Complexity of the *Neurospora crassa* Circadian Clock System: Multiple Loops and Oscillators

R.M. DE PAULA,* M.W. VITALINI,* R.H. GOMER,[†] AND D. BELL-PEDERSEN*

*Center for Research on Biological Clocks and Department of Biology, Department of Biology, Texas A&M University, College Station, Texas 77843; [†]Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005

Organisms from bacteria to humans use a circadian clock to control daily biochemical, physiological, and behavioral rhythms. We review evidence from *Neurospora crassa* that suggests that the circadian clock is organized as a network of genes and proteins that form coupled evening- and morning-specific oscillatory loops that can function autonomously, respond differently to environmental inputs, and regulate phase-specific outputs. There is also evidence for coupled morning and evening oscillator loops in plants, insects, and mammals, suggesting conservation of clock organization. From a systems perspective, fungi provide a powerful model organism for investigating oscillator complexity, communication between oscillators, and addressing reasons why the system has evolved to be so complex.

INTRODUCTION

A diverse group of organisms, ranging from bacteria to humans, display daily rhythms in gene expression, protein levels, physiology, and behavior that are controlled by endogenous circadian clocks. These clocks are composed of limit-cycle oscillators based on molecular feedback loops, input pathways that transduce external information to the oscillators, and output pathways that allow the oscillators to temporally organize cellular and behavioral processes to specific times of the day.

Considerable effort has gone into understanding the molecular, biochemical, and physical properties of the circadian clock. In eukaryotic organisms, the molecular oscillators contain multiple interlocked feedback loops consisting of both positive and negative elements (Glossop et al. 1999; Lee et al. 2000; Shearman et al. 2000; Cheng et al. 2001a; Glossop and Hardin 2002; Preitner et al. 2002; Cyran et al. 2003; Locke et al. 2006). Several lines of evidence point toward the existence of multiple distinct oscillators in cells and/or tissues of organisms contributing to circadian timing. First, there exist free-running rhythms with different period lengths in the same organism (Morse et al. 1994; Sai and Johnson 1999; Cambras et al. 2007), and there is residual rhythmicity in strains that are defective in known oscillator components (Loros et al. 1986; Stanewsky et al. 1998; Emery et al. 2000; Collins et al. 2005). Second, some tissue-specific oscillators are constructed differently from the core oscillators located in the brain (Stanewsky et al. 1998; Ivanchenko et al. 2001; Krishnan et al. 2001; Hardin et al. 2003; Collins et al. 2005). Thus, oscillator complexity may arise both intracellularly and intercellularly in organisms with differentiated tissues.

There are at least three possible reasons why organisms would benefit from having multiple distinct oscillators or interlocking clock loops. First, the interlocked loops might have evolved to allow the circadian system to be more robust—in other words, immune or resistant to stochastic

noise or to small perturbations of the parameters. This is supported by the observation that alteration of the levels of components of the loops results in less precise clocks (Cheng et al. 2001a; Preitner et al. 2002; Locke et al. 2006). Second, multiple coupled loops could add to the degree of flexibility of the network by providing a mechanism for the environment to entrain the different loops with different input signals, so that, for instance, there could be one clock that is reset by light input and a different clock that is reset by a temperature shift (Rand et al. 2006). Third, increased flexibility of the system could also be achieved using multiple distinct oscillators that respond differently to environmental input and regulate different phase-specific outputs, so that, for example, morning-specific outputs would be controlled by an oscillator with one set of light and temperature inputs and evening-specific outputs would be controlled by a different oscillator with a different set of light and temperature input parameters.

The circadian clock system of *Neurospora crassa* has been studied extensively and is one of the best-understood circadian models (Loros and Dunlap 2001; Liu and Bell-Pedersen 2006; Vitalini et al. 2006; Heintzen and Liu 2007). Armed with a detailed description of core *N. crassa* FRQ/WCC feedback loop, we are now in a unique position to extend our current view of the oscillator to accommodate a growing body of evidence showing residual oscillations in the absence of the core FRQ/WCC oscillator components. We suggest that similar to plants and animals, the FRQ/WCC oscillator contains multiple coupled feedback loops that are important for precision and flexibility of the system.

THE INTERLOCKED FRQ/WCC FEEDBACK LOOPS

N. crassa displays an easily observable 22-hour rhythm in asexual spore development (conidiation) when cultures are grown in constant darkness (DD) (Pittendrigh et al.

1959), as well as rhythms in gene expression (Loros et al. 1989), metabolism (Shinohara et al. 1998), pheromone production (Bobrowicz et al. 2002), stress response (Shinohara et al. 2002), and other processes (for review, see Vitalini et al. 2006).

