Modeling Reveals a Key Mechanism for Light-Dependent Phase Shifts of *Neurospora* Circadian Rhythms

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ABSTRACT Light shifts and synchronizes the phase of the circadian clock to daily environments, which is critical for maintaining the daily activities of an organism. It has been proposed that such light-dependent phase shifts are triggered by light-induced upregulation of a negative element of the core circadian clock (i.e., *frq, Per1/2*) in many organisms, including fungi. However, we find, using systematic mathematical modeling of the *Neurospora crassa* circadian clock, that the upregulation of the *frq* gene expression alone is unable to reproduce the observed light-dependent phase responses. Indeed, we find that the depression of the transcriptional activator white-collar-1, previously shown to be promoted by FRQ and VVD, is a key molecular mechanism for accurately simulating light-induced phase response curves for wild-type and mutant strains of *Neurospora*. Our findings elucidate specific molecular pathways that can be utilized to control phase resetting of circadian rhythms.

INTRODUCTION

The circadian clock is an intrinsic autonomous oscillator, which acts as an internal timekeeping device. Most of the molecular components of the circadian clock are expressed in an oscillatory manner with an approximate 24-h periodicity. These rhythms are generated by intracellular transcriptional-translational negative feedback loops in various organisms ranging from fungi to mammals (1). In the mammalian circadian clock, PERIOD1/2 (PER1/2) and CRYPTOCHROME1/2 (CRY1/2) inhibit their own transcriptional activators (BMAL1 and CLOCK) (reviewed in Partch and others (2)). Similarly, in the circadian clock of the filamentous fungus *Neurospora crassa*, the frequency (FRQ) protein takes part in inhibiting its transcriptional activator, white-collar complex (WCC), a heterodimeric complex formed by white-collar-1 (WC-1) and white-collar-2 (WC-2) (3–5). In addition to the core transcriptional negative feedback loop, the *Neurospora* circadian clock is regulated by additional feedback loops (3,6) and post-translation modifications (7–10).

Although the molecular components of the circadian clock vary among organisms, circadian clocks share fundamental properties. For one, the circadian clock exhibits temperature compensation: the circadian period is nearly constant over a range of physiological temperatures despite the fact that temperature alters molecular kinetic rates (11–14). Additionally, the circadian clock is autonomous: rhythms persist even in the absence of external cyclic stimuli (e.g., light, temperature, social cues) (13). Finally, the autonomous circadian rhythms can be entrained by external cues (e.g., light-dark or temperature cycles) so the clock can be synchronized to a 24-h periodic environment of daily rhythms (15,16). This synchronization is critical for the survival of organisms; the misalignment of the circadian clock has been linked with decreased survival rates (17). In particular, desynchronization has been linked to various physiological problems including insomnia, mood disorders, diabetes, and cancer (18–21).

Entrainment of the circadian clock to the light-dark cycles occurs because a light stimulus can advance or delay the phase of circadian clocks. The collection of light-induced phase shifts applied at different phases of the circadian cycle are summarized as a phase-response curve (PRC). Two types of PRCs have been observed, generally depending on the strength or duration of the light pulse (22–24). Typically, a strong or long light pulse leads to a
type 0 PRC, which is characterized with large phase shifts (~12 h) and a discontinuity. On the other hand, weak and short light pulses yield a continuous type 1 PRC characterized by small phase shifts. Intriguingly, the unique shape of the type 1 PRC is conserved across many organisms, including *Neurospora*, *Drosophila*, and mammals (23,25). Specifically, the PRC is characterized by a region of few to no phase shifts (i.e., a dead zone) during the subjective day, followed by a region of delays in the early subjective evening and a region of advances in the late subjective night (23,25). It is interesting to note that light-dependent type 1 PRCs are readily observed in *Drosophila melanogaster* and mammals but not in *Neurospora crassa*. In *N. crassa*, it has been shown that even short (90-s) light pulses can result in type 0 PRCs (26). Lakin-Thomas demonstrated type 1 PRCs using 1-min light pulses (20–30 μmol/m²s, 22°C) (27), whereas Elvin and colleagues observed type 1 PRCs with 5-min light pulses (21 μmol/m²s, 25°C) (28). These differences may be due to different experimental conditions including light intensity, duration of light pulse, temperature, etc. Therefore, systematic analysis will be necessary to determine detailed conditions for type 1 and type 0 PRCs in *N. crassa*.

