# The Clock Keeps on Ticking: Emerging Roles for Circadian Regulation in the Control of Fungal Physiology and Pathogenesis


**Luis F. Larrondo and Paulo Canessa**

## Contents



L. F. Larrondo · P. Canessa
Millennium Institute for Integrative Biology (iBio), Santiago, Chile

L. F. Larrondo (✉)
Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile
e-mail: llarrondo@bio.puc.cl

P. Canessa
Facultad de Ciencias de la Vida, Centro de Biotecnologia Vegetal, Universidad Andres Bello, Santiago, Chile

Current Topics in Microbiology and Immunology (2019) 422: 121–156
https://doi.org/10.1007/82_2018_143

Published online: 26 September 2018

**Abstract** Tic-tac, tic-tac, the sound of time is familiar to us, yet, it also silently shapes daily biological processes conferring 24-hour rhythms in, among others, cellular and systemic signaling, gene expression, and metabolism. Indeed, circadian clocks are molecular machines that permit temporal control of a variety of processes in individuals, with a close to 24-hour period, optimizing cellular dynamics in synchrony with daily environmental cycles. For over three decades, the molecular bases of these clocks have been extensively described in the filamentous fungus *Neurospora crassa*, yet, there have been few molecular studies in fungi other than *Neurospora*, despite evidence of rhythmic phenomena in many fungal species, including pathogenic ones. This chapter will revise the mechanisms underlying clock regulation in the model fungus *N. crassa*, as well as recent findings obtained in several fungi. In particular, this chapter will review the effect of circadian regulation of virulence and organismal interactions, focusing on the phytopathogen *Botrytis cinerea,* as well as several entomopathogenic fungi, including the behavior-manipulating species *Ophiocordyceps kimflemingiae* and *Entomophthora muscae*. Finally, this review will comment current efforts in the study of mammalian pathogenic fungi, while highlighting recent circadian lessons from parasites such as Trypanosoma and Plasmodium. The clock keeps on ticking, whether we can hear it or not.

## 1 Introduction

Consecutive sunsets and sunrises clearly illustrate the fact that we live in a highly cyclic environment, exposed to daily oscillations in several factors: light (and darkness), temperature, solar radiation, environmental humidity, as well as variations in organismal interactions. These daily fluctuations can bring upon considerable challenges, i.e., thermal and osmotic stress, DNA damage, yet, the repetitive nature of these changes makes them predictable, which has contributed to the appearance of circadian clocks. These biological clocks are molecular machines that have emerged throughout evolution allowing organisms to keep track of time, even in the absence of celestial cues, permitting, therefore, to anticipate some of these daily challenges (Dunlap et al. 2004). Relevantly, as we will discuss in this chapter, in recent years, it has become evident that circadian clocks can also help anticipating daily fluctuations in biotic variables associated with organismal interactions, as exemplified in several studies.

It is essential to stop for a minute to analyze the etiology of the adjective that defines these clocks: circadian, from the Latin *circa diem,* which implies that these clocks measure time with a period of approximately, but not exactly, 24 h. Thus, in the absence of environmental cues (as in constant darkness), circadian clocks exhibit their free-running period, which is close to (but not precisely) 24 h (i.e., 22.5 or 25 h), while in the presence of environmental cycles, they are entrained to precisely 24 h. Thus, circadian clocks are endogenous subcellular devices that

confer daily rhythms to a broad range of biological processes such as gene expression, physiology, and behavior (Dunlap et al. 2004).

Despite their relevance, circadian clock components do not share sequence similarity across phyla, and the evidence suggests that they have emerged at least three independent times throughout evolution (Rosbash 2009). Thus, although the sequence characteristics of clock elements vary across distant organisms, such as cyanobacteria, insects, fungi, or mammals, in all cases they appear to share the same basic design: a transcription-translation negative feedback loop (TTFL). In such circuitry, positive elements drive the expression of negative elements that feedback to shut down their synthesis by inhibiting the positive elements, a process that repeats itself every ~24 h (Bell-Pedersen et al. 2005; Montenegro-Montero et al. 2015; Dunlap 1999). In the case of plants, the circuitry of the central oscillator appears more complex, involving the presence of additional loops (Romanowski and Yanovsky 2015). On the other hand, in prokaryotes, most studies have focused on the cyanobacteria *Synechococcus elongatus* (Mackey et al. 2011), while no clock components have been reported in Archaea. However, rhythms in peroxiredoxins oxidation have also been described in several organisms, including Archaea, which has been postulated as evidence of a common evolutionary ancestor (of unknown molecular composition and circuitry topology) in the origin of circadian rhythms (Edgar et al. 2012). Moreover, rhythms in magnesium levels have been shown to exist in fungi, unicellular alga, and mammalian cells, pointing to the presence of additional conserved underlying clock mechanisms across taxa (Feeney et al. 2016).