In *N. crassa*, the FRQ/WCC oscillator is considered to be the core circadian oscillator necessary for generating many of the observed circadian rhythms, including the developmental rhythm. The FRQ/WCC oscillator is known to be composed of an autoregulatory, transcriptional/translational feedback loop involving the *frequency* (*frq*) and *white collar* (*wc-1*, *wc-2*) genes and their protein products (Loros and Dunlap 2001; Liu and Bell-Pedersen 2006). The WC-1 blue light photoreceptor (Froehlich et al. 2002; He et al. 2002) forms a complex with WC-2 (white collar complex; WCC) that binds the *frq* promoter and directly activates transcription of the *frq* gene (Froehlich et al. 2003). Levels of FRQ protein then slowly increase. FRQ dimerizes (Cheng et al. 2001b) and forms a complex with FRH (a FRQ-interacting RNA helicase) (Cheng et al. 2005) that binds to, and promotes the phosphorylation of, the WCC by several kinases, including casein kinase I (CKI) and CKII (Schafmeier et al. 2005; He et al. 2006). Once hyperphosphorylated, activity of the WCC is inhibited such that it is unable to activate transcription of *frq*. This inhibition results in reduced *frq* transcript levels and a decrease in FRQ protein production, forming the negative part of the feedback loop. Subsequent phosphorylation-induced decay of FRQ, in conjunction with dephosphorylation of the WCC by protein phosphatase 2A (Schafmeier et al. 2005), releases the inhibitory affect on the WCC and leads to reactivation of *frq* transcription, allowing the cycle to start again the next day (Liu et al. 2000).

FRQ also acts in a positive feedback loop, maintaining levels of *wc-2* mRNA and WC-1 and WC-2 proteins (Lee et al. 2000; Cheng et al. 2001a, 2002). To investigate the importance of the positive feedback loop on clock function, *wc-1* or *wc-2* mRNA levels were artificially induced in cells (Cheng et al. 2001a). As the concentration of WC-1 or WC-2 increased, the amplitudes of the developmental rhythm and FRQ protein cycles increased. This suggested that the positive loop confers stability and robustness to the FRQ/WCC oscillator (Lee et al. 2000; Cheng et al. 2001a; Schafmeier et al. 2006).

The conflicting roles of FRQ in positive and negative feedback are now beginning to be understood in terms of spatial location and posttranslational modification (Schafmeier et al. 2006). Negative feedback through WCC is accomplished by nuclear FRQ that is hypophosphorylated and thus begins early after FRQ is expressed. Progressive phosphorylation of FRQ appears to trigger a switch of FRQ from a nuclear repressor to a cytoplasmic activator of WC-1 protein accumulation (Schafmeier et al. 2006). This regulation may be through FRQ-mediated phosphorylation of cytosolic WC-1 or WC-2, which could enhance their assembly and stability. However, the mechanism by which FRQ regulates the levels of *wc-2* mRNA is currently not known.

Although not well understood, WC-1 and WC-2 also regulate each other to form an additional loop in the

FRQ/WCC oscillator. WC-2 stabilizes WC-1 protein by forming the WCC, and WC-1 negatively regulates transcription of *wc-2* (Cheng et al. 2002, 2003).

A NEW LOOP IN THE FRQ-WCC OSCILLATOR NETWORK?

Several labs have demonstrated residual oscillations in *N. crassa* cells in the absence of FRQ or WC-1 and WC-2 (Loros et al. 1986; Merrow et al. 1999; Ramsdale and Lakin-Thomas 2000; Correa et al. 2003; Granshaw et al. 2003; Christensen et al. 2004; He et al. 2005; de Paula et al. 2006). These data suggested the existence of FRQ-independent oscillators. The term FRQ-less oscillator (FLO) has been coined to collectively describe the putative oscillators responsible for such rhythms (Iwasaki and Dunlap 2000).

To begin to investigate the FLOs, microarrays were used to identify genes that cycle with a circadian period in the absence of FRQ (Correa et al. 2003). Three evening-specific clock-controlled genes (*ccgs*) that displayed a daily rhythm in mRNA accumulation in FRQ-null strains were identified. One of these, W06H2 (now called *ccg-16*), was confirmed by northern assays to accumulate rhythmic mRNA in the absence of FRQ and under conditions in which the conidiation rhythm is abolished, such as during growth in constant light (LL) or when *frq* is constitutively overexpressed from an inducible promoter (de Paula et al. 2006). In wild-type strains, the oscillator that is responsible for generating the *ccg-16* rhythm responds to both temperature and light cues for synchronization; however, in the absence of FRQ, the oscillator responds better to temperature cues. Surprisingly, the *ccg-16* mRNA rhythm requires functional WC-1 and WC-2, suggesting that the oscillator responsible for *ccg-16* rhythms is coupled either directly or indirectly to the FRQ/WCC oscillator. To distinguish the *ccg-16* FLO from other potential *N. crassa* oscillators, it has been called the WC-dependent FLO (WC-FLO).

Importantly, the *ccg-16* mRNA rhythm is temperature-compensated in the FRQ-null strain (Q_{10} of 0.9), suggesting that FRQ is not absolutely required for temperature compensation. However, this conclusion needs to be taken with a certain level of caution because it was based on RNA blots using 4-hour time points to measure *ccg-16* mRNA rhythms at three different temperatures. Sampling of more time points using a *ccg-16*:luciferase reporter (a generous gift from J. Dunlap and J. Loros) in both FRQ⁺ and FRQ-null strains is currently being conducted to confirm these results.

