Circadian clock regulation of the glycogen synthase (gsn) gene by WCC is critical for rhythmic glycogen metabolism in Neurospora crassa

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Circadian clocks generate rhythms in cellular functions, including metabolism, to align biological processes with the 24-hour environment. Disruption of this alignment by shift work alters glucose homeostasis. Glucose homeostasis depends on signaling and allosteric control; however, the molecular mechanisms linking the clock to glucose homeostasis remain largely unknown. We investigated the molecular links between the clock and glycogen metabolism, a conserved glucose homeostatic process, in Neurospora crassa. We find that glycogen synthase (gsn) mRNA, glycogen phosphorylase (gpn) mRNA, and glycogen levels, accumulate with a daily rhythm controlled by the circadian clock. Because the synthase and phosphorylase are critical to homeostasis, their roles in generating glycogen rhythms were investigated. We demonstrate that while gsn was necessary for glycogen production, constitutive gsn expression resulted in high and arrhythmic glycogen levels, and deletion of gpn abolished gsn mRNA rhythms and rhythmic glycogen accumulation. Furthermore, we show that gsn promoter activity is rhythmic and is directly controlled by core clock component white collar complex (WCC). We also discovered that WCC-regulated transcription factors, VOS-1 and CSP-1, modulate the phase and amplitude of rhythmic gsn mRNA, and these changes are similarly reflected in glycogen oscillations. Together, these data indicate the importance of clock-regulated gsn transcription over signaling or allosteric control of glycogen rhythms, a mechanism that is potentially conserved in mammals and critical to metabolic homeostasis.

M ost organisms possess an endogenous circadian clock mechanism that, through the regulation of gene expression, generates self-sustained rhythms in biological processes. These clocks are reset each day to synchronize to 24-h environmental cycles of light–dark and temperature. In addition, clocks present in organs involved in metabolism, including the liver, pancreas, muscle, and adipose tissue, can be reset by feeding cues (1, 2), allowing the integration of nutritional signals with the clock to maintain metabolic homeostasis throughout the organism. Consequently, misalignment between feeding cycles and the endogenous clock, or through circadian disruption, leads to metabolic imbalance that promotes increased body weight, insulin resistance, as well as liver and cardiovascular disease (3–6). Despite the importance of the clock in metabolic homeostasis, the molecular mechanisms connecting the clock and nutritional signals to metabolic homeostasis are not fully understood. To gain insights into this mechanism, we investigated molecular links between the clock and glycogen metabolism in the model filamentous fungus, Neurospora crassa.

Glycogen, a branched polymer of glucose residues, is a major form of carbon and energy storage in evolutionarily diverse organisms and is utilized in times of nutritional deprivation (7, 8). For example, in the mammalian liver, glycogen can be broken down to yield glucose to maintain blood glucose levels during the daily cycle of fasting (9). Yeast cells that accumulate glycogen stores display a growth advantage over cells that cannot, indicating a key role for glycogen in overall fitness (10). Glycogen concentration is controlled by the activities of two opposing enzymes, glycogen synthase (GS) and glycogen phosphorylase (GP). GS, which utilizes UDP-glucose, catalyzes the addition of glucose residues via α,1,4-linkages to the glycogen chain initiated by glycogenin, and branching enzyme introduces branch points via α,1,6-linkages. GS activity is inhibited by phosphorylation, but this regulation can be overcome by the allosteric activator glucose 6-phosphate (8). GP, along with the debranching enzyme, breaks down glycogen to release glucose-1-phosphate from α,1,4-linkages, and free glucose from α,1,6-linkages (7). Similar to GS, GP is controlled by allosterism and reversible phosphorylation. In yeast cells, expression of the genes encoding GS, GP, and the branching and debranching enzymes are coordinately controlled by the Protein Kinase A (PKA) pathway (10), and N. crassa GSN was shown to be regulated by PKA (11).

Previous studies revealed that GS and GP activity cycles under control of the circadian clock in mouse liver, and that glycogen levels peak near the end of the active phase (12–14). Furthermore, this oscillatory behavior is conserved in various mammals and in other eukaryotes (15, 16). For example, in the model filamentous fungus, N. crassa, circadian rhythms in glycogen metabolism are observed, and the gsn promoter is rhythmic (17–19). These studies suggest that circadian control of glycogen metabolism might be conserved across eukaryotic species. However, the mechanisms linking the circadian clock and glycogen metabolism remain largely unknown. To gain insights into this mechanism, we investigated molecular links between the clock and glycogen metabolism in the model filamentous fungus, N. crassa.

