Title

Early detection of daylengths with a feedforward circuit coregulated by circadian and diurnal cycles

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Abstract

Light-entrained circadian clocks confer rhythmic dynamics of cellular and molecular activities to animals and plants. These intrinsic clocks allow stable anticipations to light-dark (diel) cycles. Many genes in the model plant *Arabidopsis thaliana* are regulated by diel cycles via pathways independent of the clock, suggesting that the integration of circadian and light signals is important for the fitness of plants. Previous studies of light-clock signal integrations have focused on moderate phase adjustment of the two signals. However, dynamical features of integrations across a broad range of phases remain elusive. We recently found that phosphorylation of RIBOSOMAL PROTEIN OF THE SMALL SUBUNIT 6 (RPS6 or eS6), a ubiquitous post-translational modification across kingdoms, is influenced by the circadian clock and the light-dark (diel) cycle in an opposite manner. In order to understand this striking phenomenon and its underlying information processing capabilities, we built a mathematical model for the eS6-P control circuit. We found that the dynamics of eS6-P can be explained by a feedforward circuit with inputs from both circadian and diel cycles. Furthermore, the early-day response of this circuit with dual rhythmic inputs is sensitive to the changes in daylength, including both transient and gradual changes observed in realistic light intervals across a year, due to weather and seasons. By analyzing published gene expression data, we found that the dynamics produced by the eS6-P control circuit can be observed in the expression profiles of a large number of genes. Our work provides mechanistic insights into the complex dynamics of a ribosomal protein, and it proposes a previously underappreciated function of the circadian clock which not only prepares organisms for normal diel cycles but also helps to detect both transient and seasonal changes with a predictive power.

36 **Introduction**

37 Circadian clocks provide animals, plants and certain microbes with rhythmic dynamics. These circadian
38 pacemakers confer fitness advantages to the organisms by establishing stable anticipation of diel light-
39 dark cycles (1, 2) as well as regulating broad morphological changes over the course of the year (3). In the
40 plant model organism *Arabidopsis thaliana (A. thaliana)*, the circadian clock consists of at least four
41 interacting gene modules that form negative feedback loops, which govern an intrinsic oscillator (4, 5).
42 The expression dynamics of several hundred genes in *A. thaliana* are controlled by this circadian oscillator
43 (6, 7). The circadian oscillator retains its essential dynamical features under constant light conditions,
44 reflecting its intrinsic nature (8), but the phase of the oscillations can be adjusted in a process called
45 entrainment by the lights-on signal at dawn (5, 9). In addition, however, the light-dark cycle regulates a
46 large number of genes in a clock-independent fashion (8, 10). Therefore, molecular activities in plants are
47 influenced by at least two periodic signals. Previous studies have found that cellular activities in *A. thaliana*,
48 such as the abundance of the regulator of flowering time CONSTANS (CO) and the cytosolic calcium
49 concentration, require signals from both the clock and the light-dark cycle to regulate timing (i.e. the
50 phase of peak activity) (8, 9, 11). For example, for many mRNA transcripts, the circadian clock leads to a
51 moderate phase shift in the onset or peak time as compared to the light cycle alone (8, 9, 11). However,
52 these paradigms only represent *one* of the many diverse co-regulation modes of clock-light signal
53 integration. It remains unclear whether the integration of circadian and diel light-dark cycles underpins
54 other paradigms of signal processing.

55 The phosphorylation of RIBOSOMAL PROTEIN OF THE SMALL SUBUNIT 6 (RPS6 or eS6) is a post-
56 translational modification that occurs in a wide range of organisms including animals and plants (12-14).
57 Mice that have only a non-phosphorylatable eS6 show abnormalities at the organismal level (e.g. muscle
58 weakness), indicating that this modification is functionally significant (15). However, while it has been
59 suggested that the eS6 phosphorylation (eS6-P) is implicated in ribosome biogenesis in mammals (12), its

60 biochemical consequence and specific activity, including its molecular role in *A. thaliana*, are largely
61 unknown (13). Interestingly, eS6-P is widely used as a bioreporter for the activity of Target Of Rapamycin
62 (TOR) kinase, a central controller of cell growth and aging (16, 17). We recently found that eS6-P in *A.*
63 *thaliana* is co-regulated by both the circadian clock and the diel cycle, as eS6-P exhibits cycling behavior
64 both in a severely clock-deficient strain and under constant light conditions (18). Unlike other extensively
65 studied cellular processes, eS6-P is controlled by the clock and light-dark cycles in a strikingly opposite
66 manner: in the wild-type strain under constant light the circadian clock drives eS6-P with a peak during
67 the subjective night, whereas in a clock-deficient strain the diel light-dark cycle drives eS6 with a peak
68 during the day (18, 19). However, the biological significance of this remarkable phenomenon at cellular
69 and organismal levels remains elusive.

70 In this study, we constructed a mathematical model to examine the signaling network that regulates eS6-
71 P in *A. thaliana*. We calibrated the model with experimental measurements of the circadian clock and eS6-
72 P under various conditions. We found that the key dynamics of eS6-P can be explained by a feedforward
73 loop that connects the periodic light signals to eS6-P with a direct arm and an indirect arm via the circadian
74 clock. Although this feedforward loop is largely incoherent at the steady state of a symmetrical light-dark
75 cycle (12-hour-light and 12-hour-dark), the amplitude of its output exhibits a high sensitivity to variations
76 in daylength, due to the interaction between the two cyclic components in the loop. Notably, we found a
77 characteristic early day peak of eS6-P that detects and anticipates long days. Furthermore, we combined
78 the model with realistic photoperiod data containing both transient perturbations and long-term
79 variations of light-dark cycles and demonstrated that the detection of daylength variations by this circuit
80 communicates information about changes of both the season and the local environment throughout the
81 year. By comparing our model with several representative competing models with various circuits
82 transmitting only light signals, we found that the robust detection of day length variations requires both
83 the circadian clock and the clock-independent light sensor. Our work demonstrates a remarkable

84 information processing capacity of a feedforward loop that integrates circadian and light-dark cycles, and
85 it reveals a previously underappreciated role of the circadian clock in anticipating and predicting the
86 changes in light conditions rather than the stable photoperiod.

87

88 **Results**

89 **A mechanistic mathematical model characterizes dynamical features of a feedforward loop controlling**
90 **eS6-P**

91 To gain a better understanding of the mechanisms underlying the dynamics of eS6-P, we built a
92 mathematical model that includes a light-entrained circadian clock and a light-dependent, clock-
93 independent signaling pathway that regulates the phosphorylation of eS6 (Fig 1A). The latter light-
94 pathway consists of TOR, a kinase that transmits light signals, and ribosomal S6 kinase (S6K), a substrate
95 of TOR (20-23). S6K has been shown to phosphorylate eS6, and the TOR-S6K axis is known as a primary
96 pathway for eS6-P in multiple organisms including plants (17). The clock component of the model is
97 described by four interacting components (LHY/CCA1, the evening complex (EC), PRR9/PRR7, and
98 PRR5/PRR1, denoted as C1, C2, C3 and C4 respectively in this study). It contains the core repressilator of
99 the plant circadian circuit and has previously been used to model the circadian clock (Fig 1A) (4, 24-26).
100 We used this model to capture the essential dynamical features of the clock rather than the molecular
101 details, so we focused on this four-component core repressilator and neglected the additional feedbacks
102 in the clock (4). Because we previously observed that eS6-P oscillates under constant light (18), we also
103 considered clock-driven regulation of eS6 phosphorylation and dephosphorylation in our model (Fig. 1A).
104 Before parameter estimation, each clock component in the model was assumed to regulate (activate or
105 inhibit) the phosphorylation or dephosphorylation of eS6 with unknown parameters. Because we
106 assumed that the clock does not receive signals from the clock-independent pathway or eS6, we first

107 obtained a parameter set for the clock component by fitting the clock model using a qualitative objective
108 function to capture the basic behaviors including cyclic variation in response to light-dark cycles
109 (entrainment) and cycling under constant light conditions (see Methods, Fig. S1). We then compared the
110 simulated clock gene dynamics to previously published expression data (27) to ensure that the model
111 properly represents the phase and shape of the time course measurements (Fig. S2).

112

113 **Fig. 1. Network structure of the eS6-P regulatory circuit and comparisons between simulations and**
114 **experiments. (A)** An influence diagram of a model of the light and clock driven feedforward regulatory
115 system that regulates eS6-P (Clock+Light Model). This model consists of two cycling systems: the light-
116 dark cycle (an oscillatory input) and the circadian clock (an autonomous oscillator). Each cycle regulates
117 cellular processes independently. The regulation by light-dark cycle is mediated by TOR-S6K pathway, and
118 the clock driven regulation is mediated by transcription factors belonging to the LHY/CCA1 (C1), Evening
119 Complex (EC,C2), PRR9/7 (C3), PRR5/1 (C4) modules which contain a repressilator circuit and additional
120 interactions. The light-dark cycle also regulates the circadian clock via the LHY/CCA1 and PRR9/7 modules
121 (entrainment), thus creating a feedforward circuit. Arrows with circle head show influence of unknown
122 directions (positive or negative) before parameter estimation. **(B)** Model predictions (black) and
123 experimental observations (red) of eS6-P under wild-type in long-days (16:8, LD). Error bars indicate
124 standard deviation of pooled measurements at various circadian times. Circadian time is relative to dawn,
125 and regions shaded with grey indicate period of darkness. Blue dots show the raw data points collected
126 prior to pooling over a period of 84 hours (18). **(C)** Model predictions (black) and experimental
127 observations (red) of eS6-P under long-days (16:8, LD) but with a deficient clock (CCA1-overexpression).
128 Error bars indicate standard deviation of pooled measurements at various circadian times. Time is
129 measured and the graph is shaded as in **(B)**. **(D)** Model predictions (black) and experimental observations
130 (red) of eS6-P under constant light. Error bars indicates standard deviation of pooled measurements at

various circadian times. Circadian time is measured relative to subjective dawn (Zeitgeber time, ZT) and regions shaded grey indicate periods of subjective night. eS6-P is shown in arbitrary units (a. u.) (see Methods). **(E)** An influence diagram of our model of eS6-P updated with the direction (triangle arrowhead: activation, flat arrowhead: repression). Note that the ambiguous (diamond) regulation of the clock by light reflects that light regulates LHY/CCA1 and PRR9/PRR7 in opposite directions (we assume that light represses CCA1 stability [49], which is important to restrict the LHY/CCA1 peak to dawn). **(F)** An overlay of clock element activities on top of the eS6-P trajectory in wild type under a long-day cycle. The eS6-P cycle is indicated by the solid black curve and the clock components are represented by colored curves (red = C1 = LHY/CCA1, blue = C2 = EC, orange = C3 = PRR9/PRR7 , green = C4 = PRR5/PRR1). Circadian time is measured relative to dawn.

