, and 8 h) there was no difference in the rate of starch accumulation rate as observed empirically (Fig. 1.2B; Sulpice et al., 2014) demonstrating that the model can simulate the observed starch dynamics without those dynamics being explicitly assumed. It was also demonstrated that the stationary levels of sucrose under light and dark conditions cannot be the same when the fraction of light period is smaller than the carbon partitioning rate (Fig. 1.2C).

The timing of the optimal starch degradation rate to achieve homeostasis changes with photoperiod, which probably involves the circadian oscillator (Graf et al., 2010; Feugier and Satake, 2013; Scialdone et al., 2013). To illustrate the importance of the adjustment of starch degradation rates with changing photoperiods, Seki et al. (2017) considered the implication on starch dynamics if the plant could not change phase of its circadian clock by light and/or sugars (Fig. 1.2D). They did this by using the endogenous oscillation of the starch degradation rate that was optimized to maintain sucrose homeostasis for a 12 h photoperiod. In a 12 h photoperiod the model achieved sucrose homeostasis with a linear diel starch profile (Fig. 1.2E, F). When the external photoperiod was lengthened beyond 12 h, the rate of nocturnal starch loss increased and when the photoperiod was shortened to 8 h the night-time starch loss rate decreased (Fig. 1.2E). These simulations suggest that even in the absence of phase adjustment of the circadian clock, the plant can appropriately respond to an unexpected early or late dusk (Lu et al., 2005; Graf et al., 2010; Scialdone et al., 2013) because the night-time starch degradation rate required for sucrose homeostasis is almost invariable regardless of photoperiod (Fig. 1.2A). However, in the absence of phase adjustment of the circadian oscillator the plant in the model cannot alter its starch accumulation rate during the day (Fig. 1.2E), which contradicts the empirical findings that starch accumulation rates change flexibly in response to the photoperiod (Gibbon et al., 2004; Lu et al., 2005; Graf et al., 2010). The failure to adjust the starch accumulation rate in the day is accompanied by a loss of sucrose homeostasis (Fig. 1.2F). The theoretical results suggest that in changing photoperiods circadian adjustment is required to readjust the starch degradation rate to regulate starch accumulation during the day and maintain sucrose homeostasis.

1.2.3 Optimal phase shifts for sustaining sucrose homeostasis

Because it was found above that adjustment of circadian phase in the light is required
for sucrose homeostasis, Seki et al. (2017) extended their model by incorporating the phase-dependent responses of the circadian clock to light and sugar. The most reliable indicators of solar day length are dawn and dusk, and most organisms have evolved to use these transitions as their primary zeitgeber for circadian phase adjustments (Roenneberg and Foster, 1997). In addition, sugar adjusts the phase of the A. thaliana circadian oscillator by phase advance in the subjective morning (Haydon et al., 2013).

In an extended model, the starch degradation rate is given as the function of the phase \( \varphi \) of the circadian oscillator, which can be formalized by the following phase oscillator dynamics:

\[
\frac{d\varphi(t)}{dt} = \omega + Z_L(\varphi)f_L(L) + Z_S(\varphi)f_S(S),
\]

(1.5)

where \( \omega \) is the angular frequency, and \( Z_L(\varphi) \) and \( Z_S(\varphi) \) are the phase-dependent sensitivity functions for light and sugar signals, respectively. \( f_L(L) \) and \( f_S(S) \) indicate input from light and sucrose signals denoted as \( L \) and \( S \), respectively (see Seki et al. 2017 for more details). It was assumed that light pulses at dawn and dusk set the phase of the oscillator so that it equals the external time. The period of the oscillator was set to be 24 h, although slight deviations from the 24 h period do not affect the results. The starch degradation rate \( \beta(t) \) in Eqs. (1.1) and (1.2) is replaced as a function of the phase of circadian clock: \( \tilde{\beta}(\varphi) \) (see Seki et al. 2017).

The product, \( Z_S(\varphi)f_S(S) \), in Eq. (1.5) corresponds to the general phase response curve (PRC) for the pulse of sucrose signals given at the phase \( \varphi(t) \). To explain why the empirically measured PRC had a phase advance in the morning and phase delay in the evening and night (Fig. 1.3A; Haydon et al., 2013), Seki et al. (2017) theoretically derived the PRC needed to minimize fluctuation of sucrose levels. They used a simulated experiment in which the maximum level of sucrose signal is received by the plant at a certain circadian time \( \varphi(t) = \varphi \). In order to minimize sucrose fluctuations, the plant would transiently reduce the sucrose production by down-regulating starch degrading activity. They assumed that down-regulation of starch degrading activity is derived from the phase shift of the circadian clock. When the starch degradation rate \( \tilde{\beta}(\varphi) \) has a minimum at \( \varphi(t) = \varphi^* \) (Fig. 1.3B), the phase shift from \( \varphi(t) = \varphi \) to \( \varphi(t) = \varphi^* \) is the best solution for minimizing the sucrose fluctuation because decreasing the starch degradation rate is the most effective way to mitigate a transient elevation in sugar levels (Fig. 1.3B). Thus, the optimal phase shift to the sugar pulse given at \( \varphi(t) = \varphi \) is
\( \phi^* - \phi \), which is positive (phase advance) until \( q(t) = \phi^* \), zero at \( q(t) = \phi^* \) (the break point), and negative (phase delay) after \( q(t) = \phi^* \) (Fig. 1.3A). Therefore, the assumption that the plant minimizes sucrose fluctuations naturally derives the phase advance in the subjective morning and delay at night, which is qualitatively the same as the observed phase shifts (Fig. 1.3A; Haydon et al., 2013). The position of the break point in the optimal PRC is determined by the circadian time when the starch degradation rate is at its minimum. The experimentally-derived PRC (Fig. 1.3A) suggests that the minimum starch degradation rate is attained approximately 6–11 h after the subjective dawn.

### 1.2.4 Phase adjustment of the circadian oscillator by sucrose is necessary to achieve sucrose homeostasis

Seki et al. (2017) next investigated whether the optimal phase response of the circadian oscillator to a sugar signal is effective at adjusting the rate of starch accumulation in response to changes in photoperiod. They applied the optimal PRC with the break point at 10 h after subjective dawn to the phase oscillator dynamics in Eq. (1.5) by assuming that the subjective starch degradation rate is optimized for a 10 h photoperiod. They also assumed that plants sense the rate of change in sugar levels as a signal (i.e., \( S = dS/dt \), where \( dS/dt \) indicates the time derivative of the sucrose level). When \( dS/dt \) is negative (i.e., sucrose level decreases), an opposite phase shift occurs when compared with a positive \( dS/dt \) (i.e., sucrose level increases). Since it was found that adjustment of phase affects only the diel turnover of starch during the day, and not during night (Fig. 1.2E), Seki et al. (2017) considered first only the effect of phase adjustment of the circadian oscillator by sugars during the light period.

In the numerical analyses of the model, the rate of starch loss at night decreased (Fig. 1.4A), and the amount of sucrose at the end of the day decreased on the first day (Fig. 1.4B) when plants were transferred from long day [LD; 16 h light (L): 8 h dark (D)] to short day (SD; 8L:16D). This generated a positive sugar signal in the morning because the sugar level was elevated rapidly after dawn (Fig. 1.4C), which advanced the phase of the circadian oscillator (Fig. 1.4D) and the starch degradation rate was decreased on the second day after transfer (Fig. 1.4E). This result suggests that the phase adjustment of the circadian oscillator by sugar effectively buffers the decrease in sucrose at the end of the day by increasing the starch accumulation rate on the second day after transfer (Fig. 1.4A, B). The increased starch accumulation rate did not occur when circadian phase adjustment by sugar was not incorporated (Fig. 1.4A).
In contrast, when plants were transferred from SD to LD conditions, the sucrose level was elevated by the end of the day, which generated a negative sugar signal in the morning (Fig. 1.4H) because the sucrose level was decreased via a low starch degradation rate after dawn. The negative sugar signal delayed the phase of circadian oscillator (Fig. 1.4I), and the starch degradation rate increased on the second day after the transfer (Fig. 1.4J). Delayed phase of the core clock genes in longer photoperiods have been reported previously in experimental conditions (Flis et al., 2016). The increase in starch degradation rate due to phase adjustment of the circadian oscillator decreased the starch accumulation rate during the day and effectively buffered the increased level of sucrose at the end of the night (Fig. 1.4F, G). Again, the decreased starch accumulation rate did not occur when there was no phase adjustment by sugar. These results predict an essential role for the dynamic adjustment of the circadian clock by sugar to modulate starch metabolism in changing photoperiods. The role of phase shift in response to sugar signals was also stressed in the regulation of starch metabolism under fluctuating weather conditions (see Seki et al. 2017). The predicted starch profile (decreased starch loss rate at night under lowered light intensity) resembled the observed starch dynamics in the experiment controlling irradiance (Fig. 1 of Pilkington et al., 2015).

Changing the break point of the optimal PRC from 10 to 8 h after subjective dawn had little effect on the main results, suggesting that the position of break point for PRC is flexible. In contrast, sucrose homeostasis was not improved by sugar-mediated circadian phase adjustment when it was assumed that the plants sense the sucrose concentration (i.e., $\dot{S} = S(t)$) rather than the rate of change in sucrose levels ($\dot{S} = dS/dt$; Seki et al. 2017). This implies that plants sense sugar flux rather than direct sugar concentrations.

The model was validated by comparing the predicted starch dynamics with experimental data obtained for A. thaliana wild-type (Col-0) and a mutant in which the circadian clock does not respond to sugar signals (pseudoreponse regulator 7-11; prr7-11; Haydon et al., 2013) (see Seki et al. 2017).

### 1.3 Discussion

Results by Seki et al. (2017) provide a theoretical background to explain why the experimentally-derived phase response to sucrose (Fig. 1.3; Haydon et al., 2013) is
advance in the morning and delay in the evening and night. Such a phase response to sugar signals was ideal to minimize sucrose fluctuations under changing environments. The incorporation of a theoretically-determined phase response to sucrose generated complex starch dynamics with non-intuitive predictions (Fig. 1.4). The model predicted the appropriate adjustment of starch turnover under changing photoperiods as a result of dynamic phase adjustment of the circadian oscillator and predicted the misregulation of starch dynamics in a sugar-insensitive mutant.

The results suggest that plants sense and respond to the rate of change in sucrose levels to regulate the circadian clock rather than responding to sucrose concentrations. This type of behavior has been seen in *Bacillus subtilis* in which cells are responsive to the rate at which ethanol and salt stress increases (Young et al., 2013), as well as other bacterial systems which are sensitive to changes in their inputs, rather than to absolute levels (Block et al., 1982; Alon et al., 1999). The rate responsive behavior would function as a temporal filter that responds to rapidly growing signals more effectively than gradually developing one (Young et al., 2013). This temporal filter in sucrose signal would benefit plants to filter out noisy fluctuation in sucrose levels.

Seki et al. (2017) conclude that the dynamic adjustment of the circadian system by sucrose contributes to carbon homeostasis and that the observed linear dynamics of starch turnover are an emergent behavior arising from sucrose flux sensing. Entrainment of the circadian oscillator by light signals ensures that the circadian regulation of starch degradation occurs in synchronization with the environment and adapts to changing day length. Adjustment of the phase of starch degradation by sugar signaling feedback to the circadian oscillator optimizes sucrose homeostasis (Fig. 1.2D), ensuring better carbon management for growth (and probably better entrainment to changing photoperiodic environments) than light entrainment alone (Sanchez et al., 2016). The model and data describe a new role for the circadian system in which the oscillator is more than a passive timer that synchronizes to environmental cues. The circadian system is a dynamic organizer with a plasticity of phase that contributes to carbon homeostasis and growth.