Significance

Circadian rhythms enable organisms to anticipate daily environmental cycles and control the timing of numerous biological processes, including metabolism, to optimize the health and survival of organisms. Glycogen metabolism is a conserved glucose homeostatic process; however, the molecular mechanisms linking the circadian clock and glycogen metabolism remain largely unknown. In this report, we demonstrate that circadian clock-dependent transcriptional regulation of glycogen synthase, gsn, regulates circadian oscillations of GSN protein and glycogen accumulation in the model filamentous fungus, N. crassa.

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the core clock component and transcriptional activator CLOCK in mice directly binds to the promoter of hepatic Gs2 encoding Glycogen Synthase 2 and drives its rhythmic expression (15). However, how the clock regulates the levels and activity of GS and/or GP, necessary for glucose homeostasis, remain largely unknown.

The well-studied circadian clock in N. crassa is composed of the FRQ/WCC (white collar complex) circadian oscillator, which forms a characteristic negative feedback loop that generates daily rhythms. In the FRQ/WCC oscillator, two PAS domain-containing GATA-type zinc finger transcription factors (TFs), White Collar 1 (WC-1) and White Collar 2 (WC-2) dimerize to form the White Collar Complex (WCC) (16–18). WCC functions as a positive element in the oscillator and activates transcription of the frequency (frq) gene (19–21). The negative component FRQ accumulates, enters the nucleus, interacts with FRQ-interacting RNA helicase (FRH) (22, 23) and CK1 (24), and inhibits the WCC (25–28). Progressive phosphorylation of FRQ relieves WCC inhibition, reinitiates the cycle, and leads to proteasome-dependent degradation of FRQ (29, 30). WC-1 is also a blue light photoreceptor (19, 31), and with its partner WC-2, functions to regulate light-responsive genes, as well as downstream clock-controlled genes (ccgs) (32–34). ChIP-seq in cells given a short light pulse to activate the WCC revealed that WCC binding occurs at the promoters of ∼200 genes, and TFs were enriched among these direct WCC targets, including CSP-1 and VOS-1 (34). N. crassa VOS-1 is the homolog of Aspergillus nidulans VosA involved in the control of development, metabolism, and stress responses (35, 36). CSP-1 functions primarily as a repressor to control the expression of ∼800 genes, including wc-1 (37). Of the CSP-1 targets, ∼200 genes are involved in metabolism, and deletion of csp-1 (Δcsp-1) results in the loss of circadian time-dependent membrane lipid synthesis (37). Furthermore, CSP-1 differentially regulates the expression of wc-1 depending on glucose concentrations to maintain the circadian period over a range of glucose concentrations, a process referred to as nutritional compensation (38, 39).

In this study, we show that glycogen accumulation, and gsn and gpn mRNA levels, are clock controlled. In addition, we provide several lines of evidence to support that rhythms in gsn mRNA levels are necessary for the rhythmic accumulation of glycogen. Rhythmic expression of gsn is accomplished by rhythmic binding of the WCC to the promoter of gsn. In addition, the WCC-controlled TF, VOS-1, cooperates with WCC and CSP-1 to modulate the amplitude and phase of the glycogen oscillation by regulating gsn rhythmicity.

**Results**

The N. crassa Circadian Clock Regulates Rhythmic Expression of Glycogen Metabolic Genes and Glycogen Abundance. To determine if glycogen levels are regulated by the circadian clock, WT and arrhythmic clock mutant (Δfrq) strains were cultured in constant darkness (DD), conditions in which the clock mechanism free runs with an endogenous ∼22.5-h period. Daily rhythms of glycogen abundance were observed in WT cells, with a peak during subjective night (DD32) (Fig. 1 A and SI Appendix, Table S1). In contrast, glycogen levels were low and arrhythmic in Δfrq cells, compared with WT (Fig. 1 A and B), demonstrating circadian clock control of glycogen abundance. Consistent with rhythmic glycogen levels, both gsn and gpn mRNA levels cycled in WT (Fig. 1 C and D and SI Appendix, Fig. S1A and Table S1), but not in Δfrq cells (Fig. 1 C and D and SI Appendix, Table S1), peaking at subjective dawn (DD12 and DD36). Similar results were obtained using strains containing the gsn or gpn promoters fused to the luciferase reporter. Both Pgsn-luc and Pgpn-luc levels cycled with a daily rhythm in WT, but not in Δfrq cells (SI Appendix, Fig. S1B and Table S1), demonstrating that gsn and gpn promoter activity, rather than mRNA turnover, are controlled by the circadian clock.