Next, we fit the Clock+Light model that contains both clock-dependent and clock-independent pathways regulating eS6-P to our recent measurement of eS6-P dynamics under three experimental conditions: a wild-type (WT) strain under long day (LD, 16-hour-light and 8-hour-dark) condition, the WT strain under constant light (LL) condition, and a clock-deficient strain under LD condition (CCA1-overexpression) (18). Using an evolutionary algorithm with a likelihood-based objective function, we found optimized parameter sets that produced trajectories that reasonably matched the experimental data in all three conditions (Fig. 1B-D). Importantly, the model captured the remarkable dynamical features of eS6-P: light alone drives the upregulation of eS6-P during the day (Fig. 1C) whereas the clock alone drives the upregulation of eS6-P during the subjective night (Fig. 1D). Our model also recapitulated the day-peak of the eS6-P when both clock and light-dark cycles are present, suggesting the dominant role of the light-dark cycle. Furthermore, the model predicted a peak of eS6-P shortly after dawn that was not measured experimentally. We found that this observation was not due to the choice of a particular parameter set: the majority of the top 70 (or 5%) models (based on likelihood) from multiple optimization runs generated

155 the same behavior (Fig. S3A). Conversely, the next 70 models lacked the dawn peak, but those models
156 also lacked oscillations under constant light, suggesting a loss of clock regulation (Fig. S3B). Our
157 subsequent analyses are based on the top performing model, which produced a moderate peak of eS6-P
158 after dawn.

159 We next examined how the clock and the light-dark cycles influenced eS6-P mechanistically. While the
160 clock-independent pathway had an obvious mechanism of action, in which the light signal activates a
161 cascade of two kinases that give rise to eS6 phosphorylation, the clock pathway involved a nontrivial
162 combination of molecular influences. We found that eS6-P is regulated by multiple clock-genes that peak
163 at different time points during the day (Fig 1E). Specifically, LHY/CCA1 and PRR9/PRR7 both regulated eS6
164 during the early day with different phases of activity (LHY/CCA1 at dawn and PRR9/PRR7 a few hours after
165 dawn) and opposite effects on eS6 dephosphorylation (LHY/CCA1 inhibits and PRR9/PRR7 promotes
166 dephosphorylation). In contrast, while EC and PRR5/PRR1 also have opposing influences, their activities
167 largely overlapped and canceled each other out. These clock influences collectively resulted in the stably
168 high amount of eS6-P between the early day and dusk, while the early day peak resulted from a
169 coincidence of light, the peak LHY/CCA1 activity, and the absence of peak PRR9/PRR7 activity (Fig 1F). As
170 such, our model showed that at the steady state under LD, the circadian clock and the light-dark cycle
171 influenced eS6-P in a generally opposing manner: during the day, eS6-P is promoted by light but inhibited
172 by PRRs, while during the night, the anticipatory rise of LHY/CCA1 promoted eS6-P prior to dawn, leading
173 both to rapid early rise and the early day peak under normal LD conditions, and the shift in the rise into
174 the subjective night under constant light. Considering the clock's dynamics are light-entrained, the
175 resulting model behaved as a feedforward loop that is largely incoherent at steady state.

176 In conclusion, our calibrated model captured the observed dynamics of eS6-P under multiple conditions,
177 and allowed us to make predictions about the mechanisms underlying these intriguing dynamics. Note
178 that although our discussion primarily focuses on one optimized model, we have reproduced our key

179 conclusions with distributions of parameters rather than a single parameter set (Fig. S3), and with a much
180 more detailed clock model which includes both mRNA and protein concentrations (Fig. S4) (4).

181

182 **Integration of circadian clock and light-sensing pathways detects and anticipates long daylength upon**
183 **transient perturbations of light-dark cycles**

184 We next focused on the dynamics of eS6-P after dawn, when the coincidence of clock and light signals
185 gave rise to a sharp rise and a peak in the model (Fig. 1B-D). We hypothesized that at this key time interval
186 a change in the phase of the lights-on signal significantly affect the dynamics of the model output because
187 the distinct influences of light and clock can synergize with each other depending on their relative phase.
188 We therefore focused on variations in the timing of the transition from night to day (t_{ND}), as they might
189 be used as proxies for transient changes in the local environment (such as weather)or long-term seasonal
190 changes. We ran simulations with the optimized Clock+Light model (Fig. 1E) under the 12-hour-light and
191 12-hour-dark (12L:12D) condition. After the system reached steady state (Day 0), we varied t_{ND} (+/- 4
192 hours) at the start of a single day (Day 1), and tracked the trajectories of eS6-P from the perturbed day
193 and onwards (Fig. 2A). We found that the transient variation of t_{ND} resulted in significant changes in the
194 abundance of eS6-P (response) in the early day, but the responses converged as the day continued. In
195 particular, an early night-to-day transition time ($t_{ND}<0$) gave rise to a higher response including an early
196 day peak, as previously observed with the base model under LD condition (Fig. 1B, Fig. 2B purple), whereas
197 a late transition time ($t_{ND}>0$) had the opposite effect and resulted in a trough (Fig. 2B yellow). In addition,
198 early day maximum eS6-P (defined as the maximum eS6-P over the 4 hours after dawn) varied by as much
199 as 13.8% relative to the case of $\Delta t_{ND} = 0$. We found that the early day peaks (transient responses that are
200 greater than the steady-state response during the day) appeared only when the daylength exceeded 12

hours (Fig. 2B cyan). These results show that the eS6-P control circuit detected phase variations of the lights-on signal and thus effectively sensed daylength variation at the beginning of the day.

Fig. 2. Response of eS6-P to variations of the night-to-day transition time. (A) Diagram of perturbations of the night-to-day transition and the effect of daylength relative to a 12-hour-light-12-hour-dark (12L:12D) day. The unperturbed ($\Delta t_{ND} = 0$) and perturbed (Δt_{ND} +/- 4 hours) days are labeled. The day period is shown by yellow shaded regions and the maximum extent of the change in daylength is shown by differential shading. The system reached steady state before Day 0. **(B)** Trajectories of eS6-P in response to variations of the night-to-day transition time from $\Delta t_{ND} = -4$ (purple) to $\Delta t_{ND} = 4$ (yellow) in 0.5-hour increments. All trajectories start at the dusk of the previous day (Day 0, unperturbed), and end at the dusk of the current day (Day 1, perturbed). Dotted lines show trajectories in the absence of light. Thick solid curves show trajectories in the early day (first four hours after dawn), and thin solid curves show trajectories in the remaining hours of the day. Short line segments at the bottom show the time of dawn for each trajectory. **(C)** Side by side plots of clock perturbations in response to varying night to day transition and the resulting effects on eS6-P. For simplicity, we only show the $\Delta t_{ND} = -4$ (purple), $\Delta t_{ND} = 0$ (red), $\Delta t_{ND} = 4$ (yellow) in the bottom figure. In the top panel, color indicates clock factor (red = C1 = LHY/CCA1, blue = C2 = EC, orange = C3 = PRR9/PRR7, green = C4 = PRR5/PRR1), while shade of color represents the timing of dawn (dashed, $\Delta t_{ND} = -4$; regular solid, $\Delta t_{ND} = 0$; semitransparent solid, $\Delta t_{ND} = 4$). Dashed lines show the corresponding times while circles on the clock factors' curves highlight how the ratio between LHY/CCA1 and PRR9/PRR7 corresponds to peak height of the response. In all panels, Circadian time is shown in hours relative to normal time of dawn (i.e. +/- 0 hours).

223 We found that the sensitivity of eS6-P to changes in the light-dark cycle was associated with the altered
224 relative phase of clock-gene activities with respect to the dawn (Fig. 2C, gray lines) upon perturbations of
225 the light-dark cycle. For example, the earlier dawn allowed synergy between light signal, peak of CCA1/LHY
226 and relatively low activities of PRR modules to give rise to a strong eS6-P response (Fig. 2C). This change
227 of relative phase occurred despite the influence of the transient phase shift on the clock gene dynamics
228 as a form of entrainment (Fig. 2C upper panel). Variation in photoperiod length has been previously shown
229 to affect the absolute phase of circadian genes, altering both their timing relative to dawn and the
230 intervals between peak expression of different circadian genes, with the gap between CCA1/LHY and PRR9
231 growing as the photoperiod becomes longer (26, 28). These results suggest that the circadian clock plays
232 an essential role in the regulation of eS6-P, particularly with regard to its early day dynamics.

233 We therefore asked whether the circadian clock is required for the eS6-P circuit (Fig. 1E, Clock+Light model)
234 to detect long daylength with an early day peak. To this end, we constructed three alternative models
235 that describe various modes in which eS6-P may respond to the light signal in the absence of the clock
236 (Fig. 3A, see Table S2 for parameters). These models are: 1) a linear circuit that transmits light signals to
237 eS6-P (Fig. 3A, top panel), 2) an incoherent feedforward loop (IFFL) that produces an early day peak similar
238 to what the Clock+Light model does under LD condition (Fig. 3A and B, middle panels), and 3) a coherent
239 feedforward loop (CFFL) that allows slow decline of eS6-P upon the withdrawal of light signals (Fig. 3A,
240 lower panel). Note that none of these three models were able to fully fit the observed data (e.g. Fig. 1D)
241 due to the lack of clock regulation. Rather than evaluating these models in terms of their fit to data, we
242 focused on their performance in terms of detecting daylength variations upon dawn.

243

244 **Fig. 3. Response of alternative models of eS6-P circuit to variations of the night to day transition time.**
245 **(A)** Influence diagrams of the three alternative models of eS6-P circuit. In each diagram, the regulatory

factors are indicated by the lettered black circles, and regulatory interactions are denoted by colored lines (blue = activation, red = repression). Right panels show simulation trajectories with these models under the LD condition. Gray regions show the period of night. **(B)** Trajectories of eS6-P in response to variations of the night to day transition time from Δt_{ND} = -4 (purple) to Δt_{ND} = 4 (yellow) in 0.5-hour increments. All trajectories start at the dusk of the previous day (Day 0, unperturbed), and end at the dusk of the current day (Day 1, perturbed). Dotted lines show trajectories in the absence of light. Thick solid curves show trajectories in the early day (first four hours after dawn), and thin solid curves show trajectories in the remaining hours of the day. Short line segments at the bottom show the time of dawn for each trajectory. **(C)** Left: peak metric (ratio of the early day maximum of eS6-P to the eS6-P levels at the dusk) with respect to the perturbations of the daylength. Right: activation time of the eS6-P after dawn with respect to the perturbations of the daylength. Activation time is defined as the time for eS6-P to reach $x_0 + 0.9(x_s - x_0)$, where x_0 is the eS6-P level at the dawn and x_s is the eS6-P level at the dusk.

Each of the three alternative models generated a constant level of eS6-P upon the perturbations of daylength (Fig. 3B cyan). Particularly, the early day peak produced by the IFFL model did not distinguish short and long daylengths (Fig. 3B middle). This insensitivity to daylength variations with the alternative models was reflected in the stable maximum early responses of eS6-P regardless of their ratios to steady state (or end-of-day) eS6-P levels (Fig. 3C left). In addition, we found that the daylength-sensitive early response with the Clock+Light model was anticorrelated with the time for eS6-P to reach its steady state level, e.g. an early dawn accelerated the response of eS6-P (Fig. 3C right). These results suggest that detection of long daylength with early day peaks is a feature that requires the integration of the circadian clock and the clock-independent light-sensing pathway.