### References


Figure 1.1. The model describing the feedback between the circadian clock and carbon metabolism. The circadian clock regulates the starch degradation process and thus influences the diel sucrose profile. Furthermore, feedback from the sucrose status can adjust the phase of circadian clock.
Figure 1.2. (A) Ideal profiles for the diel starch degradation rate ($\beta$). (B) Starch and (C) sucrose profiles for different light and dark cycles. Results from different light and dark cycles were plotted with different colours. (D) An illustration of phase shift of the circadian clock in response to changing sucrose levels. When the plant cannot change phase of its circadian clock by sugars, the arrow from sucrose to the clock disappears. (E) Starch and (F) sucrose profiles when a plant with a starch degradation rate optimized for 12L:12D conditions (black) is transferred to a long (16L:8D; purple) or short (8L:16D; blue) photoperiod.
Figure 1.3. (A) Comparison between the experimentally derived phase response curve (PRC) and the theoretically derived PRC. Dots represent the Col-0 CCA1:LUC phase response curve to pulses of 90 mM sucrose in constant 10 µmol m\(^{-2}\) s\(^{-1}\) red/blue light. The phase difference in the oscillation of CCA1:LUC for the circadian cycle following sucrose pulse is plotted. Data represent the mean ± SEM (n = 8). The phase response is normalized to a 24 h period. A line represents the theoretically derived PRC when the minimum starch degradation rate occurs at a circadian time of 10 h after subjective dawn and the strength of sugar input signal [\(f_S(S)\) in Eq. (1.5)] is 0.25. (B) Optimal phase shift for sucrose homeostasis. \(\phi^*\) represents the subjective time at which the starch degradation rate is at its minimum. \(\varphi\) is the circadian time when the sugar pulse was added.
Figure 1.4. (A) Predicted starch profile, (B) sucrose profile, (C) sugar signal, (D) phase change, and (E) starch degradation rate of the plant that was transferred from long (16L:8D) to short (8L:16D) days. (F) Predicted starch profile, (G) sucrose profile, (H) sugar signal, (I) phase change, and (J) starch degradation rate of the plant that was transferred from short (8L:16D) to long (16L:8D) days. Black lines represent the plant with a phase shift, and red lines represent the plant without a phase shift. The break point of the PRC was set at 10 h. The unit for starch and sucrose is μmol C₆ g⁻¹ FW.
Chapter 2
Photosynthetic entrainment of the circadian clock facilitates plant growth by stabilizing carbon availability under environmental fluctuations

This study is published at:
2.1 Introduction

Plants are inevitably exposed to daily and seasonal variations in light environments. To continuously grow in fluctuating environments, it is crucial for plants to stably supply carbon resources for respiration and growth. Plant growth in the day is supported by the supply of photosynthates, particularly soluble sugars such as sucrose that are transported from photosynthetic leaves (source tissues) to sink tissues (e.g., roots). Plants grow even in nighttime using carbon resources accumulated during the preceding daytime. *Arabidopsis thaliana*, a model plant, partitions a large fraction of assimilated carbon into insoluble starch, which is degraded at night to produce sucrose (Smith and Stitt, 2007; Stitt and Zeeman, 2012). Because early exhaustion of starch results in carbon starvation and ensuing growth inhibition (Graf et al., 2010; Yazdanbakhsh et al., 2011), careful management of starch metabolism is essential to cope with daily and seasonal fluctuations of light conditions.

In *A. thaliana*, starch amount increases during the day at an almost constant rate and decreases almost linearly at night (Caspar et al., 1985; Gibon et al., 2004; Smith et al., 2004; Lu et al., 2005). Plants in shorter photoperiods accumulate starch more rapidly during the day and degrade it more slowly at night than in longer photoperiods (Lu et al., 2005). Plants also adjust the rate of starch degradation immediately in response to an unexpectedly early or late onset of night (Lu et al., 2005; Graf et al., 2010; Scialdone et al., 2013). The circadian clock underlying the approximately 24-h cycle of biological processes is implicated in the control of starch metabolism (Graf and Smith, 2011). Wild type *A. thaliana* (Ws) exhausts starch reserves about 24 h after the last dawn even under non-24 h light/dark cycles (T-cycles) (Graf et al., 2010), indicating that the timing of starch exhaustion is programmed by the circadian clock. In the circadian clock mutant *ccal/lhy*, in which the functional clock has a period of about 17 h (Locke et al., 2005), the depletion of starch occurs prematurely under a 24-h T-cycle but coincides with dawn under a 17-h T-cycle (Graf et al., 2010). These studies suggest that coordination of the internal timing of starch turnover with environmental cycles is necessary to avoid carbon starvation.

Phase adjustment of the circadian clock to external stimuli such as light or temperature is fundamental for synchronizing biological processes with environments (Johnson et al., 2003). In addition to signals from the external environment, endogenous signals such as
photosynthates are also important regulators of the circadian phase in *A. thaliana*. It was previously reported that phase adjustment by sugar is necessary for plants to flexibly regulate carbon metabolism in fluctuating light environments (Seki et al., 2017). Seki et al. (2017) developed a phase oscillator model describing phase regulation of the circadian clock by sucrose. This model predicted that phase adjustment of the circadian clock by sucrose is crucial for homeostatic regulation of carbon resources. These theoretical predictions were confirmed by physiological experiments using the mutant *pseudoresponse regulator 7-11* (*prr7-11*), the circadian clock of which does not show clear phase response to sucrose pulse (Haydon et al., 2013).

Whereas clock entrainment by exogenous signals such as light has been shown to be advantageous for competition and survival in several organisms (Woelfle et al., 2004; Dodd et al., 2005), the advantages of clock entrainment by photosynthetic products remain elusive. Here I theoretically evaluate the effects of clock entrainment by sugar on plant growth, a good proxy for plant fitness (Younginger et al., 2017). I extended the phase oscillator model for the circadian clock (Seki et al., 2017) by incorporating growth dynamics of sink tissues, including shoot apical meristems and roots. Growth dynamics are described by modeling phloem transportation of sucrose from source to sink tissues (Seki et al., 2015; Satake et al., 2016). I demonstrate that plant growth is facilitated by endogenous sugar entrainment under long photoperiods because the entrainment enables the stable supply of sucrose from source to sink tissues irrespective of light fluctuation. In short photoperiods, however, the effect of sugar entrainment on growth is negligibly small. These results provide important theoretical evidence that circadian-phase adjustment by endogenous signals is advantageous for plant growth.

2.2 Model and methods

To investigate the effect of clock regulation by sugar on plant growth, I integrated two previously developed models, one describing the dynamics of starch and sucrose metabolism in source leaves (Seki et al., 2017) and the other growth of sink tissues dependent on phloem transportation of sucrose from source tissues (Seki et al., 2015; Satake et al., 2016). Photosynthetic products in the source leaf are partitioned into sucrose and starch (Seki et al., 2017; Fig. 2.1A). Sucrose in the source leaf is loaded into the phloem, moves through the phloem tube, and is unloaded at sink tissues where
it is used for respiration and growth (Seki et al., 2015; Satake et al., 2016; Figs. 2.1B-D). These two models were coupled by incorporating a term that represents sucrose translocation from source to sink. I explain the detailed structures of each component of new integrated model in the following sections. I consider two sink tissues, the shoot and root apical meristems (SAM and RAM, respectively), because plant growth and development mainly occur in these organs (Fig. 2.1C). The model can be extended to the structure including multiple sinks in the complex phloem network as studied previously (Seki et al., 2015; Satake et al., 2016).

2.2.1 Sugar dynamics in source leaves and sink tissues

2.2.1.1 Source leaves

In source leaves (Fig. 2.1A), carbon is assimilated by photosynthesis at a rate $a$ during the light period. The length of the light period is given by $\tau_L$. A fraction $\gamma$ of total photoassimilates is partitioned into starch ($C$) for storage and a fraction $1 - \gamma$ is partitioned into sucrose ($S_G$). Sucrose is consumed for respiration at a rate $h_G$ and for transportation at a rate $\eta_G$. Starch is degraded into sucrose at a rate $\beta$, which is assumed to be under the control of the circadian clock and thus is a function of the phase $\phi$ of the circadian oscillator (Seki et al., 2017). These processes are formalized as follows:

\[
\frac{d}{dt}S_G(t) = aL(t)(1 - \gamma) + \beta(\phi)C^\kappa - (h_G + \eta_G)S_G, \tag{2.1}
\]

\[
\frac{d}{dt}C(t) = aL(t)\gamma - \beta(\phi)C^\kappa, \tag{2.2}
\]

where $L(t)$ indicates the light condition (defined as 1 under light and 0 under dark) and $\kappa$ is a constant. Starch reserve is accumulated during the light period at a rate determined by balance between $a\gamma$ and $\beta C^\kappa$. The starch degradation rate $\beta(\phi)$ is assumed to show diel oscillation due to regulation by the circadian clock. I assume that $\beta(\phi)$ shows a peak at dawn and a trough at the subjective dusk $\phi^*$ because a previous study showed that this oscillation pattern is ideal to minimize fluctuations in the sucrose supply to sinks (Fig. A2.1A; Seki et al., 2017).

In the previous model (Seki et al., 2017), transportation of sucrose from source leaves was assumed to be constant. In the new model, sucrose transportation is assumed to occur based on the pressure-flow hypothesis (Münch, 1930) with an assumption that the flux in phloem obeys the Hagen–Poiseuille law (Appendix 2.6.1). Sucrose dynamics are
now described both in the source leaves and phloem tubes. At the source leaves, sucrose is loaded into the adjacent phloem tube at a rate \( \eta_g \) (Fig. 2.1A). At the phloem tube, loaded sucrose is transported to sink tissues (see Appendix 2.6.1 for detailed explanation). The dynamics of sucrose concentration at the phloem tube adjacent to the source \( (g_0) \) is given by

\[
\frac{d}{dt} g_0(t) = \frac{1}{V_0} \{ \eta_g S_G(t) + g_1(t)[-J_1(t)]_+ - g_0(t)[J_1(t)]_+ \},
\]

\((2.3)\)

where \( g_1(t)[-J_1(t)]_+ - g_0(t)[J_1(t)]_+ \) describes the rate of sucrose change due to flux \( J_1(t) \) at phloem tube 1 (Figs. 2.1C, D). \( g_1 \) and \( V_0 \) represent sucrose concentration at tube 1 and the volume of tube 0, respectively (Fig. 2.1C).

Similar to the previous model (Seki et al., 2017), the phase \( \phi \) of the circadian oscillator is modeled by

\[
\frac{d}{dt} \varphi(t) = \omega + Z_L(\varphi)f_L(\varphi) + Z_S(\varphi)f_S(S_G),
\]

\((2.4)\)

where \( \omega \) is the angular frequency of the oscillator. The term \( Z_L(\varphi)f_L(\varphi) \) represents the effect of light stimulus, which is assumed to reset the phase to 0 at dawn and to \( \tau_L \) at dusk (Seki et al., 2017). \( Z_S(\varphi) \) is a phase response curve (PRC) to a sucrose pulse showing phase advance until \( \varphi = \varphi^* \) and phase delay thereafter as previously determined (Fig. A2.1B; Seki et al., 2017). Sugar input \( f_S \) (Appendix 2.6.2) is defined by the Hill function of the rate of change in sucrose level \( (i.e., S_G = dS_G/dt) \). The functions of \( Z_S(\varphi) \) and \( f_S \) have been demonstrated to be optimal for minimizing sucrose fluctuation (Seki et al., 2017). I assume that the phase shift of the circadian clock by sugar takes place only in the light period because the phase shift at night did not improve sucrose homeostasis (Seki et al., 2017).

### 2.2.1.2 Sink tissues

Translocated sucrose is unloaded into the sinks at a rate \( \eta_Y \) from the adjacent phloem tubes with sucrose concentration \( g_i \) (Fig. 2.1B). Sucrose in the sink \( (S_Y) \) is consumed for respiration and growth at rates \( h_Y \) and \( \alpha(S_Y) \), respectively. These processes are formalized as follows:

\[
\frac{d}{dt} g_i(t) = \frac{1}{V_i} \{ g_{i-2}(t)[J_i(t)]_+ - g_i(t)[-J_i(t)]_+ + \eta_Y \},
\]

\((2.5)\)
\[
\frac{d}{dt} S_Y(t) = \eta_Y g_i(t) - h_Y S_Y(t) - \alpha(S_Y),
\]

(2.6)

where \( V_i \) is the volume of component \( i \) (\( i = 4 \) for SAM and \( i = 5 \) for RAM; \( i = 1, 2, \) and \( 3 \) for connecting tubes between the source and sink; Fig. 2.1C). The first and second terms in the right-hand side of Eq. (2.5) represent the solution inflow and outflow, respectively (see Appendix 2.6.1). The function for sucrose consumption rate for growth (\( \alpha(S_Y) \)) will be explained in the next subsection. To clarify the effect of sugar entrainment on plant growth, I simplified the model by assuming that the sugar and growth dynamics of SAM and RAM are identical. Therefore, the values of \( \eta_Y \) and \( h_Y \) as well as the parameters in \( \alpha(S_Y) \) are the same in the two sinks.