Together, these data established that the oscillator driving *ccg-16* mRNA rhythms is not inhibited by high or low FRQ levels. In addition, *ccg-16* mRNA rhythms can be observed in both LL and DD, suggesting that the WC-FLO is a circadian oscillator that is not inhibited by high or low light levels. This is unlike the FRQ/WCC oscillator, which is functional only in the first 12–16 hours of LL following synchronization in light/dark cycles (Elvin et al. 2005).

Because WC-1 and WC-2 are required for rhythmic *ccg-16* mRNA levels in LL, we hypothesized that WC-1 protein levels might also be rhythmic under these same

conditions (WC-2 is not normally rhythmically expressed [Denault et al. 2001]). Indeed, a robust rhythm in WC-1 levels was also observed in wild-type strains grown in LL and in Δ FRQ strains grown in DD when the cultures were synchronized by a temperature transition (de Paula et al. 2006). Furthermore, two forms of cycling WC-1 were observed in wild-type strains, a large (~135 kD) and a small (~127 kD) form. The large form typically displayed a more robust rhythm than the small form, and only the large form was observed in Δ FRQ strains. Interestingly, the amplitude of the WC-1 rhythm was qualitatively higher in Δ FRQ strains, as compared the FRQ⁺ strains. The small form of WC-1 is likely due to alternative initiation from an in-frame methionine codon located within the WC-1 open reading frame at codon 88 (Cheng et al. 2003; Kaldi et al. 2006). The roles of the different WC-1 forms in the circadian clock are currently under investigation.

These results demonstrated that although FRQ is involved in maintaining the overall levels of WC-1 protein in the cell (Lee et al. 2000; Cheng et al. 2001a) and in generating the small form of WC-1, FRQ is not essential for rhythmic WC-1 levels. We speculate that components of the WC-FLO control WC-1 rhythms (see below). However, WC-1 rhythms were previously shown to have a long-period oscillation in the 29-hour period *frq*⁷ mutant strain (Lee et al. 2000; Cheng et al. 2001a), suggesting that when the FRQ/WCC oscillator is functional, it can influence the period of the WC-1 rhythm. This may be through coupling of the FRQ/WCC oscillator to the WC-FLO.

The function of the CCG-16 protein is unknown. The FRQ/WCC oscillator functions normally in a Δ CCG-16

strain. In addition, WC-1 protein accumulates rhythmically in DD in a Δ FRQ Δ CCG-16 strain, suggesting that CCG-16 is not a component of the WC-FLO that generates the WC-1 rhythm. Thus, *cgc-16* is likely an output of the WC-FLO.

Although the WC-FLO can function autonomously, the requirement of WC-1 and WC-2 for both the FRQ-based oscillator and the WC-FLO suggests that the two oscillators are coupled either directly or indirectly by the WC proteins. This situation is strikingly analogous to the two coupled oscillators proposed to explain the different responses of rhythmic pupal eclosion in *Drosophila* to light and temperature treatments and later used to explain splitting behavior in rodents in LL (Pittendrigh and Bruce 1959; Pittendrigh 1961; Pittendrigh and Daan 1976). In this model, a central morning oscillator (M) is light-entrainable and is coupled to a temperature-entrainable evening oscillator (E). While both oscillators are autonomous and can be directly entrained, the E oscillator requires the M oscillator for complete circadian properties and for proper phasing. Temperature directly entrains the E oscillator, and the E oscillator feeds back on the M oscillator to bring the system to equilibrium. In *N. crassa*, the M oscillator would be equivalent to the light-entrainable FRQ/WCC oscillator that controls morning-specific cgs and development, and the E oscillator would represent the WC-FLO that controls evening-specific cgs, such as *cgc-16* (Fig. 1). The WC-FLO would thus derive phase information from two sources: directly from environmental cues and indirectly through the light and temperature-responsive FRQ/WCC oscillator.

Figure 1. Speculative working model for the coupled E and M multiloop oscillator of *N. crassa*. For details of the model, see text. (Light-gray jagged arrow) Light input into the oscillator mechanism; (black jagged arrows) temperature inputs to both the FRQ/WCC oscillator and the WC-FLO; (black arrows) positive regulation; (bars) negative regulation. The dotted line between FRQ and X may explain residual developmental rhythms in the absence of FRQ or WC proteins. (Bottom left) Model of the three-loop *Arabidopsis* clock that contains M and E oscillators (Locke et al. 2006); (bottom right) intertwined E and M loop model of the mammalian clock (Leloup and Goldbeter 2004).

THE PREDICTED EVENING AND MORNING MULTILoop ARCHITECTURE OF PLANTS AND ANIMALS SUGGESTS A NEW WORKING MODEL FOR THE *N. CRASSA* CLOCK

A three-loop clock, comprising a system of intracellularly coupled M and E oscillators, was recently predicted for the *Arabidopsis* clock based on experimental evidence and mathematical modeling (Locke et al. 2006). This model accounts for the residual rhythmicity that can be observed in mutations of the core clock component TOC1 (Fig. 1). The model predicts the existence of two autonomous short-period oscillators, plus a third loop connecting the two. An M oscillator contains PRR7/9 and LHY/CCA1, and an E oscillator contains TOC1, GI, and an unknown component Y. The two oscillators are predicted to be coupled together in a loop that involves negative regulation of TOC1 by LHY/CCA and positive regulation of LHY/CCA1 from TOC1 through an unknown component X.