268 Because realistic perturbations of daylength may occur through changes of light conditions at both dusk
269 and dawn and on multiple days, we next considered the effects of multiple variations in our simulations.
270 We first allowed -/+ 2 hours of variation in t_{ND} followed by +/-2 hours of variation in the day-to-night
271 transition (t_{DN}) during dusk the previous day (Fig. 4A). Perturbations in each t_{DN}-t_{ND} pair acted in the same
272 direction to either lengthen or shorten the intervening night (Fig. 4A and B, vertical lines). With the
273 Clock+Light model, we found that consecutive changes in dusk and dawn timing gave rise to significant
274 variability in eS6-P response, similar to what was obtained with -/+ 4 hours of t_{ND} alone (Fig. 2B, Fig. 4B
275 and C). Interestingly, +/- 2 hours perturbations of t_{ND} or t_{DN} alone did not generate an early day peak (Fig.
276 4B and C). This suggests that changes in the timing of dusk can be integrated into the eS6-P response by
277 the Clock+Light circuit after dawn and serve as a predicting factor for the current daylength. The
278 incorporation of information in the previous day shows that the Clock+Light circuit can anticipate the
279 daylength changes with a memory capacity.

280 We next examined the effect of changes to t_{ND} on two consecutive days. We observed a slightly increased
281 dynamic range of early eS6-P peak on the second early day compared to the first day with the Clock+Light
282 model (Fig. S5). These results indicate that the Clock+Light circuit was able to detect changes in daylength
283 beyond the altered timing of the current dawn.

284

285 **Fig. 4. Response of eS6-P to variations in night to day transition time following changes of day to night**
286 **transition time the previous dusk. (A)** Diagram of perturbations of the day to night transition and the
287 night to day transition on two consecutive days. The first (perturbed at dusk, Δt_{ND} = -/+ 2 hours) and
288 second days (perturbed at dawn, Δt_{ND} = +/- 2 hours) are labeled. The day period with light is shown by
289 yellow shaded regions and the maximum extent of the change in daylength is shown by differential
290 shading. **(B)** Trajectories of eS6-P in response to variations of the day to night transition time and/or the

night to day transition time from Δt_{ND} = -2, Δt_{DN} = 2 (purple) to Δt_{ND} = 2, Δt_{DN} = -2 (yellow) in 0.25-hour increments. Short line segments at the bottom show the time of dawn or dusk for each trajectory. Upper: dawn perturbation only. Middle: dusk perturbation only. Bottom: both perturbations. **(C)** Peak metric (ratio of the early day (Day 1) maximum of eS6-P to the eS6-P levels at the dusk) with respect to the perturbations of the daylength under various perturbation scenarios.

Integration of circadian clock and light-sensing pathway detects gradual variations of light-dark cycles

We next asked whether the eS6-P circuit responds to progressive, long-term variations of the light-dark cycle, reflective of seasonal changes in the cycle throughout a year. We used a Solar Calculator provided by the National Oceanic and Atmospheric Administration (NOAA) to generate a data set of 'ideal' (i.e. unaffected by transient local changes) daylengths over the course of a year. We simulated the Clock+Light model based on these light-dark cycles for one year at two locations representing the extreme latitudes of the *A. thaliana* distribution, Oslo, Norway, and Praia, Cape Verde (29). As expected from the analysis of transient changes in dawn timing, our model showed a sensitivity of eS6-P to changes in daylength over the year (Fig. 5). The range of eS6-P was correlated with the degree of variation in daylength, which was broader in Oslo (6.2 to 19.0 hours, Fig. 5A) compared to Praia (11.3 to 13.0 hours, Fig. 5B). We observed variations in both the early-day maximum and early-day minimum levels of eS6-P in Oslo (Fig. 5A, orange and pink), with the early-day maxima increasing dramatically as daylength approached its yearly maximum in the summer. Starting from Day 89 of the year, the early-day maxima increased until it reached 148% of the daily steady state at Day 170. As such, we conclude that changes in the daylength over a year are able to the variations in the early-day peak of eS6-P, with the early day maxima exceeding the daily steady state level around 13.5 hours of light. In contrast, much smaller variations of the early-day eS6-P, which never exceeded the daily steady state levels, were observed with the yearly light-dark

314 cycle data in Praia (Fig. 5B), which has a maximum daylength of 13.0 hours. These results suggest that the
315 eS6-P circuit can detect seasonal changes of the light-dark cycles by varying the magnitude of the
316 responses after the dawn and thus is sensitive to changes in daylength even when they occur gradually.

317

318 **Fig. 5. Simulated amounts of eS6-P in response to seasonal changes in daylength over a year. (A)**
319 Simulation of eS6-P over a full year using daylength data for Oslo, Norway (the NOAA Solar Calculator).
320 The left figure shows the eS6-P time course over the full year (gray), overlaid with the early-day maximum
321 (green), early-day minimum (pink), and daily steady state (end-of-day, or dusk) level (black). Peak metric
322 (orange) is defined as the ratio of early-day maximum to the daily steady state level. The right figure shows
323 the eS6-P profiles of individual days selected every two weeks from Day 7 to Day 175 of the year. Time 0
324 is the actual dawn of each day. The color of each curve corresponds to the week number and is correlated
325 with the daylength from short (yellow) to long (purple). Triangles indicate the time at which the end-of-
326 day eS6-P level was measured, and blue dots indicate the position of the early-day peak. **(B)** Simulation of
327 eS6-P across a full year using daylength data for Praia, Cape Verde (the NOAA Solar Calculator). Upper and
328 lower figures are as described in **(A)**. The globe on the left of each panel shows the latitude of the position
329 that the daylength was simulated for.

330

331 We next asked whether the eS6-P circuit can detect changes in light-dark cycles due to transient changes
332 (such as weather) as well as seasonal changes. We obtained a year-long environmental radiometry data
333 from Harvard Forest (30). We normalized the measurement of downward photosynthetic radiation based
334 on the ideal daylength calculations for Boston, Massachusetts from NOAA and obtained a time-series data
335 of realistic light-dark cycles over a year (see Methods). Briefly, a threshold value was used to define
336 day/night with the Harvard Forest data, and the value was chosen to minimize the mean difference

between the inferred hours of daylight and NOAA estimations of daylength. We compared the simulation results for idealized and observed daylength data (Fig. 6) With the idealized daylengths in Boston (9.4 to 15.4 hours), we observed moderate early day peaks in the middle of the year (Fig. 6A). However, transient changes in the observed light data resulted in changes in daylength of up to 1.5 hours, which in turn gave rise to significant variation of early-day peak of eS6-P in response to the changes of sunlight due to daily weather in addition to seasonal changes of sunlight (Fig. 6B). Although transient changes mainly reduced the daylengths from the idealized day lengths throughout the year, there is a significant difference in terms of peak metric between longer (>13 hours) and shorter (<13 hours) days based on the realistic sunlight data (1.01 vs. 0.96. Welch's t-test, $p = 2.7\times10^{-34}$). Furthermore, the absolute difference in early-day peak between idealized and realistic days was greater during longer days (> 13 hours) than during shorter days (< 13 hours) by 10-fold (Welch's t-test, $p = 7.5\times10^{-32}$). As such, these weather induced changes primarily exist in long days, during which the early response of eS6-P is most sensitive to daylength changes. To further illustrate this point, we compared in simulated early day peaks between idealized and realistic days: in both simulations the peak to steady state ratio remained low during short days and increased around a daylength of 13 hours, but the ratios of the two simulations diverged as days grew longer (Fig. 6C). Together, these results show that the eS6-P circuit can detect variations in light-dark cycles when both seasonal and local, transient (weather) changes are considered.

Fig. 6. Simulated amounts of eS6-P in response to transient and seasonal changes in daylength over a year. (A) Simulation of eS6-P across a full year using daylength data for Boston, Massachusetts (the NOAA Solar Calculator). The left figure shows the eS6-P time course over the full year (grey), overlaid with the early-day maximum (green), early-day minimum (pink), and daily steady state (end-of-day, or dusk) level (black). Peak metric (orange) is defined as the ratio of early-day maximum to the daily steady state level. The right figure shows the eS6-P profiles of individual days selected every two weeks from Day 7 to Day

175 of the year. Time 0 is the actual dawn of each day. The color of each curve corresponds to the week number and is correlated with the daylength from short (yellow) to long (purple). Triangles indicate the time at which the end-of-day eS6-P level was measured, and blue dots indicate the position of the early-day peak. **(B)** Simulation of eS6-P over a year using the full year daylength data derived from Harvard Forest radiometry data [26]. The eS6-P time course over the full year (grey) is overlaid with the early-day maximum (green), early-day minimum (pink), and daily steady state (end-of-day, or dusk) level (black). Peak metric (orange) is defined as the ratio of early-day maximum to the daily steady state level. **(C)** The average peak metric (ratio of the early day maximum of eS6-P to the eS6-P levels at the dusk) for NOAA (open circle) and Harvard Forest (closed circle) days binned according to their realistic daylength (every half hour from 8 to 15 hours). Error bars indicate the standard deviation of Harvard Forest days in each bin. The color of the points and bars is correlated with daylength from short (yellow) to long (purple). The globe on the left shows the latitude of the position that the daylength was simulated for.

The sensitivity of early eS6-P responses to daylength variations raises a question whether this detection of daylength variation is robust with respect to fluctuations of light conditions due to, for example, temporary shading by taller plants, and well as fluctuations in molecular concentrations. We therefore introduced high frequency white noise to the variables describing either the amount of transmitted light or eS6-P itself. We used mutual information to quantify the signal transmitted from varied daylength to the early eS6-P response. We found that more than 50% of the mutual information (compared to the noise-free condition with a finite number of bins) was retained (>1 bit) in the presence of significant fluctuations of light or eS6-P (amplitude parameter $\mu = 5$, or about 23% coefficient of variation in light signal) (Fig. S6). This result suggests that the system is robust with respect to the rapid fluctuations of light or molecular concentrations, while it maintains its capacity to detect daylength variations. Intuitively, the peak of eS6-P in long days is driven by a clock-based, slowly increasing trajectory starting from night, and

385 this slow dynamics serves as a signal integration, or averaging method, to reduce the effect of high
386 frequency noise.

387

388 **Dynamical features of the eS6-P circuit represent expression profiles of a large number of genes**

389 Because many genes in *A. thaliana* are co-regulated by the circadian clock and light-dark cycles, we
390 hypothesized that the dynamic features of eS6-P can be observed in the expression patterns of other
391 genes. To identify genes whose expression may resemble the profile of eS6-P, we reanalyzed an *A.*
392 *thaliana* cyclic gene expression data set reported by Dalchau et al. (8), which is based on previously
393 published microarray data with expression patterns of *A. thaliana* genes under LD and LL conditions (6,
394 31, 32). By calculating the phase shift of the peak expression between measurements under LD and LL
395 conditions, we identified 126 genes where peak expression appears in the early day under LD (0-6 hours
396 after dawn) and regressed into the night under LL condition (18-24 hours after dawn), emulating the clock
397 driven sensitivity that we observed in our eS6-P model and the underlying experimental data (see
398 Methods). We next reanalyzed our previously published RNA sequencing data for *A. thaliana* genes under
399 clock-deficient condition (CCA1 overexpression) (10), and further refined the list of 126 genes by selecting
400 those that have peak expression during the day and have significantly higher expression during the day
401 than during the night under the clock-deficient condition (see Methods). With these selection criteria
402 based on the two data sets (Dalchau et al. and Missra et al. (8, 10)), we have identified 92 genes of which
403 time course expression profiles are similar to that of eS6-P (Table S3). As expected, genes identified in this
404 manner exhibited expression patterns qualitatively similar to eS6-P dynamics (Fig. 7).