In silico studies have reasoned that this particular shape of the PRC is critical for efficient and stable entrainment of the endogenous clock to light-dark cycles (25,29–32). If a circadian rhythm is already entrained to the light-dark cycle, an organism will only receive light during the day in which no phase shifts occur (i.e., the dead zone), and entrainment is maintained (23,25). On the other hand, if the intrinsic circadian rhythm is advanced (or delayed) compared to the light-dark cycle, light will be received during the phase of the delay (advance) zone so that entrainment is restored. In addition, mathematical analysis has revealed that, owing to this PRC shape, the circadian clock can be robustly entrained to light-dark cycles, which fluctuate because of environmental factors such as meteorological conditions and seasonal changes (25,29).

The light-induced upregulation of the repressor gene expression has been accepted as a determining factor of phase shifting in both mammals and *Neurospora* (16,33–35). In mammals, a light pulse during the subjective night rapidly promotes transcription of the repressor gene (*Per1* and *Per2*) via the cAMP pathway and thus leads to a phase shift (35–39). In contrast, a light pulse during the subjective day does not affect the transcription of repressors in mammals (40). In *Neurospora*, a light pulse during the subjective day or night increases the transcriptional activity of WCC and thus promotes the transcription of *frq* (34,41,42). Additional molecular targets of light have also been identified in the *Neurospora* circadian clock. For instance, light promotes transcription of *wc-1* (43,44) as well as the phosphorylation and degradation of WC-1 (45). Furthermore, light rapidly promotes transcription of the *vivid* (*vvd*) gene, which triggers photo-adaptation of the *Neurospora* clock (28,42). Specifically, VVD binds to WCC, stabilizing WCC while simultaneously inhibiting its transcriptional activity. This process allows *Neurospora* to remain sensitive to light pulses of increasing intensity (46,47) by reversing the initial strong response of repressor downregulation and preventing excess removal of WCC.

Despite the identification of diverse molecular targets of light, the majority of previous mathematical models have used upregulation of the repressor to simulate light response (15,33,46–51) because it has been commonly observed in many organisms (34,35,41–43,52–55). These models have successfully reproduced type 0 PRCs when providing long or strong light pulses, consistent with experimental data. However, the light-induced upregulation of the repressor has failed to reproduce the experimentally observed type 1 PRC (23,25) when giving a short light pulse. For instance, a mathematical model of the *Neurospora* circadian clock, in which light promotes *frq* transcription, simulates a PRC characterized by a dead zone followed by an advance region rather than a delay region (33). On the other hand, a mathematical model of the mammalian circadian clock that simulates light response by upregulating the transcription of the repressor (*Per*) (53) successfully reproduces the type 1 PRC. To simulate a dead zone, this model makes use of a gating variable, blocking the light-induced *Per* transcription during the subjective day. However, in contrast to mammals (38), light actively induces *frq* transcription during the subjective day in *Neurospora* (34,42). This inconsistency between simulations and experimental data raises the question of whether the light-induced upregulation of *frq* is the necessary and sufficient factor that governs phase shifting in the *Neurospora* circadian clock. This indicates that the fundamental molecular mechanisms leading to the correct phase shifts in response to light are still not fully understood in *N. crassa*.

In this report, we used a systematic modeling approach (56–58) to investigate key molecular mechanisms that lead to the observed type 1 PRC of *Neurospora*'s circadian clock in response to a short light pulse. After developing a simple mathematical model describing the core transcription-translational feedback loop of the *Neurospora* circadian clock, we applied the Brent’s optimization algorithm (59) to rank potential light targets by quantifying and comparing their ability to generate the type 1 PRC shape. Surprisingly, we found that the currently accepted molecular mechanism, the upregulation of the *Neurospora* circadian repressor (i.e., *frq*), was not sufficient to reproduce the type 1 PRC shape. On the other hand, the observed type 1 PRC shape is successfully simulated when light downregulates the activator WC-1. We also found that when light promotes the downregulation of the activator as opposed to the upregulation of the repressor, mathematical models with different levels of complexity can also accurately simulate both type 0 and type 1 PRCs under.
Mechanisms that Reset Circadian Rhythms