Similar to plants, there is also evidence in *Drosophila* (Stoleru et al. 2004) and mammals (Jagota et al. 2000; Daan et al. 2001) for separate control of M and E process. But unlike plants, the M and E oscillators of flies and mammals are present in different cells and are thus coupled together via cell–cell signaling. It is not known if M and E are two genetically distinct oscillators or two identical oscillators that are set to different phases using different input pathways.

Intertwined intracellular loops, similar to that proposed in plants, may also exist in mammals. Modeling of mammalian molecular clockwork revealed the existence of a new source of oscillations arising from negative regulation of *Bmal1* expression by *Rev-Erb α* (Fig. 1) (Leloup and Goldbeter 2004). In the absence of PER, oscillations of *Bmal1* mRNA persist. Furthermore, in the absence of the negative *Bmal1/Rev-Erb α* loop, BMAL1-dependent oscillations of PER and CRY also persist. The two oscillator loops are coupled through CLOCK-BMAL1. The first oscillator involves an indirect negative feedback exerted through the inhibitory binding of PER-CRY to CLOCK-BMAL1 complexes, which activates expression of *per* and *cry* mRNA. The second oscillator involves negative feedback exerted by CLOCK-BMAL1, via REV-ERB α , on the expression of *Bmal1*. Circadian oscillations that persist in *Rev-Erb α* knockout mice (Preitner et al. 2002), along with observations of restored rhythmicity by an extended light pulse in *mPer1/mPer2*-deficient mice (Bae and Weaver 2007), provide experimental support for the multiloop model.

On the basis of similarities of the FRQ/WCC oscillator and the WC-FLO to the coupled M and E oscillators of plants, flies, and mammals, we speculate that the WC-FLO constitutes a temperature-responsive evening loop of the FRQ/WCC oscillator (Fig. 1). This working model proposes that the WCC represses an unknown component X, which in turn activates the WCC. We predict that this activation is in part due to new synthesis of the large form of WC-1 protein by rhythmically active X protein because large WC-1 accumulates rhythmically in the absence of FRQ. The model predicts that the levels or activity of X

would be low when the WCC is active and high before the peak in WC-1 levels. X would also be predicted to peak antiphase to FRQ, providing an opportunity to regulate outputs at the opposite phase of the cycle (Fig. 1). When FRQ is overexpressed in cells, *cgc-16* mRNA and WC-1 rhythms are maintained (de Paula et al. 2006), and the levels of WCC increase. But most of the WCC would be phosphorylated and inactive for positive regulation of *frq* (He et al. 2006; Schafmeier et al. 2006). However, phosphorylation of the WC proteins might facilitate repression of X, allowing the WC-FLO cycle to continue. For this reason, we favor the WCC inhibiting X rather than WCC activating X, and X inhibiting the WCC to form the WC-FLO feedback loop. The levels of WC proteins are lower in FRQ-null strains; thus, FRQ is thought to positively regulate WC protein levels posttranscriptionally. In addition, the levels of *wc-2* mRNA are lower in FRQ-null strains, suggesting FRQ is somehow involved in transcriptional regulation of *wc-2*. Because it is hard to imagine how a single protein might carry out both of these activities directly, we wildly speculate that the WC-FLO would have one of these roles, such as in transcriptional repression of *wc-2*. When FRQ is absent, the levels of WCC would decrease. This would result in an increase in the activity of X, which would in turn result in lower levels of *wc-2* transcription. Finally, we predict that X would be responsive to a temperature input, allowing the WC-FLO to be directly responsive to temperature. Obviously, to test this model, we need to identify X and experiments are currently in progress to do this.

Coupling of the FRQ/WCC oscillator and WC-FLO through the WCC proteins would likely add additional stability to the system while allowing each oscillator the flexibility to control phase-dependent outputs. In the absence of external light and temperature cycles, the integrity of the circadian system would depend on this coupling to maintain identical frequencies and the appropriate phasing of the oscillators. To begin to test if both the FRQ/WCC oscillator and the WC-FLO are required for stable *cgc-16* mRNA rhythms, the phase, amplitude, and period of the *cgc-16* rhythms in wild type versus a Δ FRQ strain grown in DD or LL were compared (R. de Paula et al., unpubl.). As predicted, in the Δ FRQ strain, the amplitude of the *cgc-16* mRNA rhythms showed a significantly increased variation between experiments as compared to wild-type strains grown in DD (Fig. 2). These data support the notion that when the circadian oscillator network is intact, the precision of the *cgc-16* rhythms is increased.