405

406 **Fig. 7. Genes in *A. thaliana* with eS6-P like expression patterns. (A)** The distribution of the peak phase
407 shift between LD and LL conditions for 92 genes for which phases of expression profiles are similar to

408 those of eS6-P (blue). Equivalent phase shifts for all cyclic genes in the list generated by Dalchau et al. (8)
409 are in gray. **(B-D)** The mRNA expression patterns of 92 genes with phases of expression profiles similar to
410 those of eS6-P for wildtype under LD (B), wildtype under LL (C) and CCA1-overexpression under LD (D)
411 conditions. Time course expression profiles of individual genes are shown as colored curves while the solid
412 black line shows the average expression of all 92 genes. The dotted black line is the average expression
413 pattern of all cyclic genes in the list generated by Dalchau et al. (8). Expression values in all panels were
414 normalized from 0 to 1, such that 0 corresponds to the minimum and 1 correspond to the maximum for
415 each individual gene. **(E)** Distributions of the difference in average mRNA expression level between day
416 and night of 92 eS6-P like genes (blue) and all cyclic genes in the list generated by Dalchau et al. (8) (gray)
417 under CCA1 overexpression condition. Positive values indicate greater average expression during the day
418 and negative values indicate greater average expression during the night.

419

420 Dalchau et al. (8) classified genes with cyclic expression patterns into three categories based on whether
421 the clock or the light-dark cycle dominates the amplitude of the oscillation in mRNA levels: clock-dominant,
422 light-dominant and co-regulated. Note that all three types of genes can be influenced by both the clock
423 and the light-dark cycles, and the category 'co-regulated' here has a more stringent definition in terms of
424 the amplitudes (see Methods). Not surprisingly, the time course profile of eS6-P is categorized as 'co-
425 regulated' based on the definition of Dalchau et al. (see Methods). We found that out of the 92 eS6-P like
426 genes, 66 are classified as 'clock-dominant', while 25 are 'co-regulated' and only 1 is 'light-dominant',
427 suggesting the clock plays a prime role in generating this pattern of expression.

428 We next performed gene ontology (GO) enrichment analysis with the 92 eS6-P like genes using the set of
429 all annotated genes in *A. thaliana* as a background. We found that eS6-P like genes genes are enriched for
430 light response, photosynthetic regulation, and chloroplast localization, as expected from their

431 responsiveness to light (Table S4). Many of the same biological process and cellular compartment terms
432 are also enriched amongst the set of all genes that show cyclic expression patterns (Table S5). However,
433 the enrichment of specific molecular functions regarding memebrane transport (zinc, ferrous iron, and
434 protons) and activities (ATPpase and thioredoxin-disulfide reductase) is unique to eS6-like genes,
435 suggesting they represent a more specialized subset of the light-clock regulated genes. Overall, this
436 suggests that eS6-P like behavior, a photoperiod sensitive shift in peak activity around dawn, is associated
437 with specific light-dependent metabolic functions.

438 These results suggest that the dynamics of eS6-P may represent a broad range of transcriptional, post-
439 transcriptional and post-translational activities in *A. thaliana,* particularly in association with light driven
440 and responsive metabolic functions. While eS6-P is co-regulated by both the light and clock, the phase
441 variation in our model is driven by the clock, and this is consistent with the observation that this pattern
442 is associated with many clock-dominant genes. The similarity of dynamical features among these cellular
443 activities does not indicate a causal relationship, but it raises the possibility that the daylength detection
444 and anticipation features of such dynamics may be used by a large system of molecules in *A. thaliana*. This
445 suggests that the cyclic phosphorylation of eS6 may serve as a key signaling factor or an effector
446 integrating ribosomes into this detection and anticipation system.

447 **Discussion**

448 **Robustness and sensitivity of the clock with respect to light conditions**

449 In this study, we built a mathematical model that describes the dynamics of eS6-P, a ribosomal post-
450 translational modification that occurs in many eukaryotic species. A feedforward regulatory loop connects
451 light signals to eS6-P through a clock dependent pathway and a clock independent pathway. We found
452 that the response of eS6-P is sensitive to the changes of the light-dark cycles, e.g. the daylength variations
453 that are reflected in the time of the dawn. The circuit therefore enables cells to detect and anticipate such
454 variations which may result from changes in season and/or transient changes in weather. We showed that
455 the circadian clock plays important roles in this information processing function. This feature is different
456 from the well-known function of the clock, which ensures robust anticipation of light-dark cycles even
457 under fluctuating light conditions (33, 34). Our study shows that the clock can be used to robustly detect
458 the variations of light conditions at the beginning of a day, which is a function in addition to its traditional
459 role in generating stable rhythmic cues that counteract environmental fluctuations. The circuit achieves
460 this because light and clock signals synergize with each other only at the early phase of the light-dark
461 cycles. This property of the circuit adds to the remarkably diverse ways that the clock may be used by
462 organisms. Furthermore, it might be a fitness advantage for plants to stabilize one group of molecular
463 activities with the clock, while making other activities sensitive to the light conditions using the clock as a
464 reference.

465

466 **Physiological functions of external coincidence**

467 Although the sensitivity of eS6-P to light around dawn has not been investigated comprehensively,
468 experimental data showed that eS6-P rises quickly in response to light (18, 22), as anticipated by our
469 model. The general dynamical feature of eS6-P as a result of clock-light signal integration is consistent

470 with other known signaling events in which the coincidence of internal, clock-derived signals and external
471 light signals drives prominent peaks of molecular activities in plants (8, 9, 35). For example, according to
472 the classic 'external coincidence' mechanism for photoperiodic (seasonal) flowering, the clock drives up
473 the expression of the regulator of flowering, CONSTANS, late in the day. In long-day plants, if CO happens
474 to be exposed to light late in the day, CO is activated and triggers flowering, whereas if CO is met by
475 darkness, as is the case in the winter, flowering remains suppressed (9). Thus, the external coincidence
476 model allows plants to detect variation in photoperiod at the end of the day. Our work suggests another
477 implementation of external coincidence, where variation in light conditions is detected at the beginning
478 of the day by sensing photoperiod sensitive shifts in the phase of circadian genes(26, 28). For CO at the
479 end of the day, coincidence of clock and light signals activates CO while darkness is incoherent with the
480 clock, and represses CO. For comparison, according to our model eS6-P is most sensitive to changes in
481 light conditions at the beginning of the light period because the negative effect of the clock and the
482 potentially positive effect of light are incoherent. We propose that eS6-P helps the plant to sense variation
483 in the onset of light, as a result of cloud cover or shading by other objects in the morning, and adjust its
484 physiology accordingly.

485

486 **Versatile performance objectives of feedforward loops**

487 Previous studies have demonstrated multiple functions of feedforward loops in terms of systems
488 dynamics, including accelerating responses, fold-change detection, adaptation to constant signals and
489 filtering noise (36-39). In addition, it was shown that a feedforward loop can perform a 'counting' function
490 that transforms oscillatory input to stable signals (40). Our study shows a previously underappreciated
491 function of a particular type of feedforward loop containing an intrinsic autonomous oscillator. This
492 system not only detects the phase and period variations of the oscillatory inputs by combining the signals

493 from the external and internal oscillations, but also memorizes the altered response in the following cycles,
494 which may serve to anticipate additional perturbations. The latter feature may be useful for plants to
495 predict weather through encoding the information in the light conditions of the previous day. This result
496 further demonstrates the versatility of the functions of the feedforward loop. Future work is needed to
497 determine whether this phase-detection mechanism can be integrated with other known functions of
498 feedforward loops.

499

500 **Anticipation of changes in environmental conditions**

501 Phosphorylation of eS6 occurs in all organisms where it has been examined, including yeast, plants, and
502 humans (12-14). A previous study showed dramatically increased translation activity in mammalian cells
503 with a phosphorylation-deficient eS6, suggesting a possible role of eS6-P in controlling general resource
504 allocation in cells (15). If this potential function of eS6-P were conserved across kingdoms, then our work
505 would further suggest that eS6-P might tune some aspect of protein synthesis in response to variable light
506 condition. This function, while still to be demonstrated in plants, would not be far-fetched given that
507 translation requires a substantial input in cellular energy, which may be depleted at the end of night. This
508 detection and anticipation of daylength may help plants to prepare for days with particular patterns of
509 light exposures. It has been shown that growing yeast cells can allocate resources to anticipate favorable
510 or unfavorable environmental conditions, and the choice of these anticipations may be made with
511 rhythmic dynamics (41, 42). Regardless of the specific cellular functions of eS6-P, our work sheds light on
512 the rich dynamics of eS6-P under fluctuating environmental conditions. Moreover, the remarkable
513 information processing characteristic of the signaling circuit that controls eS6-P has the capacity to detect
514 and memorize critical cues to allow plants to adapt to a dynamical light environment.

515

516 **Methods**

517 **Framework of mathematical models**

518 We modeled gene regulatory networks that control eS6-P using ordinary differential equations (ODEs).
519 The network topologies are shown in Fig. 1. Because most interactions involving high-order molecular
520 interactions, including transcriptional regulation and multisite phosphorylation, we used a generic form
521 of nonlinear ODEs suitable for describing both gene expression and molecular interaction networks (43-
522 46). Each ODE system in the model has the form:

524 $$\frac{dX_i}{dt} = \gamma_i(F(\sigma_i W_i) - X_i)$$

525 $$F(\sigma W) = \frac{1}{(1 + e^{-\sigma W})}$$

526 $$W_i = (\omega_i^o + \sum_j^N \omega_{j\to i} X_j)$$

523 $$i = 1, \ldots, N \qquad (1)$$

527 Here, X_i is the activity of protein i. On a time scale $1/\gamma_i$, $X_i(t)$ relaxes toward a value determined by the
528 sigmoidal function, F, which has a steepness set by σ. The basal value of F, in the absence of any
529 influencing factors, is determined by ω_i^0. The coefficients $\omega_{j\to i}$ determine the influence of protein j on
530 protein i. N is the total number of proteins in the network. Activity values are scaled from 0 (absent) to 1
531 (saturation). For eS6-P, we modeled a single site phosphorylation (S237) that was assumed to be
532 independent of other phosphorylation sites, so we used first order reaction rate law that describes the
533 phosphorylation and dephosphorylation. All variables and parameters are dimensionless. One time unit
534 in the simulations corresponds to approximately 2.53 hours (9.5 unit per cycle), but we transformed the
535 time unit to 1 hour for all subsequent analyses and visualizations.