Estimation of the light process parameters in the Tseng model

To estimate an improved light response in the Tseng model, we modified light process parameters that do not affect the core clock and were not derived from experimental data (Table S2). To find optimal values for these parameters, we used the simulated annealing method (62), a global stochastic parameter search algorithm, together with the optimization algorithm discussed above. For each parameter set, we found the optimal values for the light pulse amplitude of the Tseng model (A = k26) and the circadian time of the frq mRNA peak (S) between CT0 and CT4 that minimized the error (i.e., minimizes E(A,S) in Eq. 1), as discussed above. The code is available at https://github.com/JacobBellman/PhaseResponse-Curves.

RESULTS AND DISCUSSION

A simple model of the Neurospora circadian clock

To examine the molecular mechanisms underlying phase responses to light, we have developed a simple mathematical model that describes the core transcriptional negative feedback loop of the Neurospora circadian clock (Fig. 1) (63). This model avoids complexity by only including the components of the clock necessary for assessing phase response:

diverse conditions (i.e., short pulse, long pulse, and vvd mutant). Taken together, these results indicate that WC-1 appears to be a critical factor for adjusting the circadian phase through light response.

MATERIALS AND METHODS

Estimation of the minimal error

After each parameter was increased by a 5-min pulse with amplitude A, we measured the error, E(A,S), defined by the following:

$$E(A,S) = \frac{2}{T} \sqrt{\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}(A,S))^2},$$

(1)

where y and \( \hat{y}(A,S) \) are data points of experimentally measured and simulated PRCs, respectively, and n is the number of data points. S is the circadian time (CT) of the frq mRNA maximum. Because CT must be defined by some reference, we have selected S as an input parameter to be controlled for each model. The error is divided by the maximal possible error, which is half of the period (T/2) of the endogenous clock (e.g., if T is 24 h, a 21-h phase delay is equivalent to a 3-h advance). The parameters A and S have qualitatively different effects on the error E(A,S); changing the amplitude of the pulse (A) causes an unpredictable nonlinear change in the PRC, whereas a change in S results in a shift of the PRC along the time axis with a magnitude equal to the change in S. To find the pulse amplitudes (A) and the CT of frq mRNA maximum (S) minimizing E(A,S), we first fixed A across a discrete range of reasonable values. For each value of A, we used Brent’s method, a robust one-parameter extrema-finding algorithm (59), to estimate an optimal value of S that minimizes E(A,S). We then repeated this process until all values of A across the discrete range were tested. During this process, we restricted the range of S between 0 and 4 h, as experimentally measured frq mRNA reaches a peak between CT0 and CT4 (34,60,61). For each simulation, the peak of the frq mRNA maximum was chosen in this range such that E(A,S) was minimized.

Light-induced upregulation of a repressor leads to an incorrect PRC

In both mammals and Neurospora, light upregulates the transcription of the repressor (i.e., frq in Neurospora and Per in mammals) (34,35,43,54). Accordingly, light response has been simulated by incorporating repressor upregulation.
in the majority of previous circadian clock models (15,33,48–51,53). However, these models were unable to simulate the observed type 1 PRC in response to short light pulses (42) (Fig. 2 A), which is characterized by a dead zone in the subjective day (CT6–CT12) followed by a region of delays in the early subjective night (CT12–CT22) and a region of advances in the late subjective night and continuing into the early subjective morning (CT22–CT6).

To investigate the discrepancy between the simulations of previous models and experimental data, we first tested whether the simple model (Eq. 2; Fig. 1) can simulate the experimentally observed PRC when a short light pulse upregulates frq mRNA. The light-induced upregulation of frq transcription can be implemented in the model by two approaches widely used in previous studies (15,33,48–51,53). One approach is to add \( c_f \) to the rate of change of frq mRNA in the presence of light pulse as follows, which is referred to as an additive pulse:

\[
\frac{d[F_m]}{dt} = \alpha_m \frac{[W]^m}{K^m + [W]^m} - \beta_m [F_m] + c_f. \tag{3}
\]