EVIDENCE FOR MORE FLOS IN *N. CRASSA*

In *N. crassa* cultures grown with nitrate as the sole nitrogen source, daily rhythms in nitrate reductase activity are observed in DD or LL in wild-type strains, as well as in strains that lack FRQ or WC-1 (Christensen et al. 2004). These data indicated that a nitrate reductase oscillator (NRO), which can run in the absence of a functional FRQ/WCC oscillator and is separate from the WC-FLO, generates rhythms in nitrate reductase activity. However, in the absence of WC-1, the nitrate reductase activity

Figure 2. FRQ/WCC oscillator maintains precision of the WC-FLO that regulates *ccg-16* rhythmicity. Relative mRNA levels for *ccg-16* from wild-type and Δ FRQ cultures synchronized by a shift from 30°C to 25°C and grown in DD were determined by densitometry of northern blots and plotted versus time (hours in DD). Values are mean $\pm$ S.E.M. from six separate experiments. For each strain, at each timepoint, the data were normalized to the average value at that timepoint. The standard deviation of the normalized data for wild type was 0.427, and the standard deviation for Δ FRQ was 0.554. An F-test on the normalized data showed that the variance of the *ccg-16* levels in Δ FRQ was significantly higher than the variance in wild type with $p = 0.022$.

rhythms are of low amplitude and more variable, suggesting some connection to the FRQ/WCC;WC-FLO oscillator. It is not yet known if the NRO is temperature-compensated or entrained by light or temperature.

Other evidence for multiple oscillators in *N. crassa* cells comes from observations of residual developmental rhythms in strains that lack FRQ, WC-1, or WC-2 in DD (Loros et al. 1986; Aronson et al. 1994; Lakin-Thomas 1998, 2006; Merrow et al. 1999; Lakin-Thomas and Brody 2000; He et al. 2005; Pregueiro et al. 2005). However, the rhythms are defective in one or more of the canonical circadian oscillator properties—the rhythms are not stable, do not persist, are not temperature-compensated, and are not entrained by LD cycles. These data suggest that an intact FRQ/WCC oscillator is required for complete circadian properties of the developmental rhythm. Although somewhat controversial, the developmental rhythms appear to be entrained by temperature cycles (Merrow et al. 1999; Pregueiro et al. 2005; Roenneberg et al. 2005; Lakin-Thomas 2006). Thus, another possibility is that the residual developmental rhythms arise from parts of the FRQ/WCC oscillator or WC-FLO oscillator that remain intact and respond to temperature in the mutant strains. This would be best explained if FRQ and X are connected to each other in another loop, similar to the three-loop clock of *Arabidopsis* (Fig. 1).

To date, no progress has been made in identifying the molecular constituents of the FLO(s) responsible for the residual developmental rhythmicity. This lack of progress can primarily be attributed to the inherent variability of the FLO rhythms, making it difficult to screen for mutants that disrupt the FLO oscillations. However, a recent breakthrough may change our ability to characterize the molecular components responsible for the residual oscillations. The period of the FLO conidiation rhythm can be stabilized, through an unknown mechanism, by the addition of farnesol or geraniol, two intermediates of the sterol synthesis pathway, to the growth medium (Granshaw et al. 2003). Similar to the variable rhythm on unsupplemented medium, this stabilized rhythm still lacks some of the

canonical properties of circadian oscillators, including temperature compensation and light entrainment, but the rhythm can be reset by temperature pulses.

The ability to observe a consistent developmental rhythm in the absence of a functional FRQ/WCC oscillator now paves the way for identifying FLO components. Toward this end, the influence of other known clock-affecting mutations, in combination with FRQ or WC-2 deletion mutations, on the stabilized FLO developmental rhythm is being assayed (Lombardi et al. 2007). The ability to consistently observe a rhythm in the absence of the FRQ/WCC oscillator will also allow for the application of genetic screens for mutations that alter the FLO.

CONCLUSIONS

Enormous progress has been made in understanding the molecular mechanisms of the core circadian oscillators that were originally identified in genetic screens for mutations that alter a specific rhythmic behavior. We are now in a unique position to refine our view of the clock and incorporate information demonstrating residual rhythmicity in the absence of core clock components into working models, as carried out here for the *N. crassa* clock system. We suspect that these loops were not targeted in original mutant screens because they regulate different output pathways from those assayed, such as development rhythms in fungi (Feldman and Hoyle 1973) and eclosion and locomotion rhythms in flies (Konopka and Benzer 1971).

Experimental data and modeling support an organization of the core clock as a network of coupled oscillators, which can function when some of its parts are missing to regulate some aspects of rhythmicity. On their own, the individual loops appear to be less robust; this may be in part due to the components becoming more sensitive to cellular noise. Evidence is also accumulating to suggest the existence of genetically distinct circadian oscillators that can function as core oscillators to regulate specific outputs. In microbial organisms, the complexity exists within the cell, however; in multicellular organisms, complexity can occur both between and within cells.

We clearly still have a lot more work to do, the most important of which is to identify components of the new loops. The genetically tractable *N. crassa* clock system will continue to be an important model for identifying additional components and for developing a better understanding of the roles of a multi-oscillator clock.

ACKNOWLEDGMENTS

This research was supported in part by the National Science Foundation under a grant (PHY05-51164) to the Kavli Institute for Theoretical Physics, and National Institutes of Health grants (P01 NS39546 and R01 GM58529) to D.B.-P.