GSN and GPN have opposing activities in glycogen metabolism, synthesis versus breakdown, respectively. Therefore, it was somewhat surprising to find that gsn and gpn mRNA levels peaked at the same time of day. GSN and GPN are the rate-limiting enzymes for glycogen accumulation and breakdown, respectively, suggesting that levels and/or activity of GSN and/or GPN may determine the rhythmic accumulation of glycogen. To begin to test this idea, we tagged GSN and GPN at the C terminus with a V5-epitope tag and measured GSN-V5 and GPN-V5 levels from cells harvested every 4 h in DD over 2 d using anti-V5 antibody. Total GSN and GPN levels exhibited circadian rhythms with a peak in the subjective night (∼DD32) (Fig. 1 E and F), which coincides with the peak of glycogen levels (Fig. 1A).

To determine if rhythmic glycogen abundance requires gsn or gpn, we assayed glycogen rhythms in Δgsn and Δgpn strains. The
overall levels of glycogen are low in Δgpn cells at all times of the day compared with WT cells, and while a low amplitude rhythm in glycogen levels in Δgpn cells is observed in the data, the rhythm does not meet statistical significance (Figs. 1B and 2A). Therefore, we concluded that rhythmic accumulation of glycogen is disrupted in Δgpn cells. As expected, no glycogen was detected in Δgpn cells lacking glycogen synthase (Fig. 2A). In contrast, the pro-gp-motor luciferase reporter transcriptional fusion construct (Pfrq-luc) displayed robust rhythmicity in Δgpn cells, ruling out the possibility that the loss of glycogen accumulation rhythms in Δgpn was the result of a defect in the core circadian clock mechanism (Fig. 2B). Furthermore, gsn mRNA rhythms were disrupted in Δgpn cells, and gsn mRNA rhythms were abolished in Δgpn cells, whereas the clock-controlled gene ccg-1 mRNA (40) accumulated rhythmically in the mutant strains (Fig. 2C).

The loss of rhythmic gsn mRNA and glycogen levels in Δgpn cells supported the hypothesis that cycling gsn mRNA is necessary for rhythmic glycogen accumulation. To test this hypothesis, we constructed a strain that overexpressed gsn from the tcu-1 promoter (41). Constitutive overexpression of gsn resulted in disruption of the circadian rhythm of glycogen accumulation, and an approximately threefold increase in total glycogen levels compared with WT cells (Figs. 1B and 2D and E). Further support for clock control of rhythmic gene expression being important for glycogen level rhythms is that while phosphorylated GSN accumulated rhythmically, the amount of phosphorylated GSN represented only a small fraction of total GSN (SI Appendix, Fig. S1C). Thus, under these growth conditions, signaling mechanisms that regulate GSN activity likely have only a minor role, if any, in regulating rhythmic glycogen accumulation. Taken together, these data support that circadian control of gsn is critical for rhythmic accumulation of glycogen. Therefore, we next focused on determining what controls rhythmic gsn expression, but also examined possible mechanisms of transcriptional regulation of gsn.

WCC Regulates Rhythmic Expression of gsn. WC-2 ChIP-seq from N. crassa cultures given a short light treatment to promote genome-wide WCC binding did not identify WC-2 binding sites near the gsn or gpn genes (34). However, based on the WCC-consensus binding site (19, 34, 42), we identified four putative WCC binding sites within 2 kb upstream of the translation start site of gsn (Fig. 3A). ChIP assays confirmed light-induced recruitment of WC-2 to the binding sites present in the gsn promoter, but as expected, not to gpn, which lacks WCC binding sites (Fig. 3B).