536 The clock component of our model describes four species (LHY/CCA1, EC, PRR9/7, and PRR5/1), and the
537 light-sensing pathway has two species (TOR and S6K). Together with eS6-P, our full Clock+Light model has
538 seven ODEs in total. Numerical solutions to the ODEs were obtained with Tellurium (47). Computer code
539 for simulating the models under various conditions and reproducing key figures is available at:
540 https://github.com/panchyni/eS6_Models .

541

542 **Parameter estimation**

543 Because the clock component of the model is independent of other elements except for the light input,
544 we first fit the clock components of the model (4 ODEs) by applying qualitative criteria to ensure that it
545 properly replicated features of the circadian clock [18]. We approached optimization the same way as in
546 Locke at al. (5) and De Caluwe et al. (48) by using objective functions which impose costs on deviating from
547 expected, qualitative behavior. Specifically, we penalized the model for (1) having the LHY/CCA1
548 component peak more than 1 hour before or after dawn in long (16L:8D) days, (2) having the same
549 deviation described in (1) in 12L:12D days, (3) having a period under constant light outside of 24 to 25
550 hours, (4) having a period under constant darkness outside of 24 to 28 hours, and (5) having an amplitude
551 of less than 0.1 (i.e. 10% of maximum activity) in any component. These criteria defined by intervals of
552 desired output were used to construct an objective function to evaluate the models. When calculating the
553 objective score, each of the five terms is zero if the output falls in the interval and is the squared difference
554 between the output and the boundary of the interval if the output falls outside the interval. Before
555 optimizing the model parameters, we defined a hyperbox in the parameter space that is bounded by
556 biologically plausible parameter ranges, particularly with regard to the direction of regulation and balance
557 of rates. A population of 40 vectors, each containing 24 parameters, generated by Latin hypercube
558 sampling (LHS), were used as the initial estimation. Starting with this population, we implemented

559 Differential Evolution (DE) optimization algorithm (49, 50) with a mutation rate between 0.35 and 0.65
560 and a crossover rate of between 0.75 and 0.95, and let the optimization run for 15000 generations of DE
561 or the convergence of the evolution. Similar to the approach used by De Caluwe et al. (48), we then
562 compared each component to expression data from Diurnal Database (27) which were normalized onto a
563 0 to 1 scale to ensure the estimated component activity approximated the proper phase and shape of
564 actual clock components (Fig. S2). We performed 250 runs of such optimization to ensure that the
565 performance of the selected parameter set can be reproduced. For fitting eS6-P, we selected among viable
566 models which accurately reflected clock behavior and selected the one with the greatest dynamic range
567 of among the clock components.
568
569 Next, we used the time course measurement of eS6-P to estimate the parameters controlling the
570 regulation of eS6-P by clock components (LHY/CCA1, EC, PRR97, and PRR51 which correspond to C1, C2,
571 C3, and C4 respectively) and the light induced TOR pathway (18). The data set has Western Blot
572 quantification of eS6-P for 78 hours at a 6-hour interval. In the model, the eS6-P variable describes the
573 percentage of the amount of eS6-P with respect to the total eS6. We therefore inferred the fractions of
574 eS6-P from the Western Blot quantification of the experiment and scaled these values based on an
575 approximation of the eS6-P saturation value inferred from Williams et al. (51). 2D-gel electrophoresis data
576 from this study suggest that a majority, but not all eS6 undergoes phosphorylation and that
577 phosphorylation spreads across several sites which may or may not be phosphorylated at the same time
578 (see Fig. 4 and Table 1 in (51)). As the data were not quantified in the original study and estimations based
579 on image analysis were broad (15-77% per site), we chose a conservative estimate of 0.40 as the saturating
580 value. This normalization gives the raw data a range of 0.01 to 0.4 and pooled data (described below) a
581 range of average values from 0.06 to 0.31. This procedure allows us to avoid unrealistic assumptions with
582 either no phosphorylated (eS6-P = 0) or fully phosphorylated (eS6-P = 1) in the system. Because these

583 values are approximate and mainly meant to avoid fitting to unrealistic, extreme values of eS6-P, the
584 model describes eS6-P in arbitrary units (a. u.) rather than actual percentages.

585 We regularized the transformed data by pooling the data points from the same Zeitgeber time, and we
586 used the pooled mean and variance for each time point to calculate the log likelihood. Constant light was
587 emulated in the model by fixing the value of light to 1 and the CCA1 overexpression by fixing the basal
588 production of CCA1 to 50. To examine steady state behavior under each condition, we entrained the
589 model with one day of darkness followed by ten days of the given condition, before comparing the model
590 to the experimental data on the twelfth day. The objective function for the parameter optimization was
591 defined by the log-likelihood function that quantifies the fit of the simulation results to the experimental
592 data with variabilities. Log likelihood estimates for all time points of eS6-P contribute to the objective
593 function equally in an additive manner. As with clock components, we used the DE based optimization
594 algorithm to estimate the remaining parameters. All algorithmic parameters were the same as the
595 optimization for the clock component, expect for the maximum number of generations (5000). We
596 performed 1400 optimization runs, and among the optimized parameter sets we selected the best
597 preforming model with a moderate peak to perform subsequent analyses. Note that the majority of the
598 models in the lowest 5th percentile of likelihood values exhibited the same behavior as the chosen model,
599 including cycling under all three experimental conditions and an early eS6-P peak under LD conditions (Fig.
600 S3A). Models with larger likelihood scores (the next 5 percentiles) show neither this earlier peak nor
601 cycling under constant light (Fig. S3B). A full list of model parameters can be found in Table S1.

602

603 **Applying a detailed clock to our eS6-P model**

604 In addition to our simplified 4-component clock model, we also tested our eS6-P model using a more
605 detailed circadian clock component from De Caluwe et al. (48), which has the same overall network

606 topology but explicitly considers protein and mRNA concentrations (Fig. S4). We added our equation for
607 eS6-P regulation to this full clock model, and used the set of same optimized parameters that we obtained
608 from the simplified model to simulate eS6-P with the light-dark cycle. The CCA1 constitutive
609 overexpression mutant was simulated by removing the clock and light regulated components from the
610 equations for CCA1/LHY transcription (i.e. setting the production rate to maximum). We used the protein
611 concentration of each pair of clock components, representing the mean protein concentration of the two
612 gene products, in place of our generalized clock component activity values. We scaled the concentration
613 values to a range of [0,1] using the minimum and maximum values of that protein pair. However, in the
614 case of CCA1 overexpression, we assumed that the concentration is effectively saturated at the maximum
615 wild-type value, as the average value of CCA1/LHY and PRR9/7 increased by more than five-fold. All
616 parameters for eS6 regulation were the same as in the model in Fig. 1, except for the regulatory effect of
617 the TOR pathway which was increased by 10% to avoid an unrealistic saturation point of eS6-P under wild-
618 type and CCA1 overexpression conditions.

619

620 **Alternative models**

621 To illustrate the unique performance of the Clock+Light model that contains the clock component, we
622 built three alternative models that describe plausible ways in which the eS6-P can transmit the light signal
623 to achieve detection of daylength variations at the beginning of a day. Note that it is trivial for a system
624 to detect daylength variations at the end of a day (e.g. through integration of, or slow response to, light
625 signals), and this detection may not be as useful as the early day detection in terms of anticipating
626 environmental changes. The three alternative models are: 1) a linear circuit that transmits light signals to
627 eS6-P (Fig. 3A, top panel), 2) an incoherent feedforward loop (IFFL) that produces an early day peak similar
628 to what the Clock+Light model does under LD condition (Fig. 3A and B, middle panels), and 3) a coherent

629 feedforward loop (CFFL) that allows slow decline of eS6-P upon the withdrawal of light signals (Fig. 3A,
630 lower panel). Parameter values of these models were manually chosen to give characteristic responses
631 (Fig. 3A, Table S2).

632

633 **Mutual information between daylength variations and eS6-P responses**

634 To examine the transmitted information from daylength variations (signal) to eS6-P abundance (response)
635 when the system is subject to external or internal noise, we introduced uncorrelated multiplicative white
636 noise to ODEs for the light and eS6-P as $dx/dt = f(x_1, x_2, \ldots, x_n) + \mu_x \cdot x \cdot dW$, where dW is a Wiener
637 process that can be discretized as $dW \sim N(0,1) \cdot \sqrt{dt}$ where $N(0,1)$ denotes a normally distributed
638 random variable with zero mean and unit variance, and μ_x represents the amplitude of the fluctuation.
639 To quantify the information transmission, we used mutual information between signal S and response R
640 (52). In the eS6-P system, S represents the perturbation of the time (Δt_{ND}) at which the system receives
641 light signal from a normal light-dark cycle (e.g. 12hr light and 12hr dark), and R represents the eS6-P
642 abundance. The responses were the maximum eS6-P levels in the 4-hour time window after dawn. The
643 calculation of the mutual information was performed with the discretized form (53):

644 $$I(S;R) = -\sum_{i}^{S_B} \frac{k_{s,i}}{N_T} \log \frac{k_{s,i}}{N_T} + \sum_{j}^{R_B} \frac{k_{r,j}}{N_T} \sum_{i}^{S_B} \frac{k_{i,j}}{N_T} \log \frac{k_{i,j}}{N_T} \quad (2)$$

645 Here, signals values were assigned to S_B bins, and the response values were assigned to R_B bins. By
646 binning all signals and responses, we constructed a contingency matrix of which each entry is the number
647 of observations from the simulated data that correspond to that particular signal-response pair. N_T is the
648 sum over all entries in the table, and $k_{i,j}$ is the number of instances of signal i that resulted in response j.
649 In this study, 21 signal bins and 10 response bins were used. Using more response or signal bins will likely
650 increase the mutual information, so our analysis focuses on comparisons of lower bounds of the mutual

information. The bounds of the response bins were determined by the minimal and maximal responses in the absence of the noise. For each signal value, 200 simulations were performed.

Photoperiod data processing

We obtained a copy of the NOAA Solar Calculator (https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html) and generated approximated day length information (i.e. Sunlight Duration) for a full year for three locations: Oslo, Norway (59.91 Latitude, 110.75 Longitude), Praia, Cape Verde (14.92 Latitude, -23.51 Longitude), and Boston, Massachusetts (42.35 Latitude, -71.05 Longitude). For inclusion in our model, we fit a model of day-night variation to each data set with the following form:

$$L = a + b \cdot \sin\left(\frac{2\pi d}{365} - c\pi\right) + \sin\left(\frac{2\pi t}{T}\right) \tag{3}$$

where the rightmost sine term represents a base 12-hr light/dark cycles and the leftmost sine term modulates this average day based on the time of the year. Here, d is the day of the year, t is the time of day in hours and T is the period of the day in hours. The parameters a, b, and c, are fit such that the fraction of daylight ($L > 0$) conforms to the NOAA estimations for each day (values for each location are listed in Table S6). With this idealized year-long data, the time for analysis of daily response is based the natural light condition rather than any artificially defined time. We also obtained measurement of radiation data in the Harvard Forest (30) including downward oriented photosynthetically active radiation (par.down), which we used as an approximation for daylight. A threshold value (9.0) was used to define day/night using par.down and was chosen such that it minimized the average difference between the inferred hours of daylight and NOAA estimations of day length across all days binned by month. For inclusion in our model, we smoothed radiation data from 2006 (which had the fewest missing values) using cubic splines and applied the threshold to generate binary day/night values.