### 2.2.2 Growth of sink tissues

#### 2.2.2.1 Growth dynamics

I formalized the growth kinetics of the sink tissue based on sucrose supply because a strong correlation between growth rate and sucrose supply has been reported in both the light and dark periods for \( A. \) thaliana (Sulpice et al., 2014; Mengin et al., 2017). When sugar supply is sufficient, growth is promoted by the target of rapamycin (TOR) kinase, the expression level of which correlates with \( A. \) thaliana shoot and root growth (Deprost et al., 2007; Lastdrager et al., 2014). On the contrary, Snf1-related kinase 1 (SnRK1) inhibits growth in response to low carbon availability (Baena-González and Sheen, 2008; Lastdrager et al., 2014). In addition, growth rate is likely to be saturated as sucrose supply is increased (Sulpice et al., 2014). Given these empirical findings, I assume that growth-related sucrose consumption rate is an increasing and saturating function of sucrose supply (Fig. 2.2A):

\[
\alpha(S_Y) = \alpha_{\text{Max}} \frac{S_Y}{\bar{\eta} + S_Y},
\]

(2.7)

where \( \alpha_{\text{Max}} \), \( \bar{\eta} \), and \( \bar{R} \) are constants. These values are estimated using the published data of fresh biomass in \( A. \) thaliana (Caspar et al., 1985) as explained later. The rate of increase in sink fresh biomass (\( W_Y \)) is then described by

\[
\frac{d}{dt} W_Y(t) = \lambda \alpha(S_Y) W_Y(t),
\]

(2.8)

where \( \lambda \) is the conversion rate of sucrose for growth. Therefore, growth rate is represented by \( \lambda \alpha(S_Y) \). Since dry weight and fresh weight of \( A. \) thaliana Col-0 display
qualitatively similar increase patterns (Caspar et al., 1985; Christophe et al., 2008), my model can also be applicable to the analysis of dry biomass by appropriate scaling. I tested the robustness of the results by using an alternative formalization of growth rate as a linear increasing function of sucrose (Appendix 2.6.3).

**2.2.2.2 Parameter estimation of the sucrose consumption rate for growth**

The parameter values of the sucrose consumption rate $\alpha(S_Y)$ in Eq. (7) were estimated by fitting the simulated growth curves to published data measuring fresh weight of *A. thaliana* wild type (Col-0) and the starchless *phosphoglucomutase* (*pgm*) mutant grown in a 12-h photoperiod (Caspar et al., 1985; Fig. 2.2B). Because the number of the data is limited (data at four time points per genotype), I combine the data of both genotypes for the parameter estimation. The subjective dusk $\phi^*$ was set to 12 h because both the wild type and *pgm* were likely to be completely entrained to a 12-h photoperiod in the experiment. I simulated the growth of the wild type (see next subsection) and starchless mutant using an initial value of 0.0005 at time $t = 0$ (corresponding to the first observation of plant fresh weight in the experiment). Because the *pgm* mutant does not accumulate starch, the carbon partitioning rate for starch ($\gamma$ in Eqs. (1) and (2)) was set to 0 for the mutant. The parameter values $\alpha_{\text{Max}}$, $\bar{n}$, and $\bar{R}$ in Eq. (7) were estimated by minimizing a following cost function:

$$P(\alpha_{\text{Max}}, \bar{n}, \bar{R}) = \sum_{X} \sum_{i=1}^{4} \left[ W_{\text{EXP},i} - 2W_{Y,i}(\alpha_{\text{Max}}, \bar{n}, \bar{R}) \right]^2,$$

where $W_{\text{EXP},i}$ and $W_{Y,i}$ are fresh weight at the $i$th time point in the experiment and the growth at corresponding times in the simulation, respectively (Fig. 2.2B). $X$ corresponds to the wild type and *pgm* mutant. As fresh weight is likely to be measured from whole plants in the experiment (Caspar et al., 1985), I used the sum (2$W_{Y,i}$) of the growth of SAM ($W_{Y,i}$) and RAM (also $W_{Y,i}$) for the parameter estimation.

**2.2.2.3 Simulation conditions**

To evaluate the effect of clock entrainment by sugar on growth, I simulated the growth dynamics of sugar-sensitive (wild type) and sugar-insensitive (mutant) plants in constant photoperiods (ranging from 8 h to 16 h) as well as under changing photoperiod conditions. The wild type adjusts the phase of the circadian clock by sugar as formalized in Eq. (4.4), while the sugar-insensitive mutant lacks this response to sugar (i.e., $f_{S}(S_G)$ in Eq. (4.4) is zero). Both plants respond to light signals in the same manner. Although phase regulation by sugar reduces fluctuation of carbon resources, sucrose
dynamics still deviate from homeostasis in both the wild type and mutant unless these plants precisely predict the timing of dusk \( \text{i.e., } \phi^* = \tau_L \); Seki et al., 2017). To investigate the effect of sucrose homeostasis on growth, I also consider an ideal plant that can maintain perfect homeostasis in a steady state in any photoperiod (hereafter termed the homeostatic plant). Seki et al. (2017) previously determined the optimal function for starch degradation rate to minimize sucrose fluctuation in a given photoperiod. The homeostatic plant is assumed to possess these functions in each photoperiod and is simulated by setting \( \phi^* \) being equal to \( \tau_L \) in any condition \( \tau_L \in (0, 24] \) in contrast to the fixed value of \( \phi^* \) in the wild type and sugar-insensitive mutant (summarized in a table in Fig. A2.1). Comparison of growth dynamics among these three plants enables to address the differential effects of endogenous sugar entrainment and sucrose homeostasis on growth. Because SAM and RAM grow at similar rates, I present the growth dynamics of SAM as the sink growth.

Under the constant photoperiod conditions, plant growth was simulated over 10 days at various photoperiods \( 8, 9, 10, \ldots, 16 \) h. I then compared the growth increment over 10 days among plant types by calculating the differences for the following pairs: (wild type – mutant), (homeostatic – mutant), and (homeostatic – wild type). Under the changing photoperiod conditions, plants grown for 5 days in an 8-h or 16-h photoperiod were transferred to a 16-h or 8-h photoperiod and grown for an additional 5 days (Seki et al., 2017). For the wild type and sugar-insensitive mutant, I mainly analyzed the plants with the subjective dusk \( \phi^* \) of 10 h. In addition, I also simulated 10-day growth of the plants with various values of \( \phi^* \) \( (8, 9, 11, 12 \) h) in constant photoperiods to investigate the influence of \( \phi^* \) on growth dynamics. Other parameter values are listed in Table A1. Numerical integration of the ordinary differential equations was performed with the fourth-order Runge-Kutta method using Mathematica (version 10; Wolfram Research).

2.3 Results

When the plants are grown in a constant photoperiod, the growth of the sink (SAM or RAM) of the all three types increases as the photoperiod is lengthened (Fig. 2.3A). The sugar-sensitive wild type and homeostatic plant grow significantly faster than the sugar-insensitive mutant under long days (Fig. 2.3B). When photoperiod is shorter than 12 h, the growth difference between the mutant and the others almost disappears.
Growth of the wild type is slower than that of the homeostatic plant in long photoperiods (Fig. 2.3B), suggesting that the minimization of sucrose fluctuation is the most effective strategy for efficient growth under these conditions. I confirmed the robustness of the results under different parameter values for carbon metabolism (Fig. A2.2) as well as for the phloem tube network (Fig. A2.3).

Under a long day (16 L/8 D), sucrose levels in the source and sink (SAM or RAM) are highly variable in the sugar-insensitive mutant, moderately variable in the wild type, and almost constant in the homeostatic plant (Figs. 2.4A, B). Diel patterns of growth reflect sucrose profiles in the sink, revealing a substantially lower growth rate during the evening in the mutant and a moderately lower growth rate during the evening in the wild type compared to the homeostatic plant (Fig. 2.4C). The difference in evening growth rate is the major reason for differential growth among the three plant types in a long photoperiod. The mutant accumulates the largest amount of starch, with some amount unused even at the end of night (Fig. 2.4D), indicating inefficient translocation of carbon. In contrast, the wild type accumulates less starch than the mutant and invests a larger amount of photoassimilate for growth (Fig. 2.4D).

The different sucrose, starch, and growth profiles between the wild type and sugar-insensitive mutant are caused by the clock plasticity in response to sugar. The decrease in sucrose levels at dawn (Fig. 2.4A) is sensed by the wild type as a negative sugar signal, driving a phase delay of the circadian oscillator (Fig. 2.4E). This phase delay in the morning increases the starch degradation rate during the light period (Fig. 2.4F), resulting in elevation of the sucrose level (Fig. 2.4A) and decrease of the starch level (Fig. 2.4D). Such a phase shift in the circadian oscillator does not occur in the mutant (Fig. 2.4E). Excessively high sucrose in the mutant around dawn (Fig. 2.4B) does not significantly contribute to growth (Fig. 2.4C) due to the saturating property of the growth-related sucrose consumption rate \( \alpha \) (Fig. 2.2A), while the sucrose decrease around dusk substantially reduces growth rate since at this low level \( \alpha \) is almost linearly dependent on sucrose.

Under a short day (8 L/16 D; Fig. 2.5), the wild type and sugar-insensitive mutant show the similar sugar and growth dynamics as reported previously (Seki et al., 2017). The elevation of sucrose at dawn (Fig. 2.5A) gives rise to a positive sugar signal and resultant phase advance in the wild type (Fig. 2.5E). However, this phase shift does not
substantially change the starch degradation rate (Fig. 2.5F), so the sugar and growth dynamics are almost the same for the wild type and mutant (Figs. 2.5A–D). The homeostatic plant displays distinct growth dynamics (Fig. 2.5C). Nevertheless, the difference in 10-day growth among plants is very small in the short photoperiod (Fig. 2.3B). Since all three plants fix less carbon per day compared to under long day conditions, sucrose concentration is low both in the source and sink (Figs. 2.5A, B). This leads to a slow growth rate due to low supply of sucrose. Because all three plants are limited by sucrose under short day conditions, the growth difference among them is indistinguishably small.

I examined the effect of the timing of the subjective dusk $\varphi^*$ on growth dynamics of the wild type (Figs. 2.6 and 2.7). Changes in $\varphi^*$ do not markedly alter the values of 10-day growth of the sink and its difference among plant types (Fig. 2.6). On the other hand, growth rate is strongly dependent on the timing of both the internal and external dusk. When the external dusk occurs later than the internal dusk (i.e., the length of the light period $\tau_L$ is larger than $\varphi^*$), the growth rate peaks around dawn and decreases around dusk (Fig. 2.7; see also Fig. 2.4C). As the photoperiod is shortened, amplitude of growth rate decreases and eventually becomes almost zero. When the value of $\tau_L$ is smaller than $\varphi^*$, the opposite growth pattern is observed, with a peak around dusk and a trough around dawn (Fig. 2.7; see also Fig. 2.5C).

When the plants are transferred from long to short or short to long days, the growth of the wild type and homeostatic plant are consistently higher than that of the sugar-insensitive mutant (Fig. 2.8A). All three plants are able to restore normal sugar and growth dynamics in about 2 days after the transfer, although the dynamics are complex immediately after the photoperiod change (Fig. 2.8B). Therefore, the growth differences under these conditions may reflect the results under the constant photoperiod conditions that growth of the mutant is inferior to the others especially under a long day (Fig. 2.3). Note that the dynamics of other variables such as sucrose in source were reported in a previous study (Seki et al., 2017).

2.4 Discussion

My results provide the first theoretical evidence that clock entrainment by photosynthetic products improves the fitness of higher plants. The sugar-sensitive wild
type is predicted to grow faster than the sugar-insensitive mutant under long day conditions (Fig. 2.3) because the phase shift of the circadian oscillator by sugar signals enables efficient sugar allocation for growth, while the mutant accumulates carbon as insoluble starch unusable for growth. The growth of the wild type is also higher than the mutant under changing photoperiod conditions (Fig. 2.8A). Since the wild type and mutant are assumed to possess the same entrainment property to light, the lower growth in the mutant stems solely from the lack of sugar-induced phase adjustment. These results suggest that clock entrainment by endogenous sugar signals, in addition to entrainment by exogenous light signals, optimizes plant growth in nature, where the day length gradually changes.

My results also provide important information about the internal timing of plants. My model predicts that the growth pattern of the wild type displays a maximum around dawn and a minimum around dusk in a 16-h photoperiod (Fig. 2.4C) or more generally under conditions where the value of $\tau_L$ (length of the light period) is larger than $\varphi^*$ (timing of the subjective dusk) (Fig. 2.7). When the value of $\tau_L$ is smaller than $\varphi^*$, the pattern is reversed (Fig. 2.5C where $\tau_L = 8$ h and $\varphi^* = 10$ h; Fig. 2.7). Yazdanbakhsh et al. (2011) reported, in both 16-h and 8-h photoperiods, a similar diel pattern in root elongation growth of $A.\ thaliana$ (Col-0), which possibly correlates with fresh biomass change since both growth measures consider dry matter production and water content. These findings imply that $\varphi^*$ of this accession is about 8 h. This idea is consistent with the PRC of the same accession to a sucrose pulse showing a transition from phase advance to delay, which corresponds to the phase $\varphi^*$, at 6 h to 10 h zeitgeber time (Haydon et al., 2013; Seki et al., 2017). These data suggest that a relatively short light period is subjectively anticipated by this accession. Other ecotypes of $A.\ thaliana$ that anticipate later subjective dusk ($i.e.$, large $\varphi^*$) will display the inverted growth pattern in a short photoperiod and later phase advance-to-delay transition of the sucrose-pulsed PRC.