Examination of WC-2 binding to the gsn promoter from cells grown in DD and harvested at different times of the day revealed that WC-2 is rhythmically recruited to the gsn promoter, with peak binding during the subjective day (DD14) (Fig. 3C). These data supported the idea that WCC directly regulates gsn rhythmic expression. We next examined if gsn mRNA and glycogen accumulation rhythms were altered in WT cells. As expected, the amount of phosphorylated GSN decreased in Δwc-1 cells (Fig. 3 D and E), but overall glycogen levels in the mutant were similar to WT levels (Figs. 1B and 3E). Taken together, these data indicated that the core clock component WCC directly drives rhythmic expression of gsn necessary for rhythmic glycogen accumulation.

Vos-1 Influences Rhythmic gsn and gpn mRNA and Glycogen Levels. In addition to WCC binding sites, we identified potential Vos-1 binding sites in the gsn and gpn promoter regions based on the identification of sequences similar to the consensus A. nidulans VosA DNA binding site (5′-CTGGCCCAAGGC-3′) (Fig. 3A) (43). Because vos-1 is a direct target of the WCC (34), we first examined if the circadian clock controls rhythms in the expression of vos-1. Both Pvos-luc- and Vos-1–V5 showed robust circadian oscillations, with a peak in VOS-1–V5 during the subjective night (DD28) (Fig. 4A and B). Furthermore, VOS-1 bound rhythmically to the gsn and gpn promoters, peaking in the subjective night (DD28) (Fig. 4 C and D), consistent with the nighttime peak levels of vos-1 mRNA and protein, and preceding the peak in gsn and gpn mRNA levels (Fig. 1 C and E). In the Δvos-1 strain, both Pgsn-luc and Ppgn-luc were still rhythmic, but with a significantly reduced amplitude, and with an ∼4-h phase advance of the Pgsn-luc rhythm compared with WT (Fig. 4 E and F and SI Appendix, Table S1). These data indicated that while VOS-1 is not necessary for rhythmicity of gsn and gpn, it contributes to the robustness of their rhythms. Furthermore, in Δvos-1 cells, glycogen accumulation was rhythmic, but with a lower amplitude and an ∼2-h phase advance (Fig. 4G and SI Appendix, Table S1), and the overall levels of glycogen were similar to WT levels (Fig. 1B).

CSP-1 Is Required for Rhythmic Expression of gpn, but Not gsn. CSP-1 is a direct target of the WCC (34), and previous ChIP-seq analyses indicated that CSP-1 physically binds to the gpn promoter.

Fig. 2. Rhythms in gsn mRNA accumulation are required for rhythmic glycogen levels. (A) Plot of glycogen levels from WT (black line; replotted from Fig. 1A), Δgpn (dark gray line), and Δgpn cells (light gray line) (n ≥ 2, ±SEM). Glycogen levels in Δgpn and ΔgpnE had a better fit of the data to a linear line. (B) Representative trace of Pfrq-luc in WT (black line), Δgpn (dotted dark gray line), and Δgsn (dotted light gray line) (n ≥ 3, ±SEM). Bioluminescence data were analyzed by BioDare. Arbitrary units are shown. (C) Representative Northern blots of gsn, gpn, and clock-controlled gene ccg-1 mRNA isolated from Δgpn or Δgpn cells harvested at the indicated times in DD. rRNA was used as a loading control. The data for gsn in Δgpn cells, and gpn in Δgpn cells, are plotted on the right (solid black lines, n ≥ 4, ±SEM), with both having a better fit to a linear line. (D) Northern blot of gsn mRNA from WT and Ptcu-r-gsn cells treated with low [L; 25 μM Cu or bathocuproinedisulfonic acid (BCS)], medium (M; 100 μM Cu or BCS), high (H; 250 μM Cu or BCS) levels, or untreated (U), and harvested at DD24. rRNA served as a loading control. (E) Plot of glycogen accumulation from WT (black line) and Ptcu-r-gsn cells (gray line) treated with 250 μM BCS over the indicated times in DD to consti-tutively overexpress gsn mRNA (gsnOE). Glycogen levels in gsnOE were better fit to a linear line.
and/or expression (45), and determined the levels of gpn promoter (44) or the gpn transcription and modulates the phase and amplitude of glycogen accumulation rhythms through control of gpn mRNA expression. Importantly, these data support that the circadian control of gpn expression is the primary driver of rhythmic accumulation of glycogen, because the loss of gpn rhythms in Δcsp-1 did not abolish rhythmic glycogen accumulation.