REFERENCES

- Aronson B.D., Johnson K.A., and Dunlap J.C. 1994. Circadian clock locus *frequency*: Protein encoded by a single open reading frame defines period length and temperature compensation. *Proc. Natl. Acad. Sci.* **91**: 7683.
- Bae K. and Weaver D.R. 2007. Transient, light-induced rhythmicity in mPer-deficient mice. *J. Biol. Rhythms* **22**: 85.
- Bobrowicz P., Pawlak R., Correa A., Bell-Pedersen D., and Ebbole D.J. 2002. The *Neurospora crassa* pheromone precursor genes are regulated by the mating type locus and the circadian clock. *Mol. Microbiol.* **45**: 795.
- Cambras T., Weller J.R., Angles-Pujoras M., Lee M.L., Christopher A., Diez-Noguera A., Krueger J.M., and de la Iglesia H.O. 2007. Circadian desynchronization of core body temperature and sleep stages in the rat. *Proc. Natl. Acad. Sci.* **104**: 7634.
- Cheng P., Yang Y., and Liu Y. 2001a. Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proc. Natl. Acad. Sci.* **98**: 7048.
- Cheng P., Yang Y., Gardner K.H., and Liu Y. 2002. PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. *Mol. Cell. Biol.* **22**: 517.
- Cheng P., Yang Y., Heintzen C., and Liu Y. 2001b. Coiled-coil domain-mediated FRQ-FRQ interaction is essential for its circadian clock function in *Neurospora*. *EMBO J.* **20**: 101.
- Cheng P., He Q., He Q., Wang L., and Liu Y. 2005. Regulation of the *Neurospora* circadian clock by an RNA helicase. *Genes Dev.* **19**: 234.
- Cheng P., Yang Y., Wang L., He Q., and Liu Y. 2003. WHITE COLLAR-1, a multifunctional *Neurospora* protein involved in the circadian feedback loops, light sensing, and transcription repression of *wc-2*. *J. Biol. Chem.* **278**: 3801.
- Christensen M.K., Falkeid G., Loros J.J., Dunlap J.C., Lillo C., and Ruoff P. 2004. A nitrate-induced frq-less oscillator in *Neurospora crassa*. *J. Biol. Rhythms* **19**: 280.
- Collins B.H., Dissel S., Gaten E., Rosato E., and Kyriacou C.P. 2005. Disruption of cryptochrome partially restores circadian rhythmicity to the arrhythmic period mutant of *Drosophila*. *Proc. Natl. Acad. Sci.* **102**: 19021.
- Correa A., Lewis Z.A., Greene A.V., March I.J., Gomer R.H., and Bell-Pedersen D. 2003. Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proc. Natl. Acad. Sci.* **100**: 13597.
- Cyran S.A., Buchsbaum A.M., Reddy K.L., Lin M.C., Glossop N.R., Hardin P.E., Young M.W., Storti R.V., and Blau J. 2003. vrille, Pdp1, and dClock form a second feedback loop in the *Drosophila* circadian clock. *Cell* **112**: 329.
- Daan S., Albrecht U., van der Horst G.T., Illnerova H., Roenneberg T., Wehr T.A., and Schwartz W.J. 2001. Assembling a clock for all seasons: Are there M and E oscillators in the genes? *J. Biol. Rhythms* **16**: 105.
- de Paula R.M., Lewis Z.A., Greene A.V., Seo K.S., Morgan L.W., Vitalini M.W., Bennett L., Gomer R.H., and Bell-Pedersen D. 2006. Two circadian timing circuits in *Neurospora crassa* cells share components and regulate distinct rhythmic processes. *J. Biol. Rhythms* **21**: 159.
- Denault D.L., Loros J.J., and Dunlap J.C. 2001. WC-2 mediates WC-1-FRQ interaction within the PAS protein-linked circadian feedback loop of *Neurospora*. *EMBO J.* **20**: 109.
- Elvin M., Loros J.J., Dunlap J.C., and Heintzen C. 2005. The PAS/LOV protein VIVID supports a rapidly dampened daytime oscillator that facilitates entrainment of the *Neurospora* circadian clock. *Genes Dev.* **19**: 2593.