673

674 **Identification of genes with time-series expression profiles similar to eS6-P dynamics**

675 To identify genes that show similar cyclic patterns to eS6-P dynamics under LD, LL and CCA1
676 overexpression conditions, we first selected genes identified by Dalchau et al. (8) where the peak
677 expression in long-day was during the early day (ZT 0-6 hours) but the peak expression in constant light
678 was during subjective night (ZT 18-24 hours). There were 126 genes that satisfy these conditions. We then
679 used the RNA sequencing data from Missra et al. (measurement under CCA1 overexpression condition) to
680 further filter these genes. We selected genes that had both peak expression during the day, and a
681 difference between average expression during the day and night of at least 25% of the peak value under
682 CCA1 overexpression condition. To visualize the time course profiles of these genes, we used data from
683 Diurnal Database for LD (27), Edwards et al. for LL (32), and Missra et al. for CCA1 overexpression (10).

684 For gene ontology (GO) analysis, we used all 92 eS6-P like genes and tested for enrichment against all *A.*
685 *thaliana* genes using PANTHER ((54); available through http://geneontology.org/). The same procedure
686 we used when testing all genes identified by Dalchau et al.(8). *p*-values were calculated using the Fisher's
687 exact test and multiple test correction was done using Benjamini-Hochberg method with a false discovery
688 rate threshold of 0.05.

689 Dalchau et al. classified the genes with cyclic expression patterns into three categories: light-dominant,
690 clock dominant and co-regulated (8). To examine which category the eS6-P profile would belong to, we
691 used the same approach to classifying light/clock regulation as Dalchau et al. by comparing the ratio of
692 eS6-P amplitude driven by the clock (constant light) to that driven by the light-dark cycle. Briefly,
693 amplitude difference between LL condition and LD condition was used to determine whether light or clock
694 dominates the regulation of each gene. With our eS6-P data, the amplitude under the LD condition is
695 higher than that under LL condition by 2.17 fold (difference in Bode magnitude = -3.3 decibels, dB), which

696 categorizes eS6 phosphorylation as a process co-regulated by light and clock according to the cutoffs
697 (±7dB difference in Bode magnitude), although light regulation is favored.