Alternatively, we increased the maximal transcription rate (i.e., \( \alpha_m \)) of the Hill function describing frq transcription (Eq. 2) when the light is on, which is referred to as a multiplicative pulse. Note that although these two pulse types increase frq mRNA, the magnitude of increase is different depending on the time of the pulse. That is, regardless of WCC level, the additive pulse increases the transcription of frq at the same rate. On the other hand, when WCC level is low such that the value of the Hill function is nearly zero (i.e., \( W^m/(K^m + W^m) \sim 0 \)), the multiplicative pulse has little effect on the rate of transcription. The duration of the pulse was chosen as 5 min to match the experimental protocol in (42) and (28), and the amplitudes of the additive and multiplicative pulses were chosen to simulate phase shifts that were comparable in magnitude to those in the experimental data (see Materials and Methods for details).

Through upregulation of frq transcription, neither additive nor multiplicative pulses allowed the simple model to produce accurate PRCs (Fig. 2, B and C). This is in agreement with a previously developed model that also results in an incorrect PRC shape when applying an additive pulse to frq mRNA (33). Specifically, when an additive pulse is applied, the simulated PRC does not exhibit a dead zone (Fig. 2 B). Although a dead zone appears when applying a multiplicative pulse, the dead zone is followed by an advance zone rather than a delay zone in contrast to the experimental data (Fig. 2 C). To determine whether the inaccuracy of the simulated PRCs stemmed from the simplicity of our model, we further tested more comprehensive models: the Dovzhenok model (68) and the Tseng model (46). The Dovzhenok model, composed of nine variables and 24 parameters, includes the core clock components and a circadian-clock-regulated repressor, conidial separation-1 (csp-1), which regulates various metabolic gene expressions. The Tseng model, composed of 39 reactions and 39 parameters, incorporates a detailed set of light interactions via photoreceptors WC-1 and VVD proteins in addition to the key clock components, which will be discussed in detail later. These comprehensive models also simulated incorrect PRCs similar to those of the simple model (Fig. 2, B and C) in response to either additive or multiplicative pulses promoting the transcription of frq mRNA.

Closer examination of the multiplicative and additive pulses helps explain the shapes of these PRCs. When the level of activator (WCC) is low (~CT8), the value of the Hill-function (i.e., \( \alpha_m [W]^m/(K^m + [W]^m) \)) in the simple model) describing frq transcription is consistently low in the above models. In this case, the multiplicative pulse increasing \( \alpha_m \) has little effect on frq transcription and the contour of the shaded area represents the optimal PRC shape traversed by this data. (B) PRCs simulated with an additive pulse of frq transcription for 5 min do not match the experimental data (Fig. 2 A). The magnitude of a pulse increasing the rate of frq mRNA (i.e., \( c_f \) in Eq. 3) was chosen so that the magnitudes of the resulting phase shifts were similar to the experimental data: 1, 12, and 45 (arbitrary unit hour) \(^{-1}\) in the simple model, Dovzhenok model, and Tseng model, respectively (see Materials and Methods for details). (C) The simulated PRCs with a multiplicative pulse of frq transcription for 5 min do not match the experimental data (Fig. 2 A). The magnitude of a pulse that increases the maximal transcriptional rate of the Hill function was chosen so that the magnitudes of the resulting phase shifts were similar to the experimental data (Fig. 2 A): \( \alpha_m \) is increased from 5.9 to 80.9 h \(^{-1}\) in the simple model, \( k_i \) from 1.8 to 71.8 h \(^{-1}\) in the Dovzhenok model (68), and \( k_{01} \) from 7.3 to 207.3 h \(^{-1}\) in the Tseng model (46). To see this figure in color, go online.
(Fig. S1, A–C) and thus leads to few phase shifts (i.e., a dead zone). As the activator level increases, the value of the Hill-function increases and thus the multiplicative pulse has a larger effect on frq transcription. During this time (∼CT17), the multiplicative pulse accelerates the increase of frq mRNA (Fig. S1, A–C), which advances the phase in contrast to the observed phase delay (Fig. 2, A and C). Furthermore, when frq mRNA begins to decrease (∼CT22), the multiplicative pulse slows down the loss of frq mRNA (Fig. S1, A–C), which delays the phase in contrast to the data (Fig. 2, A and C). On the other hand, when the additive pulse is given, considerable transcription upregulation occurs independent of the activator level (Fig. S1, D and F), which explains why the dead zone cannot be obtained (Fig. 2 B). These in silico data indicate that the upregulation of frq is not sufficient to reproduce the observed phase shifts. This finding is consistent with previous experimental data that indicate that vvd is required for light-dependent phase response (42).