Discussion

Many metabolic functions are under control of the clock to ensure that they are produced at the appropriate time of day, such as stimulating catabolism during the active phase to support increased energy demands (32, 46, 47). The importance of clock control of metabolism is revealed by an increased incidence of metabolic disorders in mice and humans with a disrupted clock (3, 48). The clock component and nuclear receptor REV-ERBα has shown to play a key role in connecting the clock to metabolism in mammals (49–51). Rev-erbα is expressed with a rhythmic gpn transcription and promotes rhythmic glycogen accumulation. To test this function, we assessed the expression of gpn and gsn in Δcsp-1 cells. Rhythms in Pgsn-luc were abolished (Fig. S4A), while Pgsn-luc was rhythmic with a reduced amplitude (Fig. S5B) in Δcsp-1 cells compared with WT cells. Importantly, rhythmic glycogen accumulation persisted in Δcsp-1 cells with an ~5-h phase advance (Fig. S5C). Although CSP-1 functions primarily as a transcriptional repressor (37), low levels of gpn expression in Δcsp-1 cells compared with WT cells (Fig. S5A), suggested that CSP-1 may function as an activator of gpn expression. To test this further, we constructed strains with csp-1 controlled by either the quinic acid (QA)-inducible qa-2 promoter (44) or the β-tubulin promoter to constitutively induce the csp-1 expression (45), and determined the levels of csp-1 and gpn. The β-tubulin promoter drives constitutive overexpression of a target gene of interest (45). Increased expression of csp-1 at 1 h of quinic acid induction led to a similar increase in gpn mRNA levels 1 h later, supporting that CSP-1 activates gpn transcription (Fig. S5D). Taken together, these data indicate that CSP-1 is required for
Circadian rhythm in several tissues, including the liver, adipose tissue, muscle, and pancreas, and in these tissues, modulates lipid, glucose, and bile acid metabolism (50, 52). Similarly, in N. crassa, the clock-controlled TF, CSP-1, connects the circadian clock to metabolism by regulating ~200 genes involved in metabolic pathways, including glucose homeostasis (38, 47). However, the molecular mechanisms of circadian clock-controlled glucose homeostasis remain largely unknown. We utilized N. crassa as a model to uncover potentially conserved molecular mechanisms controlling rhythmic glycogen metabolism, a critical process in glucose homeostasis. We observed circadian oscillations of glycogen, gsn mRNA, gpn mRNA, and GSN and GPN protein levels. These data are consistent with previous animal studies demonstrating circadian rhythms of glucose metabolic parameters, including glycogen abundance, plasma glucose levels, and glucose tolerance (53). Moreover, hepatic glycogen synthase (Gys2) is a direct target of the mammalian core clock protein CLOCK, and both GYS2 and glycogen abundance show dampened circadian oscillations in Clock mutant mice (15).

We observed in-phase morning-specific mRNA levels of both gsn and gpn despite their opposing functions in glycogen metabolism. However, GSN and GPN function may not only depend on mRNA abundance, but also on their protein accumulation, enzymatic activities, and localization. In animals and fungi, GS and GP are regulated by allosterism and by reversible phosphorylation (7). Phosphorylated GS becomes inactive, whereas phosphorylation is required for the activation of GP. This results in a switch-like mechanism where one enzyme is active while the other one is inactive (7). While we have not yet investigated the impact of the clock on GPN phosphorylation in N. crassa, our data reveal the importance of transcription control of gsn and little, if any, role for phosphorylation of GSN in rhythmic glycogen accumulation (SI Appendix, Fig. SIC). In addition, in yeast and skeletal muscle cells, GS and GP display differences in their cellular localization that is dependent on glycogen concentration, with GS entering the nucleus when glycogen is depleted and GP remaining cytoplasmic (54–56). The nuclear localization of GS has been suggested to provide a warning signal that fuel levels are low, which then triggers transcription of genes necessary for increasing glycogen stores (56). These data suggest the possibility that loss of rhythmic gsn expression in Δgsp cells, as well as loss of gpn rhythmicity in Δgpn cells (Fig. 2C), may be due to changes in nuclear GSN-directed transcriptional control. As such, the coordinated regulation of gsn and gpn mRNA by the clock may provide a strategy to allow the organism to efficiently shift between glycogen synthesis and breakdown, depending on the time of day to maximize energy production in the active phase, or in response to nutritional stress. Consistent with this idea, glycogen synthase (Gys1 and Gys2) and glycogen phosphorylase (Pgly) genes are robustly rhythmic in mouse liver, with similar peak expression levels during the early-to-midsubjective night (57). Future experiments will be necessary to investigate potential clock and stress control of N. crassa GPN phosphorylation and activity, as well as the potential role for nuclear GSN in rhythmic gpn and gsn transcription.