Numerous mathematical models on growth of various plants have been developed ($e.g.$, Thornley et al., 1990; Chew et al., 2014, 2017; Feller et al., 2015; Barillot et al., 2016a, 2016b). Among them, two multiscale models of $A.\ thaliana$ include the circadian clock sub-model and are able to quantitatively predict growth (Chew et al., 2014, 2017). Because the phase of the clock is dynamically regulated by sugar signals in my model
and such phase adjustment is not considered in the previous models (Chew et al., 2014, 2017), only my model enables to evaluate the impact of phase regulation by sugar on plant growth. My model correctly predicts the empirical finding that wild type *A. thaliana* (Col-0) and the mutant *prr7-11* in which phase response to sugar is abolished grow similarly in a 12-h photoperiod (Chew et al., 2017; Fig. 2.3). I suggest that this is due to the relatively weak impact of sugar entrainment on growth, as my modeling results show that the sugar-induced phase shift has less effect on growth dynamics in shorter photoperiods (Fig. 2.5). These considerations are reminiscent of the empirical finding that response of *A. thaliana* to induced-increases in trehalose-6-phosphate, a potential signal metabolite of sucrose status, was more intense in a 16-h photoperiod than in a 12-h photoperiod (Figueroa et al., 2016). My model also predicts significantly faster growth of the wild type than the sugar-insensitive mutant under long day conditions (Fig. 2.3), which will be experimentally tested using wild type *A. thaliana* and *prr7-11*. Wild type is likely to grow faster than *prr7-11* under long day conditions because the latter accumulates more starch than the former in a 16-h photoperiod (Seki et al., 2017) which implies that *prr7-11* can utilize less sucrose for growth than wild type. However, it should be noted that mutation of *PRR7* affects not only sugar signaling but also light signaling in the plant circadian system (Kaczorowski and Quail, 2003; Farre et al., 2005), and thus the growth characteristics of *prr7-11* can be influenced by variations in light as well as sugar entrainment. Nevertheless, sugar entrainment is likely to be a dominant factor because a previous study has theoretically shown that the deficient in sugar signaling only is sufficient to correctly predict patterns of starch turnover of *prr7-11* (Seki et al., 2017).

There are several possibilities to expand my model. I used a constant rate $a$ for carbon capture, which was determined in a previous study based on experiments where nutrient levels were controlled (Gibon et al., 2004; Feugier and Satake, 2013). In higher plants, however, it has been demonstrated that sugar accumulation downregulates photosynthesis, possibly through decreased activity of Rubisco (Araya et al., 2006; Ribeiro et al., 2012; Quentin et al., 2013; Lobo et al., 2015). Formalizing the carbon capture rate as a decreasing function of sucrose concentration could reduce the growth of the sugar-insensitive mutant under long day conditions due to the very high sucrose level around dawn (Figs. 2.4A, B). Regarding circadian clock properties, I considered a
circadian oscillator with a period of 24 h \((i.e., \omega = 1)\). A recent study has reported a positive correlation between the free-running period of natural populations of \textit{Mimulus guttatus} and the latitude of their geographic origin (Greenham et al., 2017). In \textit{A. thaliana}, a similar but weak correlation has been found (Michael et al., 2003), although advantages of such variation in the period remain elusive. Growth simulations of plants with fast- and slow-running clocks could potentially reveal the adaptive value of natural variation in the circadian period in terms of growth optimization. Moreover, I can consider the possibility that two or more clock genes are involved in the phase response to sugar, where each gene can have differential responsiveness. Although the crucial role of \textit{PRR7} in the response has been established (Haydon et al., 2013), other clock genes could participate in it; for instance, \textit{CCA1} will be a good candidate, mutation of which disrupts the dependency of the circadian period on sucrose concentration in growth media (Haydon et al., 2013). To examine this possibility, the single phase oscillator will be expanded to coupled two or multiple oscillators model, in which each phase oscillator is defined with different PRC to sucrose and sugar input function \((Z_s \text{ and } f_S \text{ in Eq. (2.4), respectively})\). Alternative way is to use the clock gene-regulatory network model (Fogelmark and Troein, 2014; De Caluwé et al., 2016) with the explicit formalization of the interaction between clock genes and sugar signals.

I conclude that plants optimize growth by monitoring nutrient status and utilizing endogenous sugar signals as circadian-phase regulators. Photosynthetic products act not only as direct growth substances but also as feedback signals to the clock to realize the efficient carbon-usage for growth. It is plausible that plants as sessile organisms utilize signals from metabolism because they seem more controllable than environmental signals. In this sense, the phase regulation by endogenous signals is potentially even more important for plants than animals that also utilize metabolic signals as zeitgebers (Woller et al., 2016). My data strongly support the concept that the circadian clock improves the fitness of organisms by forming complex feedback loops with the signaling pathways it controls (Sanchez and Kay, 2016).

2.5 References


Figure 2.1. Model describing sugar dynamics in source and sink tissues and translocation of sucrose through the phloem tube. (A) In a source leaf, the circadian clock and carbon metabolism are reciprocally regulated. (B) Translocated sucrose is used for growth and respiration in the sink. Each structural component is expressed by a cylinder of radius $r_X$ and height $l_X$, where $X$ is either G (source), T (tube), or Y (sink). (C) Tubal structure of the model. $p_i$ represents hydrostatic pressure at the apex of component $i$ ($i \in \{0, \ldots, 5\}$). Xylem adjacent to the phloem tube is also schematized. SAM: shoot apical meristem; RAM: root apical meristem. (D) Fluxes in the model. Red arrows ($J_i$) indicate phloem sap flow. Light blue arrows ($w_i$) indicate pure water flow due to osmosis, which occurs at the region represented by light blue circles.
Figure 2.2. (A) Sucrose consumption rate $\alpha$ for growth of the sink (SAM or RAM). The unit for sucrose is $\mu$molC$_6$ g$^{-1}$FW. (B) Time evolution of growth of the wild type (black) and the starchless pgm mutant (red) grown in a 12-h photoperiod. In (B), lines represent the sum of the simulated growth of the two sinks and circles represent the published growth data (Caspar et al., 1985).
Figure 2.3. (A) Growth of the sink (SAM or RAM) of the mutant, wild type, and homeostatic plant over 10 days under constant photoperiod conditions. (B) Growth difference between the wild type and mutant (upper), between the homeostatic and mutant (middle), and between the homeostatic and wild type (lower).
Figure 2.4. Predicted profiles of (A) sucrose in the source, (B) sucrose in the sink (SAM or RAM), (C) growth rate of the sink (SAM or RAM), (D) starch, (E) phase, and (F) starch degradation rate of the plants grown in a 16-h photoperiod. The unit for sucrose and starch is μmolC₆ g⁻¹FW. White background: light period; Gray background: dark period.
Figure 2.5. Predicted profiles of (A) sucrose in the source, (B) sucrose in the sink (SAM or RAM), (C) growth rate of the sink (SAM or RAM), (D) starch, (E) phase, and (F) starch degradation rate of the plants grown in an 8-h photoperiod. Details are as in the legend to Fig. 2.4.
Figure 2.6. (A) 10-day growth of the sink (SAM or RAM) of the mutant, wild type, and homeostatic plant, and (B) growth differences among the plants at various values of the subjective dusk $\phi^*$. 
Figure 2.7. Comparison of growth rate of the sink (SAM or RAM) of the wild type at various values of the subjective dusk $\phi^*$. In each panel, growth dynamics in various photoperiods is represented. Different colors correspond to different light conditions (upper left panel).
Figure 2.8. (A) Growth of the sink (SAM or RAM) of the mutant, wild type, and homeostatic plant transferred from an 8-h to 16-h photoperiod (left) and from a 16-h to 8-h photoperiod (right) and the growth difference among the plants. Color codes as in Fig. 2.3A. (B) Predicted profiles of sucrose in the sink and growth rate around the photoperiod change. Details are as in the legend to Fig. 2.4.
2.6 Appendix

2.6.1 Sucrose solution flux through phloem tubes

Fluxes of sucrose solution and pure water were modeled as in previous studies that addressed the phloem transport of sucrose (Seki et al., 2015) or a flowering signal (florigen) (Satake et al., 2016). Modeling was based on the pressure-flow hypothesis, which states that the difference in hydrostatic pressure between the source and sink generates the solution flux (Münch, 1930). I assume that sucrose concentration is uniform within each tube, an approximation used in the previous studies to predict the realistic grain arrangement of rice (Seki et al., 2015) and various inflorescence structures observed in Arabidopsis thaliana mutants (Satake et al., 2016). The sucrose concentration in tube $i$ ($i \in \{0, ..., 5\}$; Fig. 2.1C) at time $t$ is designated as $g_i(t)$. The phloem tubes are represented as rigid cylinders (Figs. 2.1A, B). The sets of the source leaf, tubes, and sink tissues are represented by symbols $G$, $T$, and $Y$, respectively, where $G = \{0\}$, $T = \{1, 2, 3\}$, and $Y = \{4, 5\}$ (Figs. 2.1A–C).

2.6.1.1 Sucrose flux as Hagen–Poiseuille flow

Inertia of phloem sap flow is considered negligibly small compared to its viscosity (Thompson, 2006); thus, sucrose solution flux $J_i(t)$ can be calculated using the Hagen–Poiseuille equation:

$$J_i(t) = \frac{\pi r_i^4}{8v} (p_k(t) - p_i(t)),$$

(A2.1)

where $v$ is the viscosity of phloem sap. $r_i$ and $l_i$ are the radius and length of component $i$, respectively, and correspond to $r_T$ and $l_T$ ($i \in T$) and $r_Y$ and $l_Y$ ($i \in Y$) (Fig. 2.1B). $p_k(t)$ and $p_i(t)$ are the turgor pressures at the apex of component $k$ and $i$, respectively (Fig. 2.1C). The turgor pressures are caused by the pure water flow between phloem and xylem, which is detailed below. I define the direction of the phloem sap so that it flows from component $k$ to $i$ when $J_i(t)$ is positive, while it flows from component $i$ to $k$ when $J_i(t)$ is negative.

6.1.2 Phloem-xylem flow of pure water

Pure water flows from (or toward) the phloem tube toward (or from) xylem due to osmosis, which is assumed to occur at the small side surface near the apex of each component (Fig. 2.1D). Water flux $w_i(t)$ depends on the difference between the turgor pressure and the osmotic pressure:

$$w_i(t) = mA_i(p_i(t) - g_i(t))RT,$$

(A2.2)

where $m$ is per-area permeability, and $A_i$ is the surface area of component $i$. Under the
assumption of dilute solution, the osmotic pressure is calculated as $g_i(t)RT$ by the van’t Hoff equation, where $R$ and $T$ are the gas constant and absolute temperature, respectively.

Because I assume that every cylinder is rigid, water efflux should be equal to water influx at each component (Fig. 2.1D). The conservation law of water volume is therefore held:

$$0 = J_1(t) + w_0(t),$$

(A2.3)

$$J_1(t) = J_2(t) + J_3(t) + w_1(t),$$

(A2.4)

$$J_i(t) = J_{i+2}(t) + w_i(t) \ (i \in T \setminus \{1\}),$$

(A2.5)

$$J_i(t) = w_i(t) \ (i \in Y).$$

(A2.6)

Turgor pressure $p_i(t)$ is algebraically represented by solving a set of simultaneous linear equations (Eqs. (A2.1)–(A2.6)). $p_i(t)$ depends solely on the sucrose concentration in the phloem, the dynamics of which is formalized below. Sucrose flux $J_i(t)$ is then calculated by substituting $p_i(t)$ into Eq. (A2.1).