The light-induced downregulation of an activator simulates accurate PRCs

These in silico data indicate that the upregulation of frq alone is not sufficient to reproduce the observed phase shifts (Fig. 2). Therefore, we surveyed all the parameters in the simple model by increasing each parameter for 5 min and explored which parameters are able to simulate the observed PRC. For each parameter, we identified the optimal magnitude of pulsatile increase leading to a minimal error between the simulated and observed PRCs (42,61) (see Materials and Methods for details) (Fig. 3 A). Interestingly, parameters whose pulsatile increase yields low values of minimal error can be categorized into two groups. The pulsatile increase of the first group of parameters ($K$, $\beta_m$, $\beta_p$), which are highlighted in red in Fig. 3 A, decreases the level of a repressor by reducing transcription of frq mRNA ($K$) or enhancing degradation of frq mRNA ($\beta_m$) or FRQ proteins ($\beta_p$). This is consistent with the previous modeling study showing that inhibition of frq transcription can lead to an accurate PRC (51). The pulsatile increase of the second group of parameters ($k_f$, $\beta_m$, $\beta_p$), which are highlighted in blue in Fig. 3 A, decreases the level of an activator by enhancing 1) binding of FRQ and WCC ($k_f$), 2) degradation of WCC ($\beta_m$), or 3) degradation of FRQ:WCC ($\beta_p$). Similar results were obtained when two parameters were increased simultaneously upon light response (Fig. 3 B). This indicates that combinations of these parameters do not have a significant improvement on the PRC shape.

These results indicate that light should decrease a repressor or an activator in the model to obtain the accurate PRC shape (Fig. 2 A). However, it is unlikely that light downregulates the repressor, as light has been reported to upregulate the expression of the repressor gene frq (34,41,42). On the other hand, a previous study has reported that a light pulse can trigger the phosphorylation of WC-1 protein and its degradation (45) and hence reduce the level of WCC. Furthermore, it has been shown that the induction of FRQ leads to rapid WC-1 turnover (9) and inhibition independent of light (28,69). Thus, we hypothesized that light-induced depression of the activator may play a critical role in the phase responses to light. Indeed, when a light pulse induces degradation of the activator, both the simple model (Eq. 2) and the Dovzhenok model produce more accurate PRCs (Fig. 4 A) than when light pulse promotes the transcription of the repressor (Fig. 2, B and C). Similar results are attained when pulsing WCC in a simple model of the Neurospora clock developed by Francois et al. (49) (Fig. S2).

To explore why the light-induced degradation of the activator simulates accurate PRCs, we scrutinized the effect of light on the time course of the activator in the Dovzhenok model (Fig. 4 B) and the simple model (Fig. S3). When the level of the activator is low (e.g., green triangle in
Fig. 4), the light-induced degradation has little effect on the activator level and thus yields a dead zone. When the level of the activator is increasing (e.g., purple star in Fig. 4 B), the enhanced degradation of the activator slows down the accumulation of the activator and delays the phase. On the other hand, when the level of the activator is near its peak or decreasing (e.g., pink circle in Fig. 4 B), the degradation of the activator accelerates the entrance to the decreasing phase.

Although the light-induced downregulation of the activator simulates accurate PRCs (Fig. 4 A), it decreases frq mRNA (Fig. 4 C), which conflicts with experimental data reporting the upregulation of frq mRNA in response to light (34). We hypothesized that this conflict may be due to the simplicity of the light response process in the simple model and the Dovzhenok model. This hypothesis will be investigated in the next section with the Tseng model, which contains a more comprehensive process for light response.