Previous studies demonstrated that direct targets of CSP-1 peak in the evening, antiphase to direct WCC target genes that peak in the morning (37). However, our studies revealed that the cycling mRNAs of WCC target, gsn, and CSP-1 target, gpn, peak at the same time of day. To determine a possible mechanism for coordinated phase regulation of gsn and gpn, we retrieved the previous data (47, Table S1) to identify molecular wirings and parameter space that would reproduce our experimental data (SI Appendix). Computer simulations suggest that the rhythmic expression of gpn is independently regulated by both monomeric CSP-1 and heterodimeric CSP-1/VOS-1 complex with stronger activation by CSP-1/VOS-1 complex to satisfy the in-phase relationship between gsn and gpn mRNA levels, and the loss of gpn rhythmicity in Δcsp-1. In other words, our model suggests that the phase and rhythmicity of gsn is determined by VOS-1 and CSP-1, respectively. On the other hand, the model suggests that gpn is independently regulated in WCC and VOS-1. CSP-1 or VOS-1, respectively, to reproduce the reduced expression of gsn in Δvos-1 and the loss of rhythmicity of gsn in Δcsp-1. Our simulations suggest that WCC and CSP-1 regulate the rhythmic expression of gsn and gpn, respectively, and VOS-1 regulates the abundance and phase of gsn and gpn. These model-driven hypotheses will be experimentally validated in our future experiments. Furthermore, we plan to expand this model to investigate reciprocal regulation of GSN and GPN to determine the posttranslational modifications of GSN and GPN contributing to rhythmic accumulation and breakdown of glycogen.

Our data support that rhythmic gsn mRNA levels are necessary for rhythmic accumulation of glycogen. The main driver of rhythmic gsn expression appears to be through the rhythmic binding of WCC to the gsn promoter. First, conditions that alter gsn mRNA rhythms, amplitude, and/or phase (constitutive expression, deletion of wc-1, vos-1, or csp-1) similarly affect glycogen rhythms, whereas conditions that abolish gsn rhythms (deletion of csp-1) maintain rhythms in glycogen. Validation of this idea will require mutating the WCC binding sites in the promoter of gsn and assaying rhythms in gsn mRNA and glycogen levels. The role of CSP-1 and VOS-1, and possibly other TFs, in gsn regulation may be necessary for processing various inputs from the environment to adjust the timing of glycogen metabolism for maximum energy benefit. Interestingly, csp-1 transcription was previously shown to be activated under high glucose (2%) conditions (37), identical to our growth conditions. It is therefore also of interest to determine if glycogen rhythms and phase are altered in different glucose conditions. The WCC is not only a circadian TF, but also functions as a blue-light photoreceptor (29), and Aspergillus VosA is involved in stress responsive pathways, fungal development, and carbohydrate metabolism (43). Thus, similar to WCC light responsiveness, VOS-1 likely responds to environmental signals, such as nutrient stress, to modulate the expression of gsn.

In conclusion, we demonstrate that gsn mRNA rhythms are necessary for the daily cycle of glycogen abundance in the simple
model organism *N. crassa*. The complex mechanism of *gcn* and *gpn* promoter regulation, which appears to be conserved in mammalian cells (14), as well as possible feedback regulation of GSN on *gpn*, and GPN on *gcn*, equips the fungus with the ability to anticipate daily environmental stress (clock control), while at the same time providing flexibility to deal with acute stress, including nutrient availability, to coordinate energy production and other physiological processes under normal and stressful conditions.

### Materials and Methods

#### Strains and Culture Conditions

Strains for this study are described in *SI Appendix, Table S3*. Mutant strains were created as previously described (38). Primers for plasmid construction are listed in *SI Appendix, Table S3*. For circadian time course experiments, subjective day and night bars on the plots in the figures were determined based on the period of the rhythm (*SI Appendix, Table S1*) as previously described (40).

#### Other Methods

Culture conditions, RNA extraction, Northern blotting, protein extraction, Western blot assays, bioluminescence assay, ChIP-qPCR, glycan quantification, and data analysis are described in *SI Appendix, Materials and Methods*.

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