2.6.1.3 Sucrose dynamics in the phloem tube

To describe the sucrose dynamics in the phloem, I introduce the symbol $[x]_+$ meaning $\max\{0, x\}$. Sucrose in component $k$ is transported to component $i$ when $J_i(t)$ is positive. The amount of sucrose transported from $k$ to $i$ per unit time is given by $g_k(t)J_i(t)$. In contrast, when $J_i(t)$ is negative, sucrose is transported from component $i$ to $k$ at an amount given by $g_i(t)(-J_i(t))$. These two cases are integrated into the term $g_k(t)[J_i(t)]_+ - g_i(t)[-J_i(t)]_+$ (respectively, $-g_k(t)[J_i(t)]_+ + g_i(t)[-J_i(t)]_+$), which denotes the rate of the sucrose change due to the flux $J_i(t)$ at component $i$ (respectively, component $k$). By applying this consideration to each component, sucrose dynamics in the phloem are described by the following equations:

$$\frac{d}{dt}g_1(t) = \frac{1}{V_1}\{g_0(t)[J_1(t)]_+ + g_2(t)[-J_2(t)]_+ + g_3(t)[-J_3(t)]_+ - g_1(t)([-J_1(t)]_+ + [J_2(t)]_+ + [J_3(t)]_+)\},$$

(A2.7)

$$\frac{d}{dt}g_i(t) =$$

$$\frac{1}{V_i}\{g_1(t)[J_i(t)]_+ + g_{i+2}(t)[-J_{i+2}(t)]_+ - g_i(t)([-J_i(t)]_+ + [J_{i+2}(t)]_+) \ (i \in T \setminus \{1\})\},$$

(A2.8)

where $V_i$ represents the volume of the source ($i \in G$), the tubes ($i \in T$), and the sinks ($i \in Y$). The dynamics of $g_i(t) \ (i = 0, 4,$ and $5)$ are described by Eqs. (2.3) and (2.5).
2.6.2 Parameter estimation of the sugar input function

Similar to a previous study (Seki et al., 2017), I define the sugar input function \( f_S \) by the Hill equation:

\[
f_S(S_G) = \text{sgn}(S_G) \frac{\frac{|S_G|^n}{K + |S_G|^n}}{n}, \tag{A2.9}
\]

where \( \text{sgn} \) is a sign function, and \( n \) and \( K \) are constants. The parameter values of \( K \) and \( n \) are determined for various values of the subjective dusk \( \varphi^* \) using the procedure described in a previous study (Seki et al., 2017).

2.6.3 Alternative formalization of growth rate

In subsection 2.2.2.1, I define growth rate \( \lambda a(S_Y) \) of the sink tissues as a nonlinear function of sucrose concentration (Eqs. (2.7) and (2.8)). Here I test the alternative formalization of growth rate as a linear function of sucrose. I assume that sucrose is consumed for growth at a rate \( \alpha_1 \) and that growth is repressed when sucrose falls below a threshold level. Based on these assumptions, the dynamics of sucrose \( S_Y \) and plant fresh weight \( W_Y \) are described by

\[
\frac{d}{dt} S_Y(t) = \gamma Y g_1(t) - h_Y S_Y(t) - \alpha_1 S_Y(t), \tag{A2.10}
\]

\[
\frac{d}{dt} W_Y(t) = \lambda (\alpha_1 S_Y - \alpha_2 [S_Y^* - S_Y^+]) W_Y(t), \tag{A2.11}
\]

where \( \alpha_2 \) is a parameter, \( S_Y^* \) is a threshold constant, and the symbol \( [x]^+ \) is defined as in subsection 2.6.1.3 above. The term \( \lambda (\alpha_1 S_Y - \alpha_2 [S_Y^* - S_Y^+]) \) in Eq. (A2.11) is considered as the growth rate (Fig. A2.4A) and is referred to as “linear growth rate”. The growth rate in the main text is correspondingly referred to as “nonlinear growth rate”. When sucrose concentration is relatively low, the linear growth rate becomes negative (Fig. A2.4A), which corresponds to death of living tissues due to insufficient maintenance respiration. I estimated the values of \( \alpha_1 \) and \( \alpha_2 \) using procedures similar to those explained in Section 2.2.2.2. I set \( S_Y^* = 0.9 \).

Similar to the results in the main text assuming the nonlinear growth with sucrose (Fig. 2.3), 10-day growth of the mutant is lower than the wild type and homeostatic plant under constant photoperiod conditions (Fig. A2.4B). However, the growth of all three plants in long photoperiods are closer using the linear growth rate than when computed using the nonlinear growth rate. Growth patterns are significantly altered by the change in formalization of the growth rate (Figs. A2.4C and D; Figs. 2.4C and 2.5C), while the sugar dynamics are not (data not shown). Under a long day (16 L/8 D) with the linear growth rate, the very high sucrose level around dawn (Fig. 2.4B) strongly contributes to growth of the mutant, in contrast to the case using the nonlinear growth rate (Fig.
A2.4C; Fig. 2.4C), because growth rate keeps increasing with sucrose concentration. This reduces the growth difference between the mutant and the others. Under a short day (8 L/ 16 D) with the linear growth rate, the overall growth trend is unchanged, but its baseline is higher compared to that with the nonlinear growth rate (Fig. A2.4D; Fig. 2.5C) simply because of the higher growth rate at a sucrose level of about 0.8 (Fig. A2.4A and Fig. 2.5B).

When the plants are transferred from a short to a long photoperiod, the growth of the wild type and homeostatic plant are still greater than the mutant (Fig. A2.5A) as in the case for the nonlinear growth model. However, the mutant grows faster than the others when the plants are transferred from a long to a short photoperiod (Fig. A2.5B). Transient dynamics of the growth rates immediately after the photoperiod change from short to long (Fig. A2.5C) are similar to those assuming the nonlinear growth rate (Fig. 2.8B). On the other hand, when photoperiod changes from long to short (Fig. A2.5D) the growth rate is transiently higher in the mutant (around $t = 0$), lower in the wild type (around $t = 24$), and almost the same in the homeostatic plant compared to the dynamics assuming the nonlinear growth rate (Fig. 2.8B). These changes in transient growth dynamics of the plants exposed to the long-to-short day transition, in addition to the small growth difference among the plants in constant conditions, distinguish the overall growth from those described in the main text (Fig. A2.5B and Fig. 2.8A).
Figure A2.1. (A) Starch degradation rate and (B) phase response curve to sucrose optimal for realizing sucrose homeostasis (Seki et al., 2017). Different colors correspond to different values of the subjective dusk $\phi^*$. How $\phi^*$ is treated in the sugar-insensitive mutant, wild type, and homeostatic plant is summarized in the right table.
Figure A2.2. Growth difference among the mutant, wild type, and homeostatic plant under the change of parameter values. Changed parameters are represented above each group of three figures. Different colors correspond to the different extents of the parameter change shown at the top.
Figure A2.3. (A), (B), (C) Configurations of the phloem tubes used for computation of growth in the main result, (D), and (E), respectively. Black letters in each tube stand for the ratio of the tube lengths. (D), (E) 10-day growth of the sink of the mutant, wild type, and homeostatic plant. (F), (G) Growth difference among the plants based on the data in (D) and (E), respectively. In (D) and (F), the result for shoot apical meristem (SAM) only is shown because that of the root apical meristem (RAM) is almost the same, despite the length difference between tubes 2 and 3.
Figure A2.4. (A) Growth rate linearly dependent on sucrose (solid line). The growth rate formalized in the main text is also shown (dashed line). (B) 10-day growth of the sink (SAM or RAM) of the mutant, wild type, and homeostatic plant with the linear growth rate under constant photoperiod conditions, and the growth difference among the plants. (C), (D) Predicted profiles of the linear growth rate of the plants in a 16-h (C) and 8-h (D) photoperiod. The unit for sucrose is µmolC₆ g⁻¹FW. White background: light period; Gray background: dark period.
Figure A2.5. (A), (B) Growth of the sink (SAM or RAM) of the mutant, wild type, and homeostatic plant with the linear growth rate transferred from an 8-h to 16-h photoperiod (A) and from a 16-h to 8-h photoperiod (B) as well as the growth difference among the plants. Color codes as in Fig. A2.4B. (C), (D) Predicted profiles of the linear growth rate around the photoperiod change. Details are as in the legend to Fig. A2.4.
### Table A1. Summary of parameters, variables, and functions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Carbon capture rate</td>
<td>µmolC6/gFW/hour</td>
<td>6 (Feugier and Satake, 2013)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Carbon partitioning rate for starch</td>
<td></td>
<td>0.68 ($\varphi^* = 8h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.64 ($\varphi^* = 10, 11, 12h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Constant (starch degradation occurs in proportion to $C^*$)</td>
<td></td>
<td>2/3 (Seki et al., 2017)</td>
</tr>
<tr>
<td>$h_G$</td>
<td>Respiration rate in source</td>
<td>l/hour</td>
<td>0.79 (Feugier and Satake, 2013)</td>
</tr>
<tr>
<td>$h_Y$</td>
<td>Respiration rate in sink</td>
<td>l/hour</td>
<td>0.79</td>
</tr>
<tr>
<td>$\eta_G$</td>
<td>Sucrose loading rate</td>
<td>l/hour</td>
<td>1.98 (Feugier and Satake, 2013)</td>
</tr>
<tr>
<td>$\eta_Y$</td>
<td>Sucrose unloading rate</td>
<td>m$^3$/hour</td>
<td>$1.58 \times 10^{-9}$ (Satake et al., 2016)</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency of the circadian clock</td>
<td></td>
<td>1 (Seki et al., 2017)</td>
</tr>
<tr>
<td>$\varphi^*$</td>
<td>Timing of subjective dusk</td>
<td>Hour</td>
<td></td>
</tr>
<tr>
<td>$\tau_L$</td>
<td>Photoperiod</td>
<td>Hour</td>
<td></td>
</tr>
<tr>
<td>$K$</td>
<td>Half saturation constant of $f_S$</td>
<td></td>
<td>0.1 ($\varphi^* = 8h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1 ($\varphi^* = 9h$) †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1 ($\varphi^* = 10h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 ($\varphi^* = 11h$) †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.3 ($\varphi^* = 12h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td>$n$</td>
<td>Constant determining the shape of $f_S$</td>
<td></td>
<td>0.5 ($\varphi^* = 8h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1.4 ($\varphi^* = 9h$) †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0 ($\varphi^* = 10h$) (Seki et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.2 ($\varphi^* = 11h$) †</td>
</tr>
<tr>
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<td>-------</td>
</tr>
<tr>
<td>( \alpha_{\text{Max}} )</td>
<td>Saturation constant of ( \alpha )</td>
<td>( \mu \text{molC}_6/\text{gFW/hour} )</td>
<td>7.6 ( \times ) 10^{-6} †</td>
</tr>
<tr>
<td>( \tilde{K} )</td>
<td>Half saturation constant of ( \alpha )</td>
<td>( \mu \text{molC}_6/\text{gFW} )</td>
<td>9.9 ( \times ) 10^{-2} †</td>
</tr>
<tr>
<td>( \tilde{n} )</td>
<td>Constant determining the shape of ( \alpha )</td>
<td></td>
<td>6.9 †</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>Constant in the linear growth rate</td>
<td>1/hour</td>
<td>0.17 †</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>Constant in the linear growth rate</td>
<td>1/hour</td>
<td>0.23 †</td>
</tr>
<tr>
<td>( S_Y^* )</td>
<td>Threshold constant in the linear growth rate</td>
<td>( \mu \text{molC}_6/\text{gFW} )</td>
<td>0.9</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>Conversion rate of sucrose to growth</td>
<td>1/( \mu \text{molC}_6/\text{gFW} )</td>
<td>1</td>
</tr>
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<td>( r_G )</td>
<td>Cylinder radius of a tube in the source</td>
<td>m</td>
<td>1.0 ( \times ) 10^{-4}</td>
</tr>
<tr>
<td>( r_T )</td>
<td>Cylinder radius of a connecting tube</td>
<td>m</td>
<td>1.0 ( \times ) 10^{-4}</td>
</tr>
<tr>
<td>( r_Y )</td>
<td>Cylinder radius of a tube in the sink</td>
<td>m</td>
<td>1.0 ( \times ) 10^{-5}</td>
</tr>
<tr>
<td>( l_G )</td>
<td>Cylinder height of a tube in the source</td>
<td>m</td>
<td>1.0 ( \times ) 10^{-2}</td>
</tr>
<tr>
<td>( l_T )</td>
<td>Cylinder height of a connecting tube</td>
<td>m</td>
<td>3.0 ( \times ) 10^{-2} or 6.0 ( \times ) 10^{-2} (Fig. A2.3)</td>
</tr>
<tr>
<td>( l_Y )</td>
<td>Cylinder height of a tube in the sink</td>
<td>m</td>
<td>2.0 ( \times ) 10^{-2}</td>
</tr>
<tr>
<td>( V_X )</td>
<td>Volume of cylinder of type X</td>
<td>( \text{m}^3 )</td>
<td>( \pi r_X^2 l_X )</td>
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<tr>
<td>( A_X )</td>
<td>Side surface area of cylinder of type X</td>
<td>( \text{m}^2 )</td>
<td>( 2 \pi r_X l_X )</td>
</tr>
<tr>
<td>( \nu )</td>
<td>Solution viscosity</td>
<td>Pa s</td>
<td>8.9 ( \times ) 10^{-4} (Seki et al., 2015)</td>
</tr>
<tr>
<td>( m )</td>
<td>Per area membrane permeability</td>
<td>( \text{m/Pa/s} )</td>
<td>5.0 ( \times ) 10^{-14} (Seki et al., 2015)</td>
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<tr>
<td>( R )</td>
<td>Gas constant</td>
<td>J/K/mol</td>
<td>8.31</td>
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<tr>
<td>Variables</td>
<td>$T$</td>
<td>Air temperature</td>
<td>K</td>
</tr>
<tr>
<td>-----------</td>
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<td>-----------------</td>
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</tr>
<tr>
<td>$S_G$</td>
<td>Sucrose concentration in the source</td>
<td>µmolC6/gFW</td>
<td></td>
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<tr>
<td>$S_Y$</td>
<td>Sucrose concentration in the sink</td>
<td>µmolC6/gFW</td>
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<tr>
<td>$C$</td>
<td>Starch concentration</td>
<td>µmolC6/gFW</td>
<td></td>
</tr>
<tr>
<td>$\phi$</td>
<td>Phase of the circadian clock</td>
<td>Hour</td>
<td></td>
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<tr>
<td>$W_Y$</td>
<td>Fresh weight of the sink</td>
<td>g</td>
<td>$W_Y(0) = 5.0 \times 10^{-4}$</td>
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<td>$g_i$</td>
<td>Sucrose concentration of component $i$</td>
<td>µmolC6/gFW/m³</td>
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<tr>
<td>$p_i$</td>
<td>Turgor pressure of component $i$</td>
<td>Pa</td>
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<table>
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<tr>
<th>Functions</th>
<th>$L$</th>
<th>Light condition (1 under light; 0 under dark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>Starch degradation rate</td>
<td>1/hour</td>
</tr>
<tr>
<td>$Z_S$</td>
<td>Continuous phase response curve to sugar signal</td>
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<tr>
<td>$f_S$</td>
<td>Transformation function of sucrose signal</td>
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<tr>
<td>$\alpha$</td>
<td>Sucrose consumption rate for growth</td>
<td>µmolC6/gFW/hour</td>
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Note: Parameters in general use (e.g., gas constant) are represented in SI units for clarity, which are used in numerical simulations with conversion of the units. The values marked with † are estimated in the current study. The remaining values are arbitrarily chosen because of the lack of empirical data.
2.6.4 References