The downregulation of activators via VVD leads to accurate light responses under diverse conditions

Unlike the simple model and Dovzhenok model, the Tseng model includes VVD, the photoreceptor responsible for photoadaptation in the Neurospora clock. In the model, light converts the transcriptionally inactive hypophosphorylated WCC to light-activated WCC (laWCC). laWCC promotes transcription of multiple genes with light-response-element motifs, including wc-1, frq, and in particular vvd. Next, the translated VVD protein binds to laWCC in the nucleus to form a transcriptionally inactive complex laWCC:VVD, which results in photoadaptation (Fig. 5 A). When the complex dissociates, laWCC is converted to hypophosphorylated WCC, which can be converted to transcriptionally active WCC (aWCC). Although this light-induced molecular process is consistent with a recent experimental study of photoadaptation (70) and promotes expression of frq mRNA consistent with experimental data (34) (Fig. S4 A), the Tseng model was unable to simulate the observed PRC with its default parameter set (Fig. 5 B).

The source of this inaccuracy can be described by simulating time courses of transcriptionally active activators (i.e., laWCC and aWCC) and total activators (WC-1) in response to light (Fig. 5, C and D). After light input, the activators are transcriptionally upregulated, which leads to VVD-dependent photoadaptation. However, the activators are not downregulated (Fig. 5, C and D), which may explain why the Tseng model is unable to simulate the accurate PRC, as we found that the downregulation of the activator is required to simulate the accurate PRC with other models (Figs. 3 and 4).

To identify a new set of light process parameters in the Tseng model that can simulate the observed PRC (Fig. 2 A), we used simulated annealing, a global stochastic optimization algorithm (see Materials and Methods for details) (62). After searching more than $10^5$ parameter sets, the simulated annealing method only converged to a few parameter sets, and interestingly, these parameter sets were all similar. From this analysis, we identified nine new photoadaptation parameters (Fig. 5 E; Table S2), which produce the accurate PRC (Fig. 5 F). Surprisingly, with the new parameter set, both of the transcriptionally active activators (i.e., laWCC and aWCC) decrease considerably in response to the light (Fig. 5 G). This indicates that the downregulation of the activator is also critical for the Tseng model to accurately simulate the observed PRC, which is consistent with the other models (Figs. 3 and 4). In the modified Tseng model (Fig. 5 E; Table S2), the light-induced downregulation of the activator is enhanced because of a faster degradation rate of laWCC and faster sequestration of laWCC by VVD. In particular, the rapid sequestration by VVD is triggered by the increased rates of transcription, translation, and nucleus translocation of VVD and a stronger binding between VVD and laWCC. Furthermore, the degradation rate of the complex laWCC:VVD is also decreased, which is in agreement with recent experimental findings (70). In the modified Tseng model, unlike the original Tseng model, a light pulse decreases the level of WC-1 protein (Fig. 5, D and H), as is
observed in experimental data (45). Finally, the increased transcriptional activity of laWCC leads to the upregulation of frq transcription in response to light (Fig. S4B), which is in agreement with experimental data (34).

The modified Tseng model, which downregulates the activator in response to light, also simulates more accurate PRCs under different conditions. Strong light stimulus with a long duration (~15 min) yields a discontinuous type 0 PRC (Fig. 6A), which qualitatively differs from a continuous type 1 PRC (Fig. 2A). When 15-min light pulses are given, the modified Tseng model more accurately simulates a type 0 PRC than the original Tseng model (Fig. 6A).

Photoadaptation is disrupted when vvd is mutated. Thus, even when the same light pulse leading to the type 1 PRC in wild type (Fig. 5A) is applied, the VVD mutant shows a type 0 PRC (Fig. 6B). When we set the rate of vvd translation to 0 in the model to simulate the VVD mutant and gave the same light pulse used in Fig. 5, A and D, the modified Tseng model, but not the original Tseng model, accurately simulated a discontinuous type 0 PRC (Fig. 6B). Taken together, the accurate phase responses of the modified Tseng model under diverse conditions indicate that the downregulation of the activator is indeed the major molecular pathway...
leading to phase shifts of the Neurospora circadian clock in response to light.