- Emery P., Stanewsky R., Hall J.C., and Rosbash M. 2000. A unique circadian-rhythm photoreceptor. *Nature* **404**: 456.
- Feldman J.F. and Hoyle M.N. 1973. Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* **75**: 605.
- Froehlich A.C., Loros J.J., and Dunlap J.C. 2003. Rhythmic binding of a WHITE COLLAR-containing complex to the frequency promoter is inhibited by FREQUENCY. *Proc. Natl. Acad. Sci.* **100**: 5914.
- Froehlich A.C., Liu Y., Loros J.J., and Dunlap J.C. 2002. White Collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. *Science* **297**: 815.
- Glossop N.R. and Hardin P.E. 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. *J. Cell Sci.* **115**: 3369.
- Glossop N.R., Lyons L.C., and Hardin P.E. 1999. Interlocked feedback loops within the *Drosophila* circadian oscillator. *Science* **286**: 766.
- Granshaw T., Tsukamoto M., and Brody S. 2003. Circadian rhythms in *Neurospora crassa*: Farnesol or geraniol allow expression of rhythmicity in the otherwise arrhythmic strains *frq¹⁰*, *wc-1*, and *wc-2*. *J. Biol. Rhythms* **18**: 287.
- Hardin P.E., Krishnan B., Houl J.H., Zheng H., Ng F.S., Dryer S.E., and Glossop N.R. 2003. Central and peripheral circadian oscillators in *Drosophila*. *Novartis Found. Symp.* **253**: 140.
- He Q., Cheng P., He Q., and Liu Y. 2005. The COP9 signalosome regulates the *Neurospora* circadian clock by controlling the stability of the SCFFWD-1 complex. *Genes Dev.* **19**: 1518.
- He Q., Cha J., He Q., Lee H.C., Yang Y., and Liu Y. 2006. CKI and CKII mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the *Neurospora* circadian negative feedback loop. *Genes Dev.* **20**: 2552.
- He Q., Cheng P., Yang Y., Wang L., Gardner K.H., and Liu Y. 2002. White collar-1, a DNA binding transcription factor and a light sensor. *Science* **297**: 840.
- Heintzen C. and Liu Y. 2007. The *Neurospora crassa* circadian clock. *Adv. Genet.* **58**: 25.
- Ivanchenko M., Stanewsky R., and Giebultowicz J.M. 2001. Circadian photoreception in *Drosophila*: Functions of cryptochrome in peripheral and central clocks. *J. Biol. Rhythms* **16**: 205.
- Iwasaki H. and Dunlap J.C. 2000. Microbial circadian oscillatory systems in *Neurospora* and *Synechococcus*: Models for cellular clocks. *Curr. Opin. Microbiol.* **3**: 189.
- Jagota A., de la Iglesia H.O., and Schwartz W.J. 2000. Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. *Nat. Neurosci.* **3**: 372.
- Kaldi K., Gonzalez B.H., and Brunner M. 2006. Transcriptional regulation of the *Neurospora* circadian clock gene *wc-1* affects the phase of circadian output. *EMBO Rep.* **7**: 199.
- Konopka R.J. and Benzer S. 1971. Clock mutants of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **68**: 2112.
- Krishnan B., Levine J.D., Lynch M.K., Dowse H.B., Funes P., Hall J.C., Hardin P.E., and Dryer S.E. 2001. A new role for cryptochrome in a *Drosophila* circadian oscillator. *Nature* **411**: 313.
- Lakin-Thomas P.L. 1998. Choline depletion, *frq* mutations, and temperature compensation of the circadian rhythm in *Neurospora crassa*. *J. Biol. Rhythms* **13**: 268.
- . 2006. Circadian clock genes *frequency* and *white collar-1* are not essential for entrainment to temperature cycles in *Neurospora crassa*. *Proc. Natl. Acad. Sci.* **103**: 4469.