698

699

700 **Author Contributions**

701 Designed research: TH. Performed research: NP and TH. Analyzed data and wrote the manuscript: NP,
702 AGV and TH.

703

704 **Conflict of Interest**

705 The authors declare no conflict of interest.

706

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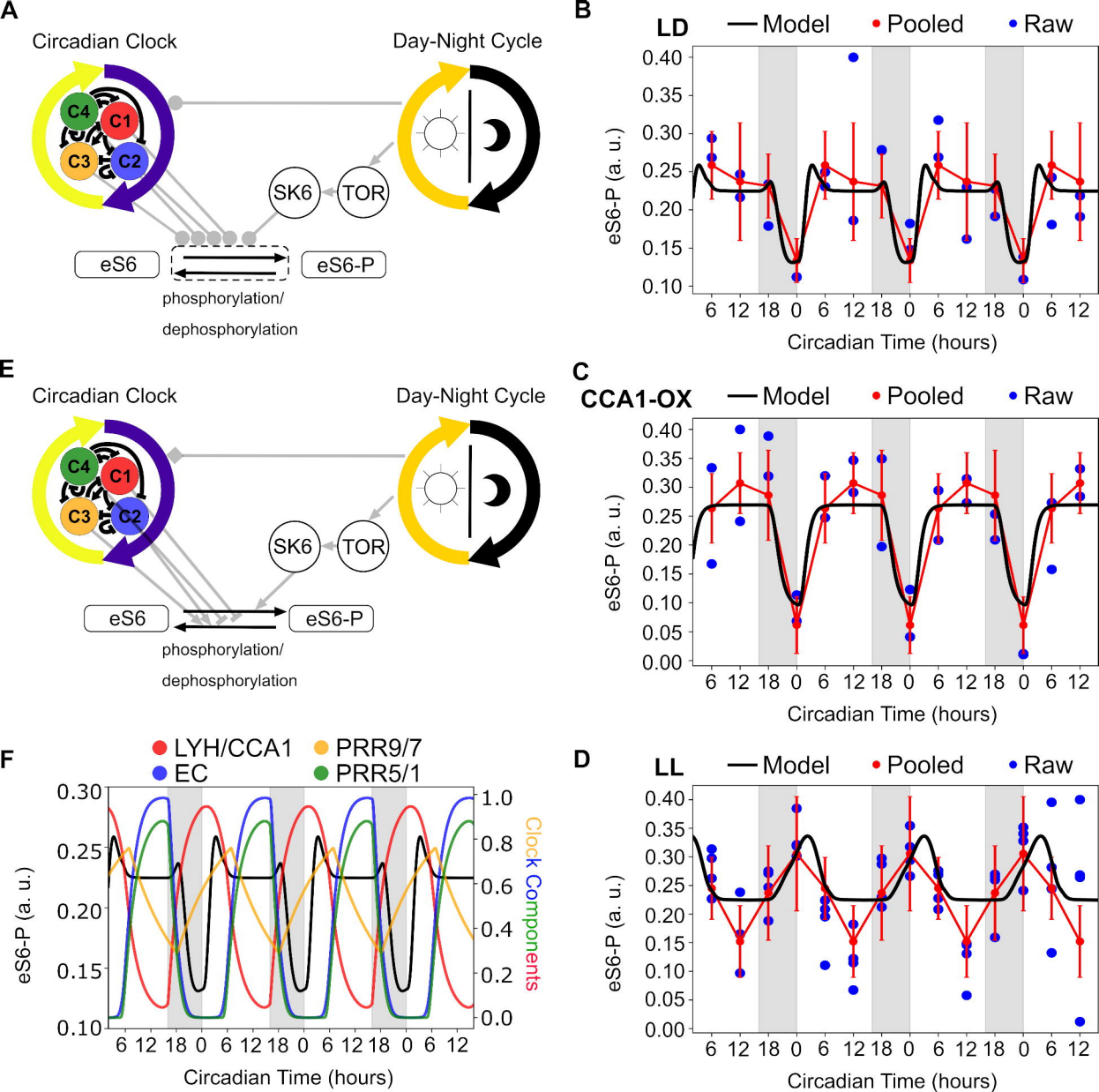
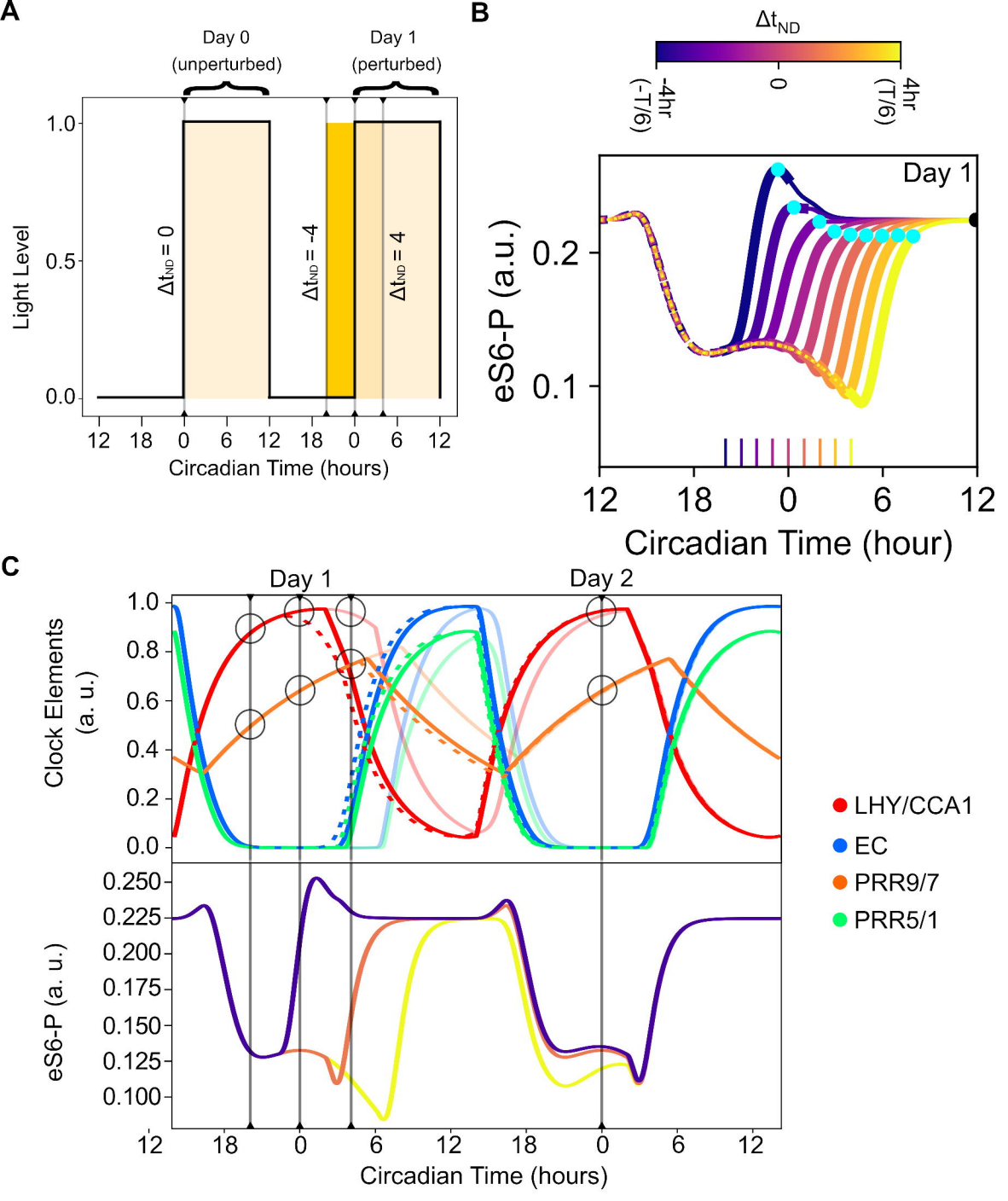
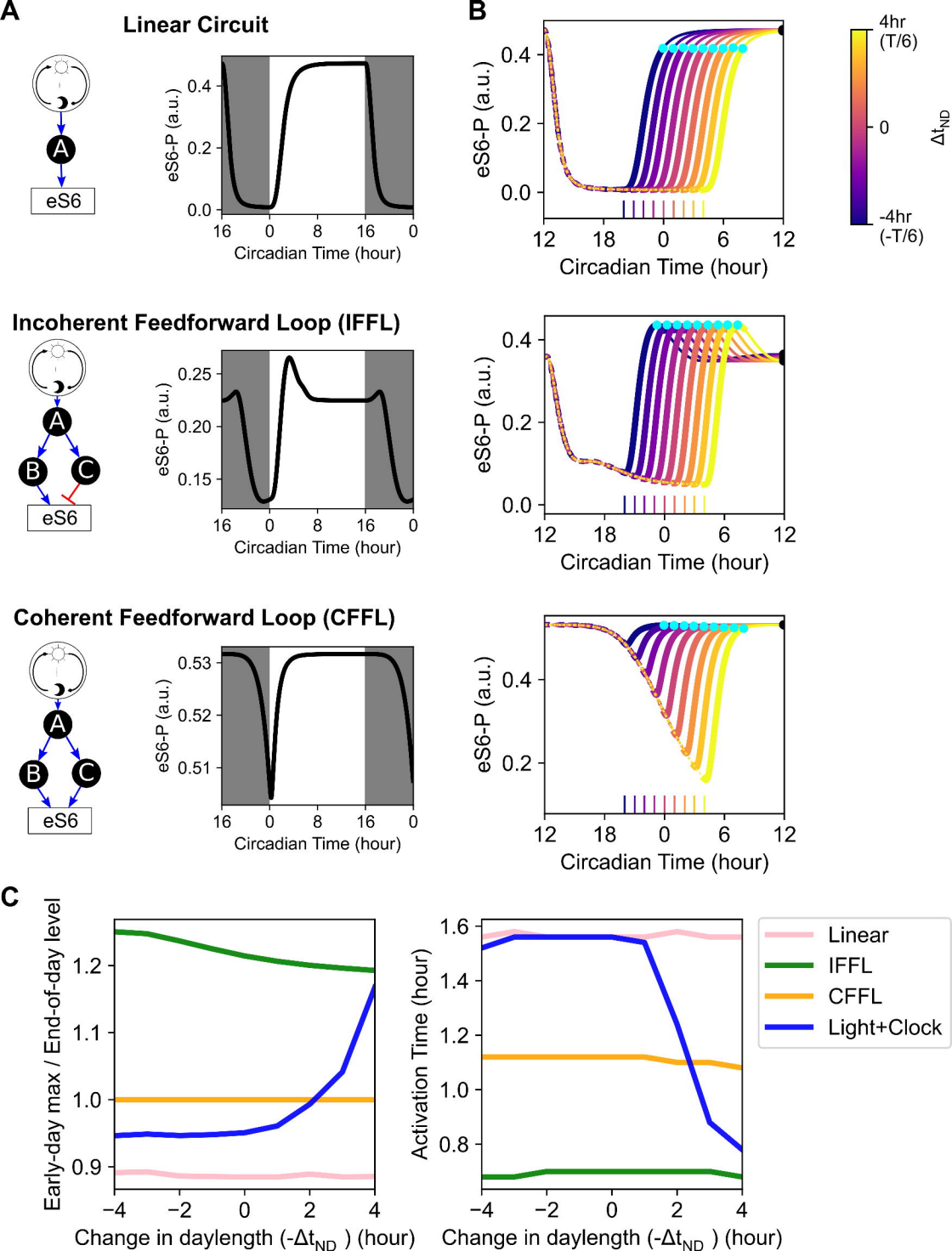
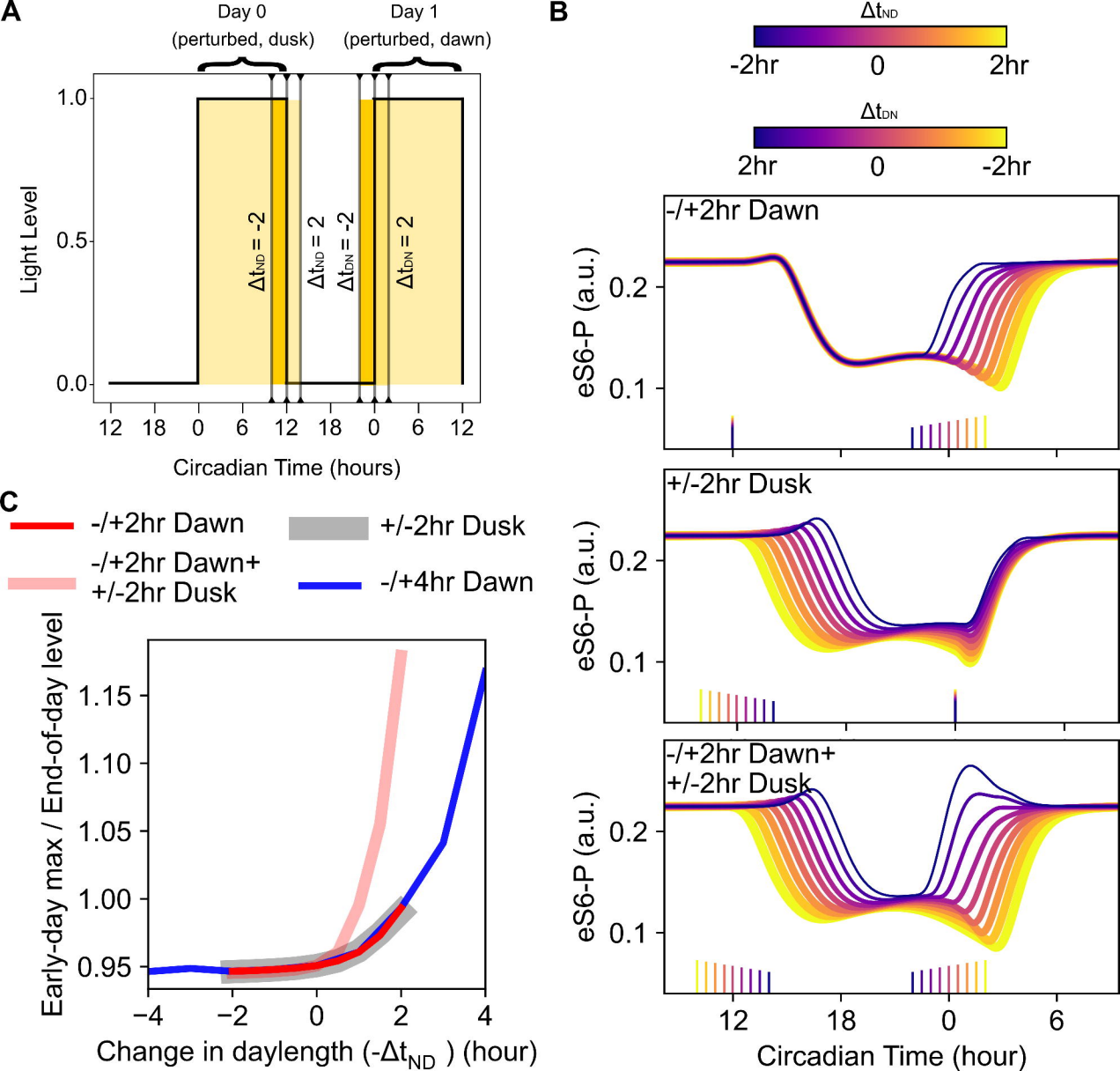
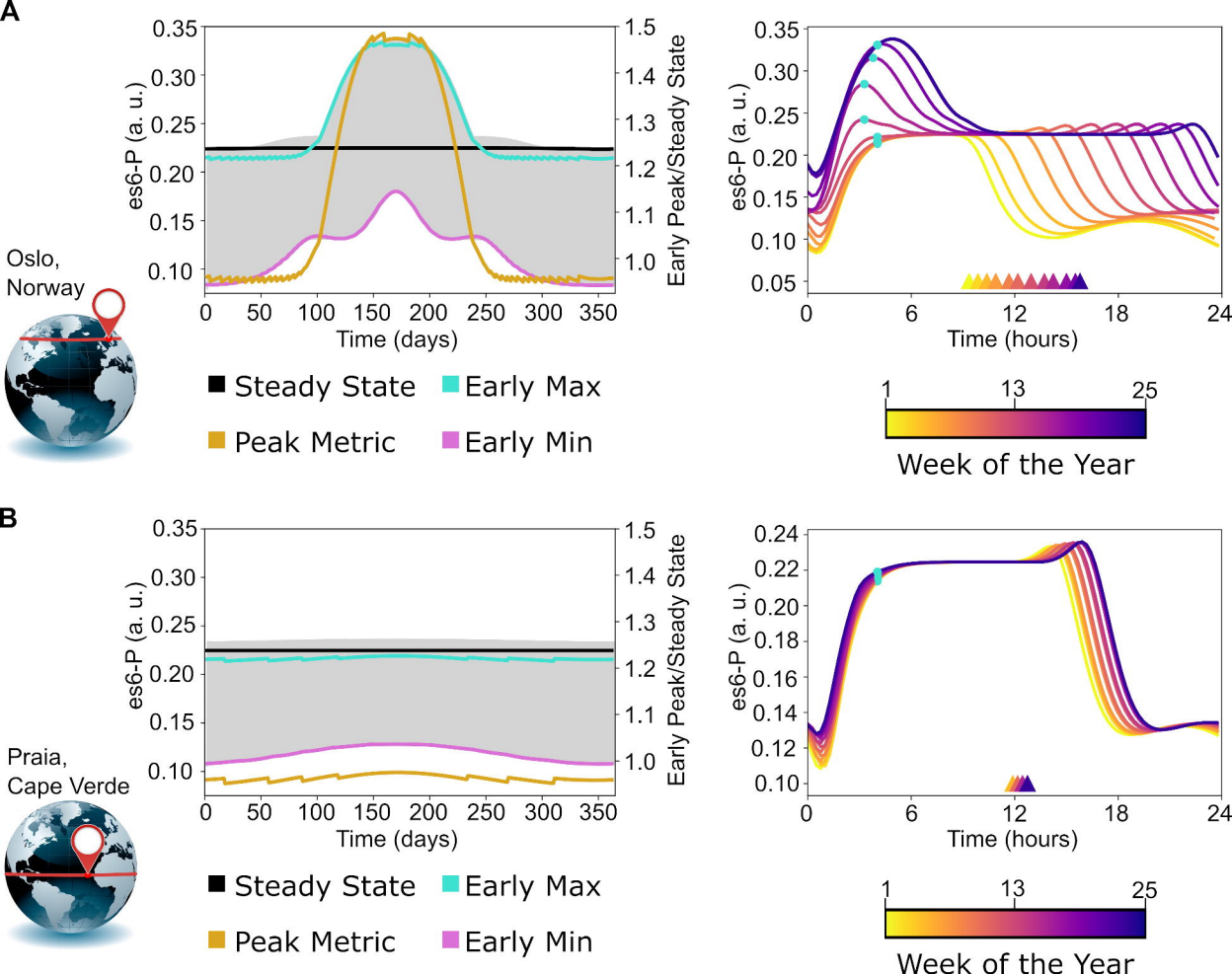
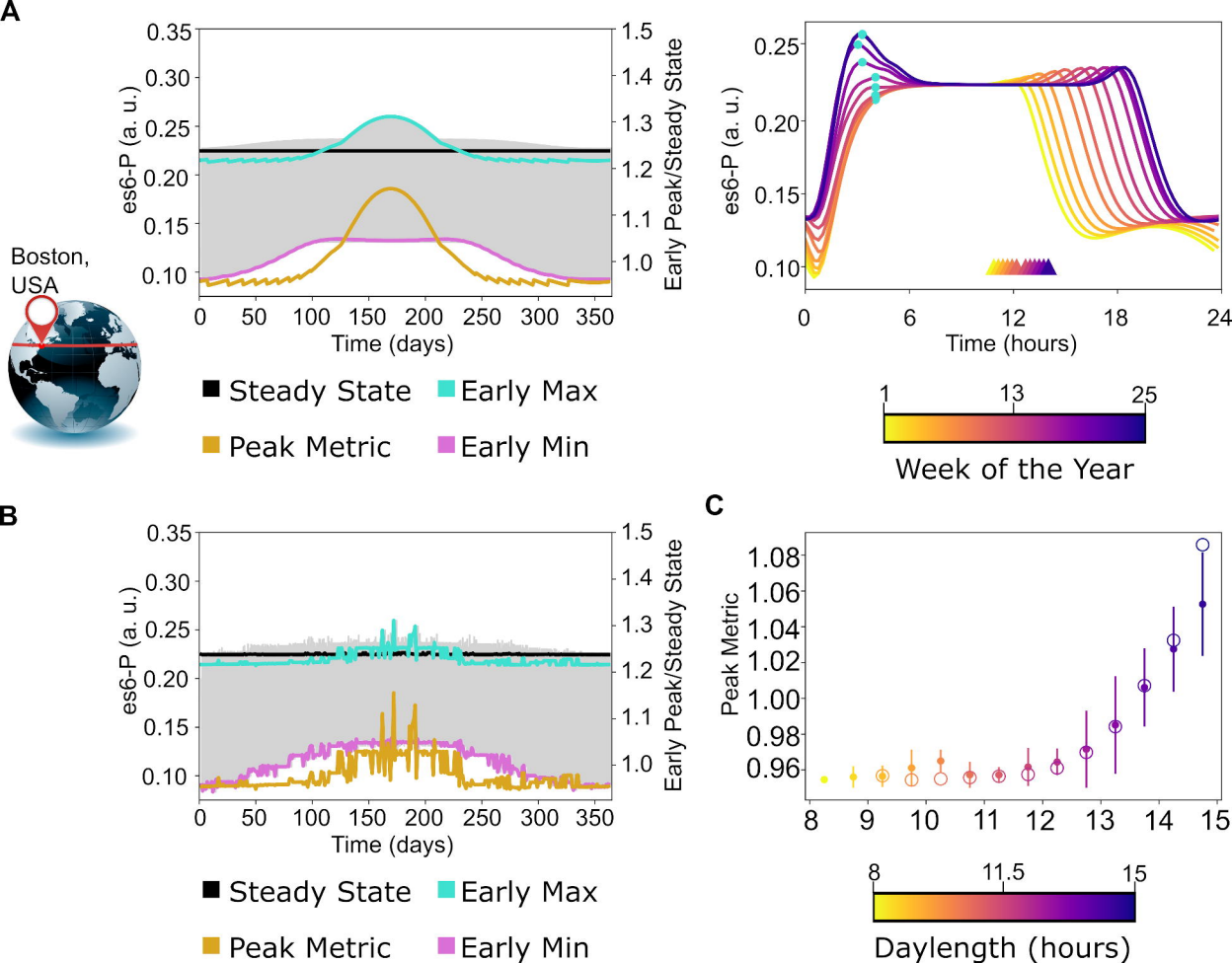