CONCLUSIONS
The phase of a circadian clock should be adjustable by light to time regular daily activities and adapt to environmental changes such as seasonal day-length changes (16). The shape of the PRC in response to light is shared across organisms (Fig. 2 A), which appears to allow for effective and robust entrainment to light-dark cycles (25,29–32). Using a systematic mathematical modeling approach (Fig. 3), we have investigated key molecular mechanisms that lead to the unique shape of the type 1 PRC in the Neurospora circadian clock. Unexpectedly, we found that light-induced upregulation of the transcriptional activator, FRQ, which has been considered as a major light signal target (16,33–35) and thus has been used in the majority of previous modeling studies (15,26,33,48–51,53), does not simulate the observed PRC (Fig. 2). On the other hand, light-induced downregulation of the transcriptional activator, WCC, can simulate the observed PRCs (Fig. 4). Furthermore, when incorporating this mechanism into previous comprehensive mathematical models, it is capable of simulating accurate PRCs of both wild-type (Fig. 5) and vvd mutant strains (Fig. 6). Intriguingly, previous studies have demonstrated that induction of FRQ results in rapid turnover of WC-1 (9) and inhibition of WCC (28,69), which suggests that light-induced FRQ may downregulate WC-1 sufficiently to create the observed PRCs. It is also important to highlight previous results demonstrating that VVD plays a critical role in setting the phase of the circadian clock at the light/dark boundaries. Specifically, VVD negatively modulates the activity of WCC to regulate the degree of induction of frq mRNA from dark to light transition and the decrease of frq mRNA at dusk (28,42,71). This is consistent with our findings that the depression of WCC is a key mechanism in Neurospora for light-induced phase shifting. On the other hand, Froehlich and colleagues showed an altered phase of conidiation in the Neurospora mutant strain lacking the proximal light-regulatory element in the endogenous frq promoter, which drives the acute light response (41). This suggests that the determination of the circadian phase is a complex function of both transcriptional regulation on the frq promoter and post-translational depression of WC-1. Considering our computational analysis, it will be important to systematically reassess the roles of frq induction and WCC depression for clock resetting.

Our systematic mathematical modeling studies (Fig. 3) have revealed that the shape of the light-induced PRC (Fig. 2 A), which is shared by many organisms (25), can be simulated if light downregulates either 1) the transcriptional activator or 2) the transcriptional repressor of the core transcriptional negative feedback loop in the circadian clock (Fig. 3). Consistent with our modeling prediction, experimental studies have revealed that light downregulates the transcriptional activator of Neurospora, WC-1, through the phosphorylation and degradation of WC-1 (42,45,70). Intriguingly, a short light exposure triggers rapid reduction of BMAL1 in the rat suprachiasmatic nucleus (72), which suggests a potentially conserved molecular mechanism for light-dependent phase shifts from the Neurospora to the mammalian system. Based on our systematic in silico analysis and experimental data supporting the light-induced downregulation of the transcriptional activator, it will be important to reassess the current paradigm of light-dependent induction of the negative elements (i.e., frq, Per1, and Per2) as the molecular mechanism for phase shifting circadian rhythms. Furthermore, in the circadian clock of Drosophila, light promotes degradation of the Timeless (TIM) protein via cryptochrome (CRY), resulting in downregulation of the transcriptional repressor, PER (73–78), which is in agreement with our modeling prediction.

The misalignment between the circadian clock and environmental daily cycles increases the risk of various diseases such as cancer, mood disorders, and diabetes (18). To manipulate the circadian phases and treat misalignment, various pharmacological compounds have been developed, but the shape of their PRC considerably differs to that of light. For instance, PF-670462, a key kinase inhibitor of the mammalian circadian clock, delays the phase regardless of dose timing and thus leads to a PRC that only consists of phase delays (15,79). The melatonin PRC consists of an advance zone in the subjective morning, delay zone in the subjective evening, and a dead zone in the subjective night, in contrast to the light PRC (80–83). Our studies indicate that downregulation of either the activator or the repressor element of the circadian clock can mimic the effect of light response, which can be a potential drug target for effective and natural phase resetting of a malfunctioning circadian clock.

SUPPORTING MATERIAL
Four figures and two tables are available at http://www.biophysj.org/biophys-supplemental/S0006-3495(18)30916-0.

AUTHOR CONTRIBUTIONS
J.B., J.K.K., S.L., and C.I.H. designed the model and the computational experiments. J.K.K. designed the optimization algorithms. J.B. performed the computer simulations. J.B., J.K.K., S.L., and C.I.H. analyzed the computational results and wrote the manuscript.

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