Chapter 3
Mathematical modeling of the phase response to photosynthetic sugars in the plant circadian system
3.1 Introduction

The circadian clock is an endogenous timekeeper generating approximately 24-h rhythmicity of a wide range of biological processes. The circadian rhythm allows organisms to coordinate their physiology and behavior under periodic fluctuation of environment. The circadian oscillator in *Arabidopsis thaliana* consists of interlocking transcriptional feedback loops and regulates important processes such as metabolism and growth (Hsu and Harmer, 2014). Metabolites such as photosynthetic sugars and nitrogen in turn modulate the circadian properties (Haydon et al., 2013; Yuan et al., 2016), forming further feedback loops between the circadian oscillator and metabolic status to improve fitness (Sanchez and Kay, 2016).

To keep the appropriate phase relationship between the circadian oscillator and day-night environmental cycles, the circadian phase needs to be adjusted in response to external environmental signals (Johnson et al., 2003). The magnitude and direction of the phase shift of the circadian oscillator in response to the signal is dependent on the timing when the signal is perceived as represented by a phase response curve (PRC). Previous studies in *A. thaliana* mainly focused on the PRC in response to light signals. Light pulses have been reported to advance and delay the phase during the latter and former half of the subjective night, respectively (Covington et al., 2001; Locke et al., 2005b; Ohara et al., 2015a). When the circadian clock was appropriately entrained to external light-dark cycles, plants biomass was increased, suggesting that entrainment is advantageous (Dodd et al., 2005; Graf et al., 2010).

Besides the signals from external environments, endogenous signals such as photosynthetic sugars also affect the circadian phase (Haydon et al., 2013; Seki et al., 2017). Interestingly, the *A. thaliana* circadian oscillator differentially responds to sugar and light signals—the PRC for sucrose signals has phase advances in the subjective morning (Haydon et al., 2013; Seki et al., 2017). Recent theoretical studies have revealed the importance of the clock entrainment by endogenous sugar on sucrose homeostasis and growth. Using an integrated model of starch metabolism and phase dynamics of the circadian clock, Seki et al. (2017) predicted that fluctuation of sucrose level was minimized when the circadian phase was advanced in the morning in response to sucrose signals. Another theoretical study demonstrated that adjustment of circadian phase to maintain carbon homeostasis is advantageous for plant growth (Ohara and Satake 2017). These studies based on the phase oscillator models unraveled a
non-intuitive relationship between starch metabolism, the circadian oscillator, and growth. However, these models did not contain mechanistic detail about how sugars adjust the phase of the circadian oscillator.

Computational approaches using clock gene-regulatory network models have been useful to understand the mechanisms underlying the phase response to light in the plant circadian system. Based on a clock model with a detailed structure of the regulatory network, Pokhilko et al. (2012) have predicted the important process that could cause discontinuous changes of phase shifts in the *A. thaliana* circadian oscillator in response to red light pulses. Ohara et al. (2015b) have developed a model including multiple photoreceptors and predicted the essential receptor for the oscillator to properly respond to light-to-dark transition. However, there has been no trial *in silico* that evaluates the effect of clock gene regulation by sugar on the phase response property of the plant circadian oscillator. The lack of the computational studies hampers our understanding about which clock genes can be the target of sugar signals and what is the mechanism generating significant phase advance of the circadian oscillator in the subjective morning.

Here I extended a clock-gene regulatory network model (De Caluwé et al., 2016) by incorporating the effect of sugar on the clock genes and simulated phase response dynamics of the system to sucrose signals to address following two questions: (1) which genes in the circadian oscillator are targets of sucrose signals? and (2) How the potential target genes should be regulated by sucrose (e.g. activation or repression) to realize observed phase advance in the subjective morning? I also used an earlier version of the model based on a single feedback-loop (Locke et al., 2005a), to elucidate the common mechanism underlying the phase advance in the subjective morning.

### 3.2 Models and Methods

#### 3.2.1 Clock-gene network models

To identify the target clock gene and regulatory property (activation or repression) of sugar signals that generate the phase advance in the subjective morning, I used two types of gene-regulatory network models with different complexities. Comparison of these models enables us to unravel both realistic and common mechanisms underlying the phase advance of the circadian oscillator in the subjective morning.

#### 3.2.1.1 Interconnected feedback loop model
The first model (De Caluwé et al., 2016; hereafter referred to as DC2016) describes the dynamics of mRNA and protein of eight clock genes: two redundant MYB-like transcription factors \textit{Circadian Clock Associated 1} (CCA1) and \textit{Late Elongated Hypocotyl} (LHY), which peak around dawn; four members of \textit{Pseudo-Response Regulator} (PRR) gene family \textit{PRR9}, \textit{PRR7}, \textit{PRR5}, and \textit{Timing of CAB Expression 1} (TOC1; also known as PRR1), which sequentially express from morning to afternoon; and \textit{Early Flowering 4} (ELF4) and \textit{Lux Arrhythmo} (LUX), which peak around dusk and are constituents of the evening complex (Nusinow et al., 2011). In the model, dynamics of eight clock genes were reduced by merging two genes (CCA1/LHY, PRR9/PRR7, PRR5/TOC1, ELF4/LUX, respectively) into a single variable (CL, P97, P51, EL, respectively) based on the similarities of their roles and expression patterns (Fig. 3.1a). The model was capable of reproducing key features of the \textit{A. thaliana} circadian clock such as phenotypes of clock mutants and the light response (De Caluwé et al., 2016) despite its relatively compact network structure compared to the highly intricate model (Pokhilko et al., 2012; Fogelmark and Troein, 2014). The model describes the mRNA dynamics of \textit{CCA1/LHY} (CL), \textit{PRR9/PRR7} (P97), \textit{PRR5/TOC1} (P51), and \textit{ELF4/LUX} (EL) as follows:

\[
\frac{d[CL]_m}{dt} = \frac{v_1 + v_2 \cdot L \cdot [P]}{1 + \left(\frac{[P97]_p}{K_1}\right) + \left(\frac{[P51]_p}{K_2}\right)} - \left(k_{1L} \cdot L + k_{1D} \cdot (1 - L)\right) \cdot [CL]_m, \tag{3.1}
\]

\[
\frac{d[P97]_m}{dt} = \frac{v_2 \cdot L \cdot [P] + v_2A \cdot V \cdot [CL]_p}{1 + \left(\frac{[P51]_p}{K_4}\right) + \left(\frac{[EL]_p}{K_5}\right)} - k_2 \cdot [P97]_m, \tag{3.2}
\]

\[
\frac{d[P51]_m}{dt} = \frac{v_3}{1 + \left(\frac{[CL]_p}{K_6}\right) + \left(\frac{[EL]_p}{K_7}\right)} - k_3 \cdot [P51]_m, \tag{3.3}
\]

\[
\frac{d[EL]_m}{dt} = \frac{L \cdot v_4}{1 + \left(\frac{[CL]_p}{K_8}\right) + \left(\frac{[P51]_p}{K_9}\right) + \left(\frac{[EL]_p}{K_{10}}\right)} - k_4 \cdot [EL]_m, \tag{3.4}
\]

where \([X]_m\) and \([X]_p\) represent mRNA and protein levels of component X (X corresponds to CL, P97, P51, and EL, respectively). \([P]\) represents the protein level of a putative light-effect mediator \(P\), \(L\) represents the light condition (defined as 1 under light and 0 under dark), and \(v_i, K_i,\) and \(k_i\) are constant. The temporal dynamics of clock proteins and \(P\) have been formalized by ordinary differential equations (De Caluwé et al., 2016) and I used the same formalism in this study. The parameters for the model were set to the optimized values fitted to experimental data reported in the original
3.2.1.2 Single feedback loop model

The second model (hereafter referred to as L2005 model) is characterized by a single positive-negative feedback loop, which is based on the earliest plant clock model (Locke et al., 2005a). In the L2005 model there is an activator (TOC1) and a repressor (LHY) with the phase of the activator being about 6 hours ahead of the repressor. Therefore I renamed TOC1 as EA (early-phased activator) and LHY as LR (late-phased repressor; Fig. 3.1b). The mRNA dynamics of EA and LR are formalized as:

\[
\frac{d[EA]_m}{dt} = \frac{n_2[EA]_m}{g_2^2 + [LR]_n^2} - \frac{m_4[EA]_m}{k_4 + [EA]_m}, \tag{3.5}
\]

\[
\frac{d[LR]_m}{dt} = q_1 [P] + \frac{n_1[EA]_m}{g_1^2 + [EA]_m^2} - \frac{m_1[LR]_m}{k_1 + [LR]_m}, \tag{3.6}
\]

where \([X]_n\) represents the protein level in the nucleus of component X (X corresponds to LR and EA), and \(a, b, q_1, n_i, g_i, m_i, \) and \(k_i\) are constant. \([P]\) and \(L\) are the same as in the DC2016 model. Note that Locke et al. (2005a) separately formalized the clock-protein level in the cytoplasm and nucleus, and I used the similar formalism. Locke et al. (2005a) reported two parameter sets for the model; one is optimally fitted to experimental data and is accompanied with damped oscillation and the other yields the suboptimal solution with limit cycle oscillation. Because the DC2016 model displays sustained oscillation, I adopted the latter parameter sets to facilitate the comparison of the results between two models.

It should be noted that the rate of the change in the mRNA level is defined by the balance between its production and degradation in both models. Therefore, the peak timing of mRNA, which corresponds to the transition from the rising stage to the declining stage of mRNA, is advanced either when production becomes low or degradation becomes high, and vice versa. The peak timing of mRNA was used to quantify the level of phase-shift of the oscillator in response to sugar signals as explained later.

3.2.2 Modeling regulation of circadian oscillator by sucrose

I extended the DC2016 and the repurposed L2005 (see Appendix A) models by incorporating the effects of sucrose on the clock-gene expression or on the mRNA degradation activity because there is strong experimental evidence that sucrose affects transcript levels of several clock genes (Haydon et al., 2013). I considered three
situations by which sucrose might regulate the *A. thaliana* circadian oscillator.