A
Circadian Clock
Day-Night Cycle
C4
C1
C3
C2
SK6
TOR
eS6
eS6-P
phosphorylation/
dephosphorylation
B
LD
Model
Pooled
Raw
eS6-P (a. u.)
Circadian Time (hours)
E
Circadian Clock
Day-Night Cycle
C4
C1
C3
C2
SK6
TOR
eS6
eS6-P
phosphorylation/
dephosphorylation
C
CCA1-OX
Model
Pooled
Raw
eS6-P (a. u.)
Circadian Time (hours)
F
LYH/CCA1
PRR9/7
EC
PRR5/1
eS6-P (a. u.)
Clock Components
Circadian Time (hours)
D
LL
Model
Pooled
Raw
eS6-P (a. u.)
Circadian Time (hours)

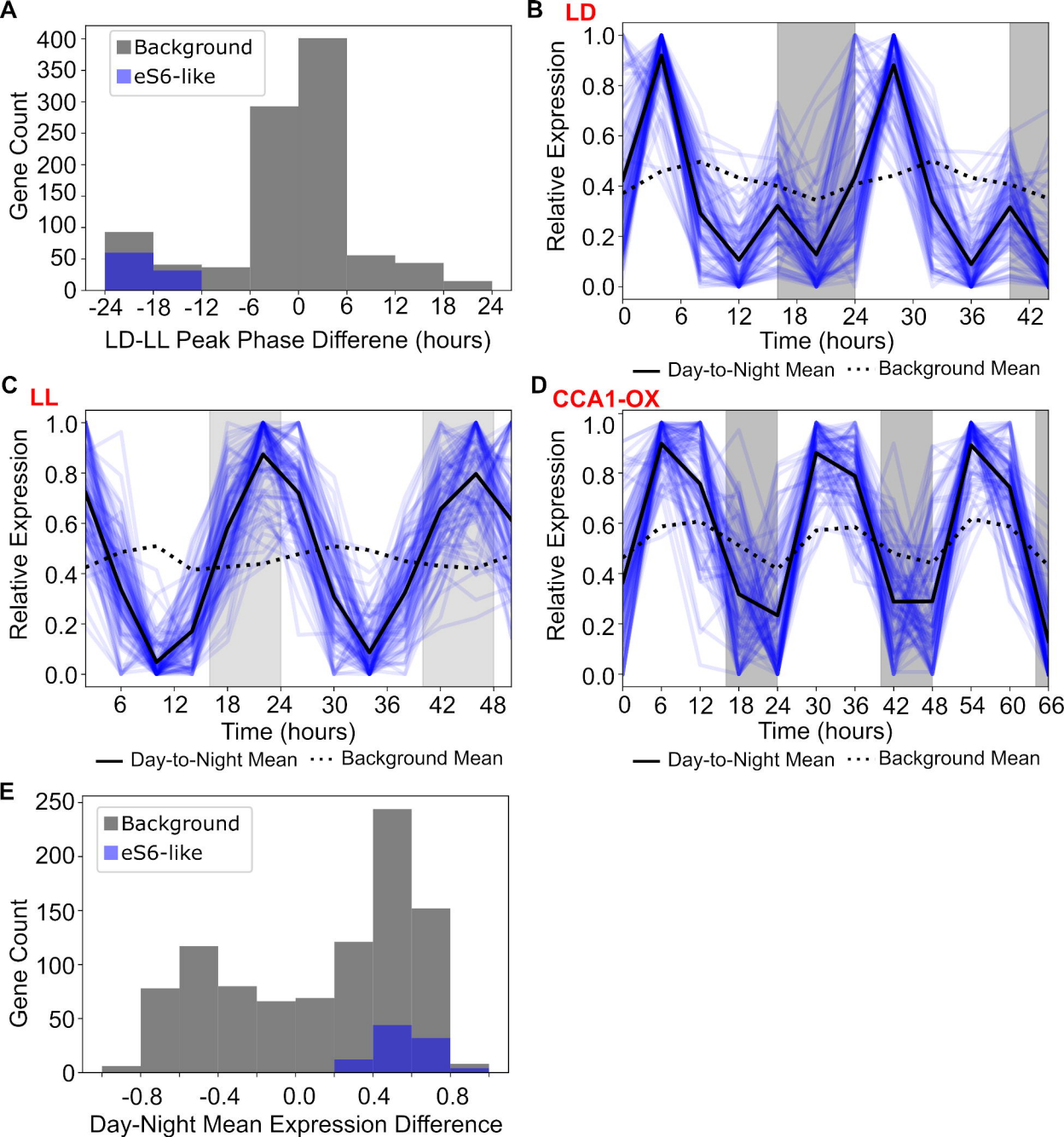

A
Day 0
(unperturbed)
Day 1
(perturbed)
Light Level
1.0
0.5
0.0
Δt_ND = 0
Δt_ND = -4
Δt_ND = 4
12 18 0 6 12 18 0 6 12
Circadian Time (hours)
B
Δt_ND
-4hr
(-T/6)
0
4hr
(T/6)
Day 1
eS6-P (a.u.)
0.2
0.1
12 18 0 6 12
Circadian Time (hour)
C
Day 1
Day 2
Clock Elements
(a. u.)
1.0
0.8
0.6
0.4
0.2
0.0
LHY/CCA1
EC
PRR9/7
PRR5/1
eS6-P (a. u.)
0.250
0.225
0.200
0.175
0.150
0.125
0.100
12 18 0 6 12 18 0 6 12
Circadian Time (hours)


A
Linear Circuit
A
eS6
eS6-P (a.u.)
Circadian Time (hour)
B
4hr (T/6)
0
-4hr (-T/6)
Δt_{ND}
Incoherent Feedforward Loop (IFFL)
A
B
C
eS6
Coherent Feedforward Loop (CFFL)
C
Early-day max / End-of-day level
Activation Time (hour)
Change in daylength ($-\Delta t_{ND}$) (hour)
Linear
IFFL
CFFL
Light+Clock


A
Day 0
(perturbed, dusk)
Day 1
(perturbed, dawn)
Light Level
1.0
0.5
0.0
Δt_ND = -2
Δt_ND = 2
Δt_DN = -2
Δt_DN = 2
12 18 0 6 12 18 0 6 12
Circadian Time (hours)
B
Δt_ND
-2hr 0 2hr
Δt_DN
2hr 0 -2hr
-/+2hr Dawn
+/-2hr Dusk
-/+2hr Dawn+
+/-2hr Dusk
eS6-P (a.u.)
0.2
0.1
12 18 0 6
Circadian Time (hour)
C
-/+2hr Dawn
+/-2hr Dusk
-/+2hr Dawn+
+/-2hr Dusk
-/+4hr Dawn
Early-day max / End-of-day level
1.15
1.10
1.05
1.00
0.95
−4 −2 0 2 4
Change in daylength (-Δt_ND) (hour)


A
Oslo,
Norway
es6-P (a. u.)
Early Peak/Steady State
Time (days)
Time (hours)
Steady State
Early Max
Peak Metric
Early Min
Week of the Year
B
Praia,
Cape Verde
es6-P (a. u.)
Early Peak/Steady State
Time (days)
Time (hours)
Steady State
Early Max
Peak Metric
Early Min
Week of the Year


A
Boston, USA
es6-P (a. u.)
Early Peak/Steady State
Time (days)
Steady State
Early Max
Peak Metric
Early Min
Time (hours)
Week of the Year
B
C
Peak Metric
Daylength (hours)


A
Background
eS6-like
Gene Count
LD-LL Peak Phase Differene (hours)
B
LD
Relative Expression
Time (hours)
Day-to-Night Mean
Background Mean
C
LL
Relative Expression
Time (hours)
Day-to-Night Mean
Background Mean
D
CCA1-OX
Relative Expression
Time (hours)
Day-to-Night Mean
Background Mean
E
Background
eS6-like
Gene Count
Day-Night Mean Expression Difference