- Lakin-Thomas P.L. and Brody S. 2000. Circadian rhythms in *Neurospora crassa*: Lipid deficiencies restore robust rhythmicity to null *frequency* and *white-collar* mutants. *Proc. Natl. Acad. Sci.* **97**: 256.
- Lee K., Loros J.J., and Dunlap J.C. 2000. Interconnected feedback loops in the *Neurospora* circadian system. *Science* **289**: 107.
- Leloup J.C. and Goldbeter A. 2004. Modeling the mammalian circadian clock: Sensitivity analysis and multiplicity of oscillatory mechanisms. *J. Theor. Biol.* **230**: 541.
- Liu Y. and Bell-Pedersen D. 2006. Circadian rhythms in *Neurospora crassa* and other filamentous fungi. *Eukaryot. Cell* **5**: 1184.
- Liu Y., Loros J., and Dunlap J.C. 2000. Phosphorylation of the *Neurospora* clock protein FREQUENCY determines its degradation rate and strongly influences the period length of the circadian clock. *Proc. Natl. Acad. Sci.* **97**: 234.
- Locke J.C., Kozma-Bognar L., Gould P.D., Feher B., Kevei E., Nagy F., Turner M.S., Hall A., and Millar A.J. 2006. Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana*. *Mol. Syst. Biol.* **2**: 59.
- Lombardi L., Schneider K., Tsukamoto M., and Brody S. 2007. Circadian rhythms in *Neurospora crassa*: Clock mutant effects in the absence of a *frq*-based oscillator. *Genetics* **175**: 1175.
- Loros J.J. and Dunlap J.C. 2001. Genetic and molecular analysis of circadian rhythms in *Neurospora*. *Annu. Rev. Physiol.* **63**: 757.
- Loros J.J., Denome S.A., and Dunlap J.C. 1989. Molecular cloning of genes under control of the circadian clock in *Neurospora*. *Science* **243**: 385.
- Loros J.J., Richman A., and Feldman J.F. 1986. A recessive circadian clock mutation at the *frq* locus of *Neurospora crassa*. *Genetics* **114**: 1095.
- Marrow M., Brunner M., and Roenneberg T. 1999. Assignment of circadian function for the *Neurospora* clock gene *frequency*. *Nature* **399**: 584.
- Morse D., Hastings J.W., and Roenneberg T. 1994. Different phase responses of the two circadian oscillators in *Gonyaulax*. *J. Biol. Rhythms* **9**: 263.
- Pittendrigh C.S. 1961. Circadian rhythms and the circadian organization of living things. *Cold Spring Harbor Symp. Quant. Biol.* **25**: 159.
- Pittendrigh C.S. and Bruce V.G. 1959. Daily rhythms as coupled oscillator systems and their relation to thermoperiodism and photoperiodism. In *Photoperiodism and related phenomena in plants and animals* (ed. Withrow), p. 475. AAAS, Washington, D.C.
- Pittendrigh C. and Daan S. 1976. A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. *J. Comp. Physiol.* **106**: 333.
- Pittendrigh C.S., Bruce B.G., Rosensweig N.S., and Rubin M.L. 1959. Growth patterns in *Neurospora*. *Nature* **184**: 169.
- Pregueiro A.M., Price-Lloyd N., Bell-Pedersen D., Heintzen C., Loros J.J., and Dunlap J.C. 2005. Assignment of an essential role for the *Neurospora frequency* gene in circadian entrainment to temperature cycles. *Proc. Natl. Acad. Sci.* **102**: 2210.
- Preitner N., Damiola F., Lopez-Molina L., Zakany J., Duboule D., Albrecht U., and Schibler U. 2002. The orphan nuclear receptor REV-ERB α controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* **110**: 251.
- Ramsdale M. and Lakin-Thomas P.L. 2000. sn-1,2-diacylglycerol levels in the fungus *Neurospora crassa* display circadian rhythmicity. *J. Biol. Chem.* **275**: 27541.
- Rand D.A., Shulgin B.V., Salazar J.D., and Millar A.J. 2006. Uncovering the design principles of circadian clocks: Mathematical analysis of flexibility and evolutionary goals. *J. Theor. Biol.* **238**: 616.
- Roenneberg T., Dragovic Z., and Mellow M. 2005. Demasking biological oscillators: Properties and principles of entrainment exemplified by the *Neurospora* circadian clock. *Proc. Natl. Acad. Sci.* **102**: 7742.
- Sai J. and Johnson C.H. 1999. Different circadian oscillators control Ca(2+) fluxes and lhcb gene expression. *Proc. Natl. Acad. Sci.* **96**: 11659.
- Schafmeier T., Kaldi K., Diernfellner A., Mohr C., and Brunner M. 2006. Phosphorylation-dependent maturation of *Neurospora* circadian clock protein from a nuclear repressor toward a cytoplasmic activator. *Genes Dev.* **20**: 297.
- Schafmeier T., Haase A., Kaldi K., Scholz J., Fuchs M., and Brunner M. 2005. Transcriptional feedback of *Neurospora* circadian clock gene by phosphorylation-dependent inactivation of its transcription factor. *Cell* **122**: 235.
- Shearman L.P., Sriram S., Weaver D.R., Maywood E.S., Chaves I., Zheng B., Kume K., Lee C.C., van der Horst G.T., Hastings M.H., and Reppert S.M. 2000. Interacting molecular loops in the mammalian circadian clock. *Science* **288**: 1013.
- Shinohara M.L., Loros J.J., and Dunlap J.C. 1998. Glyceraldehyde-3-phosphate dehydrogenase is regulated on a daily basis by the circadian clock. *J. Biol. Chem.* **273**: 446.
- Shinohara M.L., Correa A., Bell-Pedersen D., Dunlap J.C., and Loros J.J. 2002. *Neurospora clock-controlled gene 9 (ccg-9)* encodes trehalose synthase: Circadian regulation of stress responses and development. *Eukaryot. Cell* **1**: 33.
- Stanewsky R., Kaneko M., Emery P., Beretta B., Wager-Smith K., Kay S.A., Rosbash M., and Hall J.C. 1998. The *cry^b* mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* **95**: 681.
- Stoleru D., Peng Y., Agosto J., and Rosbash M. 2004. Coupled oscillators control morning and evening locomotor behaviour of *Drosophila*. *Nature* **431**: 862.
- Vitalini M.W., de Paula R.M., Park W.D., and Bell-Pedersen D. 2006. The rhythms of life: Circadian output pathways in *Neurospora*. *J. Biol. Rhythms* **21**: 432.

Cold Spring Harbor Symposia on Quantitative Biology

Complexity of the *Neurospora crassa* Circadian Clock System: Multiple Loops and Oscillators

R. M. de Paula, M. W. Vitalini, R. H. Gomer, et al.

Cold Spring Harb Symp Quant Biol 2007 72: 345-351

Access the most recent version at doi:[10.1101/sqb.2007.72.002](https://doi.org/10.1101/sqb.2007.72.002)

References

This article cites 66 articles, 36 of which can be accessed free at:
<http://symposium.cshlp.org/content/72/345.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to *Cold Spring Harbor Symposia on Quantitative Biology* go to:
<http://symposium.cshlp.org/subscriptions>