**Case (1): Sucrose directly affects the clock gene expression**

Sucrose directly activates or represses the expression of a clock gene. This corresponds to a situation that, for instance, a sucrose-sensitive protein interacts with the clock-gene promoter independently from clock proteins acting as a transcription factor. If CCA1/LHY (CL) is the target of sucrose that activates the gene expression, its mRNA dynamics (Eq. (3.1)) become:

\[
\frac{d[CL]_m}{dt} = \frac{v_1 + v_1 L + S(t)}{4 \left( \frac{[P97]^P}{k_1} + \left( \frac{[P51]^P}{k_2} \right)^2 \right)} - (k_{1L} * L + k_{1D} * (1 - L)) * [CL]_m, \tag{3.7}
\]

where \(S(t)\) stands for sucrose input defined later. When sucrose represses the gene expression, Eq. (3.1) is modified as:

\[
\frac{d[CL]_m}{dt} = \frac{v_1 + v_1 L + S(t)}{4 \left( \frac{[P97]^P}{k_1} + \left( \frac{[P51]^P}{k_2} \right)^2 \right)} - (k_{1L} * L + k_{1D} * (1 - L)) * [CL]_m. \tag{3.8}
\]

If other clock components (P97, P51, EL) are assumed to be the target of sucrose, \(S(t)\) is similarly added to numerator or denominator of the first term in the right-hand side of Eqs. (3.2)–(3.4). Sucrose input \(S(t)\) is defined as:

\[
S(t) = \begin{cases} 
S_{\text{pulse}} & \text{if } t_{\text{onset}} \leq t < t_{\text{onset}} + t_{\text{dur}}, \\
0 & \text{otherwise}
\end{cases} \tag{3.9}
\]

where \(S_{\text{pulse}}\) stands for the intensity of a sucrose pulse, and \(t_{\text{onset}}\) and \(t_{\text{dur}}\) indicate the onset time and duration of the pulse, respectively. In the DC2016 model, there are eight situations to examine because two regulatory relationships (active or repressive) are possible between sucrose and each of four clock components (Fig. 3.2).

**Case (2): Sucrose indirectly affects the clock gene expression through modulation of clock protein activity**

Sucrose status modulates the activity of a clock protein (e.g. by affecting its phosphorylation state), resulting in the alteration of the expression of its target clock gene. The effect of sucrose on the gene expression depends on the abundance of the clock protein that is modulated by sucrose. If sucrose affects the expression level of *CCA1/LHY (CL)* through interaction with the PRR9/PRR7 (P97) protein, mRNA dynamics of CL become:

\[
\frac{d[CL]_m}{dt} = \frac{v_1 + v_1 L + [P]}{1 + S(t)} - (k_{1L} * L + k_{1D} * (1 - L)) * [CL]_m. \tag{3.10}
\]

In general, when sucrose affects the expression of component X through interaction
with the protein Y, the effect of sucrose is formalized by replacing \([Y]_p\) with \(S(t)[Y]_p\) in the equation for mRNA dynamics of X (X and Y correspond to CL, P97, P51, and EL). Here sucrose input \(S(t)\) is defined as:

\[
S(t) = \begin{cases} 
S_{\text{pulse}}, & t_{\text{onset}} \leq t < t_{\text{onset}} + t_{\text{dur}}, \\
S^*, & \text{otherwise}
\end{cases}
\]  

(3.11)

where \(S^*\) is constant that stands for the effects of sucrose on a clock protein in the absence of external sucrose pulse. I considered the following two situations; (i) \(S_{\text{pulse}} > S^*\); a clock protein is up-regulated by the sucrose pulse. (ii) \(S_{\text{pulse}} < S^*\); a clock protein is down-regulated by the sucrose pulse. The value of \(S^*\) was chosen so that the systems show the self-sustained oscillation and is listed in Table 1. In the DC2016 model, there are twenty situations to examine because two regulatory relationships (up- or down-regulation) are possible between sucrose and clock proteins (Fig. 3.3): PRR9/PRR7 and PRR5/TOC1 proteins for \(CCA1/LHY; CCA1/LHY, PRR5/TOC1,\) and \(ELF4/LUX\) proteins for \(PRR9/PRR7; CCA1/LHY\) and \(PRR5/TOC1\) proteins for \(PRR5/TOC1; CCA1/LHY, PRR5/TOC1,\) and \(ELF4/LUX\) proteins for \(ELF4/LUX\).

**Case (3): Sucrose affects mRNA stability**

Sucrose activates or represses degradation of mRNA. If sucrose affects mRNA degradation of \(CCA1/LHY (CL)\), its mRNA dynamics become:

\[
\frac{d[CL]_m}{dt} = \frac{v_1+v_1L*p}{(k_{1L}*L+k_{1D}*(1-L))}*S(t)*[CL]_m
\]

(3.12)

where \(S(t)\) is defined as

\[
S(t) = \begin{cases} 
S_{\text{pulse}}, & t_{\text{onset}} \leq t < t_{\text{onset}} + t_{\text{dur}}, \\
1, & \text{otherwise}
\end{cases}
\]  

(3.13)

When \(S_{\text{pulse}} > 1\), degradation of mRNA is activated by sucrose. On the contrary, when \(S_{\text{pulse}} < 1\), sucrose suppresses mRNA degradation. If other clock component X (X corresponds to P97, P51, and EL) is assumed to be the target of sucrose, the effect of sucrose is similarly formalized by replacing \([X]_m\) in the second term in the right-hand side of Eqs. (3.2)–(3.4) with \(S(t)[X]_m\). Similar to the Case 1, in the DC2016 model, there are eight situations to examine (Fig. 3.4).

The L2005 model was extended similar to the DC2016 model by incorporating the effects of sucrose on the clock-gene expression or on the mRNA degradation activity (see Appendix A).

3.2.3 Phase response curve
The PRC was generated in the similar way used in a previous study (Ohara et al., 2015b). I mainly used 3-h sucrose pulse to stimulate the circadian oscillator. The value of $S_{\text{pulse}}$ is listed in Table 1. The sucrose pulse was added in the continuous light condition after the system converges to the limit cycle. I used the peak mRNA timing of $CCA1/LHY$ as an indicator of phase-shift determination and considered it as the subjective dawn in the DC2016 model. Because LR in the L2005 model corresponds to LHY in the original study (Locke et al., 2005a), I considered the peak mRNA timing of $LR$ as the subjective dawn and used to estimate phase shifts. I call the former half of one circadian cycle subjective morning and the latter half subjective night. The phase shifts were evaluated at six circadian cycles after the pulse perturbations to avoid transients. Numerical integration of the ordinary differential equations was performed with the fourth-order Runge-Kutta method using Mathematica (version10; Wolfram Research).

### 3.3 Results

#### 3.3.1 Potential target genes of sucrose signal are $PRR5/TOC1$, $ELF4/LUX$, and $PRR9/PRR7$

**3.3.1.1 The direct effect of sucrose on clock gene expression in the DC2016 model**

When sucrose directly affects the clock gene expression, among the eight possible situations examined, phase advance was predicted in the subjective morning when sucrose activates $PRR5/TOC1$ or $ELF4/LUX$ (Fig. 3.2c, d) or represses $PRR9/PRR7$ (Fig. 3.2f). Phase advance was detected only around the dawn when the $CCA1/LHY$ expression was repressed (Fig. 3.2e). Even when signal intensity ($S_{\text{pulse}}$) and duration ($S_{\text{dur}}$) were altered, the PRCs had phase advance and delay at similar phases of the pulse (Fig. 3.2; Fig. A3.1), suggesting that the major determinant of the direction of phase shifts (i.e. advance or delay) is determined mainly by the timing of the sucrose pulse. In contrast, the magnitude of phase shifts was strongly affected by the signal intensity and duration (Fig. 3.2; Fig. A3.1).

**3.3.1.2 The effect of sucrose on regulation of the clock protein activity in the DC2016 model**

When sucrose regulates the activity of clock proteins, among the twenty situations examined, phase advance was predicted in the subjective morning if sucrose indirectly activates $PRR5/TOC1$ (Fig. 3.3f–g) or $ELF4/LUX$ (Fig. 3.3h–j) or represses $PRR9/PRR7$
(Fig. 3.3c–e). These results support the findings in the Case 1 that phase advance in the subjective morning is possible when sucrose up-regulates PRR5/TOC1 or ELF4/LUX or down-regulates PRR9/PRR7. In addition, phase advance around dawn was also detected when the CCA1/LHY expression was indirectly repressed (Fig. 3.3a, b), that is similar to the result from the Case 1 (Fig. 3.2e).

3.3.1.3 The effect of sucrose on mRNA degradation in the DC2016 model

Similar results were obtained when sucrose regulates the mRNA degradation. Among the eight situations examined, phase advance was predicted in the subjective morning when the mRNA degradation of PRR9/PRR7 is activated (Fig. 3.4b) or that of PRR5/TOC1 or ELF4/LUX is repressed (Fig. 3.4c, d). When sucrose activates mRNA degradation of CCA1/LHY, phase advance was also predicted in the subjective morning as well as in the later night. These analyses demonstrate that there are commonly three possible clock regulations by sucrose for the phase advance in the subjective morning—sucrose up-regulates PRR5/TOC1 or ELF4/LUX mRNA or down-regulates PRR9/PRR7 mRNA. Only in the Case 3, sucrose pulse down-regulating CCA1/LHY mRNA induced phase advance in the morning. Overall, the effects of signal intensity and duration on the PRCs observed in Case 1 were similar between Cases 1–3 (data not shown).

3.3.2 Mechanisms underlying the phase-advance in the subjective morning

Because I defined the peak mRNA timing of LR as the subjective dawn in the L2005 model, declining and rising stages of LR mRNA correspond to the subjective morning and night, respectively (Fig. 3.5a, d). When sucrose directly represses LR expression, sucrose pulse added in the subjective morning advanced the phase (Fig. 3.5a, b). In contrast, the pulse in the subjective night delayed the phase (Fig. 3.5d, e). This is because during the subjective morning the pulse-induced decrease of LR mRNA (Fig. 3.5a) was followed by the premature rise of EA mRNA (Fig. 3.5b), leading to the advanced phase of the oscillator (Fig. 3.5a, b). When the pulse was added during the subjective night, the pulse-induced decrease of LR mRNA (Fig. 3.5d) was followed by the slow decline of EA mRNA (Fig. 3.5e), resulting in the delayed phase of the oscillator (Fig. 3.5d, e).

These results can be explained intuitively using the phase plane representations (Fig. 3.5c, f). The sucrose perturbation to the system during the subjective morning moved state points toward the same direction as the rotation of the limit cycle (Fig. 3.5c), resulting in the advancement of the phase of the oscillator. During the night, the pulse
moved state points in the opposite direction (Fig. 3.5f), shifting the phase of the oscillator backward. The resultant PRC showed clear phase advance in the subjective morning and delay in the subjective night (Fig. 3.6a). When sucrose directly activates LR expression, opposite phase response was predicted based on the same argument, namely phase delay in the subjective morning and phase advance in the subjective night (Fig. A3.2a; Fig. 3.6a). The results from the Case 2 (Fig. A3.2b; Fig. 3.6c) and Case 3 (Fig. A3.2c; Fig. 3.6e) were similar to those in the Case 1 (Fig. 3.5c) because of the same effect of perturbation on the system. I also examined the situation where sucrose directly represses EA expression and found that phase advance was induced in the subjective morning because sucrose pulse moved the state point in the same direction as the rotation of the limit cycle (Fig. A3.2d; Fig. 3.6b). Again, the results from the Case 2 (Fig. 3.6d) and Case 3 (Fig. 3.6f) were similar to those in the Case 1 (Fig. 3.6b) when the sucrose stimuli have the same effect of perturbation on the system.

3.4 Discussion

The analyses of the realistic gene regulatory network model (the DC2016 model) demonstrated that target genes of sugar signal could be PRR5/TOC1, ELF4/LUX, and PRR9/PRR7. I predicted that either sugar-induced down-regulation of PRR9/PRR7 mRNA (Fig. 3.2f; Fig. 3.3c–e; Fig. 3.4b) or up-regulation of PRR5/TOC1 (Fig. 3.2c; Fig. 3.3f, g; Fig. 3.4c) or ELF4/LUX (Fig. 3.2d; Fig. 3.3h–j; Fig. 3.4d) mRNA is necessary to induce phase advance consistently in the subjective morning. CCA1/LHY could also be a candidate, but phase advance in most part of the morning was detected only through the specific process (i.e. activation of mRNA degradation; Fig. 3.4a). Among these possibilities, the regulation of PRR9/PRR7 by sucrose is of great interest. Haydon et al. (2013) showed that photosynthetically derived sugars decrease transcript levels of PRR7 and PRR5, increase CCA1 and LHY, and does not affect PRR9. The prr7 mutant is deficient in the clear phase response to sugars (Haydon et al., 2013). These results suggest that suppression of PRR7 would be sufficient to realize phase advance in the subjective morning.

The contribution of the evening components to the clock response to sugars have not been empirically investigated partly because mutations of LUX (Hazen et al., 2005; Onai and Ishiura, 2005), ELF4 (Doyle et al., 2002; McWatters et al., 2007), and another
member of the evening complex \textit{ELF3} (Hicks et al., 1996; Covington et al., 2001; Thines and Harmon, 2010) cause arrhythmic or very weak circadian oscillation in continuous light or dark condition. Therefore, it is difficult to examine whether they are target for sugar signals by measuring e.g. phase shifts of the mutant clock in response to sucrose pulse in the constant light condition.

Among the potential regulatory-relationships between clock genes and sucrose, down-regulation of \textit{PRR9/PRR7} might have an advantage for plant growth. It has been experimentally suggested that \textit{PRR7} and 5 (Nakamichi et al., 2012; Liu et al., 2013) and the evening complex (Nusinow et al., 2011) repress the expression of \textit{PHYTOCHROME INTERACTING FACTOR4 (PIF4)} and \textit{PIF5}, both of which encode the basic helix–loop–helix transcription factor that is essential for hypocotyl elongation growth in \textit{A. thaliana} seedlings. Because sucrose pulse in the phase-response-curve experiments transiently increases carbon availability for \textit{A. thaliana} seedlings, sucrose-induced down-regulation of \textit{PRR7} followed by increased expressions of \textit{PIF4} and \textit{PIF5} (Liu et al., 2011) would be advantageous for effective usage of excess carbon and facilitating plant growth.

In conclusion, I have theoretically elucidated the regulatory mechanisms by which endogenous sugar signals regulate the plant circadian oscillator. Although the previous theoretical studies have revealed the importance of the dynamical feedback from sucrose to the plant clock for flexible regulation of carbon metabolism and optimization of sink growth (Feugier and Satake, 2013; Seki et al., 2017; Ohara and Satake, 2017), how the circadian phase is shifted in response to the sucrose feedback has been elusive due to the lack of a comprehensive study using computational approaches. The current study using the clock-gene regulatory network models elucidated possible regulatory relationships between clock genes and sucrose stimuli that induce significant phase advance during the subjective morning. My computational approaches can be applied to investigate how sugar signaling works at a whole plant scale to realize carbon homeostasis. A previous experimental study has suggested that the circadian oscillator in \textit{A. thaliana} roots have distinct network structure from that in shoots, and can be entrained by photosynthetic sugars transported from the source leaf (James et al., 2008). Although the effects of sugar signals on carbon homeostasis have been examined mainly in the photosynthetic leaves (Seki et al., 2017), it would be possible that the translocated sucrose also affects homeostatic regulation of carbon metabolism in roots.
The clock model with the modified network structure will predict how the phase of the root clock can be shifted by the sucrose stimulus. This knowledge will be incorporated into the previous model describing sucrose translocation from the source to sink (Ohara and Satake, 2017) in order to explicitly formalize the circadian-phase regulation by sugar in roots. Such extended model will be helpful to investigate the impact of the circadian entrainment by sugar signaling at a whole plant scale.

3.5 References


Figure 3.1. Clock-gene network structures of the (a) DC2016 and (b) L2005 models. The DC2016 model includes a positive-negative feedback loop consisting of CCA1/LHY (activator) and PRR9/PRR7 (repressor) and a negative-negative feedback loop between CCA1/LHY (repressor) and PRR5/TOC1 (repressor).
Figure 3.2. Phase response curves simulated by the DC2016 model. Clock gene expression is directly activated (a)–(d) or repressed (e)–(h) by sucrose stimulus. Target genes are (a), (e) *CCA1/LHY*, (b), (f) *PRR9/PRR7*, (c), (g) *PRR5/TOC1*, and (d), (h) *ELF4/LUX*. Different colors correspond to different stimulation strengths. The values of $S_{\text{pulse}}$ for strong and weak stimulations are the double and half of that for moderate
stimulation, respectively. Illustrations above the PRCs display the stimulation schemes where rectangles represent clock genes and solid lines with arrows and bars represent activation and repression effects, respectively. Stars indicate the conditions that yield phase advance in the subjective morning.
Figure 3.3. Clock gene expression influenced by sucrose though up- (red) or down-regulation (blue) of the activity of clock proteins in the DC2016 model. (a), (b) *CCA1/LHY* expression is influenced through the regulation of (a) PRR9/PRR7 or (b) PRR5/TOC1. (c)–(e) PRR9/PRR7 expression is influenced through the regulation of (c) CCA1/LHY, (d) PRR5/TOC1, or (e) ELF4/LUX. (f), (g) PRR5/TOC1 expression is influenced through the regulation of (f) CCA1/LHY or (g) PRR5/TOC1. (h)–(j) ELF4/LUX expression is influenced through the regulation of (h) CCA1/LHY, (i) PRR5/TOC1, or (j) ELF4/LUX. The values of $S^*$ for up- and down-regulation effect of sucrose are 0.5 and 2.0, respectively. Illustrations above the PRCs display the stimulation schemes where rectangles and ellipses represent clock genes and proteins, respectively, and solid lines with arrows and bars represent activation and repression.
effects, respectively. Stars indicate the conditions that yield phase advance in the subjective morning.
Figure 3.4. Sucrose affects degradation of mRNA in the DC2016 model. The mRNA degradation of (a) CCA1/LHY, (b) PRR9/PRR7, (c) PRR5/TOC1, and (d) ELF4/LUX are activated (red) or repressed (blue). Illustrations above the PRCs display the stimulation schemes where rectangles represent mRNA. Stars indicate the conditions that yield phase advance in the subjective morning.
Figure 3.5. Phase shifts obtained by the sucrose pulse added during (a)–(c) the subjective morning (white background) and (d)–(f) the subjective night (gray background) in the L2005 model. LR expression is directly repressed by the pulse. (a), (d) Time series of LR mRNA concentration. (b), (e) Time series of EA mRNA concentration. Two vertical dashed lines represent the onset and offset of 3-h sucrose pulse. Subjective dawn corresponds to the peak mRNA timing of LR. (c), (f) Limit cycles in phase planes, the coordinates of which indicate LR and EA mRNA concentrations. Arrows represent the direction of rotation. Flashes and thick lines on the limit cycles represent the onset and the 3-h period of sucrose stimulations, respectively. Black circles and squares represent subjective dawn and dusk, respectively.
Figure 3.6. Phase response curves simulated by the L2005 model. (a) LR and (b) EA expressions are directly activated (red) or repressed (blue) by the sucrose pulse. (c) LR and (d) EA expressions are influenced through up- (red) or down-regulation (blue) of EA and LR proteins, respectively. (e) LR and (f) EA mRNA degradations are activated (red) or repressed (blue). Illustrations above the PRCs display the stimulation schemes where rectangles, ellipses, and diamonds represent the clock gene, protein, and mRNA respectively. Stars indicate the conditions that yield phase advance in the subjective morning.
Table 3.1 Summary of the values used for $S_{\text{pulse}}$ and $S^*$.

<table>
<thead>
<tr>
<th># of Figure</th>
<th>$S_{\text{pulse}}$ Value</th>
<th># of Figure</th>
<th>$S^*$ Value</th>
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<tr>
<td>3.2a–d</td>
<td>1.0 (Moderate)</td>
<td>3.3</td>
<td>0.5 (Up-regulation)</td>
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<td>2.0 (Strong)</td>
<td></td>
<td>2.0 (Down-regulation)</td>
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<tr>
<td></td>
<td>0.5 (Weak)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.2e–h</td>
<td>8 (Moderate)</td>
<td>3.6c, d</td>
<td>0.95 (Up-regulation)</td>
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<tr>
<td></td>
<td>16 (Strong)</td>
<td></td>
<td>1.50 (Down-regulation)</td>
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<td>4 (Weak)</td>
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<tr>
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<td>2.0 (Up-regulation)</td>
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<td>0.2 (Down-regulation)</td>
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<td>3.4</td>
<td>1.2 (Activation)</td>
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<td>0.8 (Repression)</td>
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3.6 Appendix

Here I describe the formalization of the effects of sucrose on the circadian oscillator based on the L2005 model.

Case (1): Sucrose additively affects the clock gene expression

If LR or EA is the target of sucrose that activates the gene expression, mRNA dynamics become:

\[
\frac{d[L_R]_m}{dt} = q_1 \times [P] \times L + \frac{n_{1+}[E_A]_m}{g_1^b + [E_A]_m} + S(t) - \frac{m_{1+}[L_R]_m}{k_{1+}[L_R]_m}, \tag{A3.1}
\]

\[
\frac{d[E_A]_m}{dt} = \frac{n_{2+}g_2^b}{g_2^b + [L_R]_m} + S(t) - \frac{m_{4+}[E_A]_m}{k_4+[E_A]_m}. \tag{A3.2}
\]

When sucrose represses the gene expression, mRNA dynamics become;

\[
\frac{d[L_R]_m}{dt} = \left(q_1 \times [P] \times L + \frac{n_{1+}[E_A]_m}{g_1^b + [E_A]_m}\right) \times \frac{1}{1+S(t)} - \frac{m_{1+}[L_R]_m}{k_{1+}[L_R]_m}, \tag{A3.3}
\]

\[
\frac{d[E_A]_m}{dt} = \frac{n_{2+}g_2^b}{g_2^b + [L_R]_m} \times \frac{1}{1+S(t)} - \frac{m_{4+}[E_A]_m}{k_4+[E_A]_m}. \tag{A3.4}
\]

There are four situations to examine because two regulatory relationships (active or repressive) are possible between sucrose and each of two clock components (Fig. 3.6a, b).

Case (2): Sucrose affects the clock gene expression through modulation of clock protein activity

If sucrose affects LR expression through the interaction with EA protein, mRNA dynamics become;

\[
\frac{d[L_R]_m}{dt} = q_1 \times [P] \times L + \frac{n_{1+}S(t)\times [E_A]_m}{g_1^b + S(t)\times [E_A]_m} - \frac{m_{1+}[L_R]_m}{k_{1+}[L_R]_m}. \tag{A3.5}
\]

If sucrose affects EA expression through the interaction with LR protein, mRNA dynamics become;

\[
\frac{d[E_A]_m}{dt} = \frac{n_{2+}g_2^b}{g_2^b + S(t)\times [L_R]_m} - \frac{m_{4+}[E_A]_m}{k_4+[E_A]_m}. \tag{A3.6}
\]

There are four situations to examine because two regulatory relationships (up- or down-regulation) are possible between sucrose and each of two clock proteins (Fig. 3.6c, d).

Case (3): Sucrose affects mRNA stability

If sucrose affects mRNA degradation of LR or EA, mRNA dynamics become;
\[
\frac{d[LR]_m}{dt} = q_1 \cdot [P] \cdot L + \frac{n_1 *[EA]^a}{g_1 + [EA]^a} - S(t) \cdot \frac{m_1 *[LR]_m}{k_1 + [LR]_m} \tag{A3.7}
\]

\[
\frac{d[EA]_m}{dt} = \frac{n_2 g_2}{g_2 + [LR]_m} - S(t) \cdot \frac{m_4 *[EA]_m}{k_4 + [EA]_m} \tag{A3.8}
\]

There are four situations to examine because two regulatory relationships (active or repressive) are possible between sucrose and mRNA degradation for each of two clock components (Fig. 3.6e, f).

These models were used to explore the underlying mechanism of phase shift in the subjective morning.
**Figure A3.1.** Phase response curves simulated by the DC2016 model for various pulse durations. Clock gene expression is directly (a)–(d) activated or (e)–(h) repressed by sucrose stimulus. Target genes are (a), (e) *CCA1/LHY*, (b), (f) *PRR9/PRR7*, (c), (g) *PRR5/TOC1*, and (d), (h) *ELF4/LUX*. Different colors correspond to different pulse durations.
Figure A3.2. Limit cycles in phase planes for various combinations of a target gene of sucrose and its regulatory property. (a) LR expression is directly activated. (b) LR expression is indirectly repressed through down-regulation of the EA protein. (c) LR mRNA degradation is activated. (d) EA expression is directly repressed. Details are as in the legend to Fig. 3.5.
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