As mentioned, the free-running period of a circadian clock can be entrained to external rhythmic cues, being light and temperature the most relevant ones, such that its period matches the environment, which in planet Earth implies 24 h days. Thus, clocks can be synchronized or "entrained," allowing temporal control of several processes, optimizing them to certain times of the day (Dunlap 1999; Bell-Pedersen et al. 2005; Montenegro-Montero et al. 2015). Three basic requirements are generally considered in order to classify an oscillation as truly circadian: (1) It should persists in the absence of environmental cues [i.e., in constant light or constant darkness (free-running conditions)] with a period of *circa* 24 h; (2) it must be entrained by cyclical temperature or light signals; and finally, (3) it should exhibit temperature and nutritional compensation (Montenegro-Montero and Larrondo 2013; Dunlap 1999). Such characteristics allow distinguishing circadian-based rhythms from cell-cycle-regulated or environment-driven ones, and from other metabolic or developmental rhythms (Dunlap et al. 2004).

Accumulative evidence has helped to highlight the importance of clock regulation in organismal physiology and fitness. Their transversal relevance has been recently recognized with the 2017 Nobel Prize in Medicine awarded to work conducted in Drosophila, due to its implications to biology and human health (Dibner and Schibler 2017). Examples compiled in cyanobacteria, plants, and mammals, utilizing mutants with altered clocks, have revealed that when the internal circadian period matches the one in the oscillating environment improved fitness can be observed (Ouyang et al. 1998; Dodd et al. 2005; Yerushalmi and

Green 2009; Lowrey and Takahashi 2011). Several studies have shown the importance of circadian regulation in mammals, and how alterations or misalignments can lead to metabolic disorders and disease (reviewed in (Perelis et al. 2015; West and Bechtold 2015; Paschos and FitzGerald 2017; Scheiermann et al. 2018)). On the other hand, the evidence supports the effect of clock regulation of immune and defense responses not only in mammals, but also in flies and plants (Bhardwaj et al. 2011; Wang et al. 2011; Shin et al. 2012; Zhang et al. 2013; Goodspeed et al. 2012, 2013; Korneli et al. 2014; Ingle et al. 2015; Labrecque and Cermakian 2015; Lee and Edery 2008; Scheiermann et al. 2018). Therefore, the data supports the idea that circadian clocks confer an adaptive advantage, allowing anticipation of environmental changes, including interaction with other organisms and maximizing the efficiency of some processes at certain times of the day (McClung 2006; Roden and Ingle 2009).

*Neurospora crassa* is a filamentous fungus considered to be a premier model for circadian studies. In this ascomycete, daily spore production (conidiation) occurs just before dawn (a time when humidity is high, and the temperature is low) which would confer an adaptive advantage allowing enhanced spore dispersion and survival (Bell-Pedersen et al. 1996). Despite the extensive characterization of clocks mechanisms in Neurospora, there is scarce molecular information regarding circadian clocks in other fungal models including pathogenic ones. Therefore, how these clocks could modulate microbial virulence, and converse with the clock of the host, are questions that remain largely unexplored.

In the next pages, we will provide a basic view of clock mechanisms in Neurospora, to then review recent molecular and phenotypic description of clocks in other fungi, including pathogenic ones. Importantly, we will also cover emergent information of clock regulation on other pathogenic organisms.

## 2 Circadian Clocks: Neurospora and Beyond

The fungal kingdom comprises an enormous diversity of organisms, most of which remain uncharacterized. Conservative estimations indicate that it harbors at least 5.1 million species (Blackwell 2011), distributed in five main phyla: Chytridiomycota, Zygomycota, Glomeromycota, Basidiomycota, and the Ascomycota (James et al. 2006). The latter hosts the unicellular Saccharomycotina and Taphrinomycotina subphyla as well as the Pezizomycotina subphylum of filamentous fungi (Hibbett et al. 2007). Within the Pezizomycotina, one can distinguish groups such as the Dothideomycetes, Eurotiomycetes, Leotiomycetes, Sordariomycetes, Pezizomycetes, and the Orbiliomycetes, being the last two the most basal filamentous fungi (Traeger et al. 2013; James et al. 2006). Nevertheless, despite their great diversity and abundance, molecular work describing the mechanistic details of circadian clocks has concentrated exclusively in one species, the ascomycete *N. crassa*.

## 2.1 Molecular and Phenotypical Rhythms in Neurospora

Along with Drosophila, work in Neurospora paved the road to the molecular dissection of circadian clocks, thanks to its straightforward genetics and the ease to visualize a circadian phenotype: daily conidiation (Dunlap 2008). After almost 30 years of the cloning of the first clock components in both organisms, this is what we know so far. In Neurospora, the circadian TTFL (also called the FRQ-WCC oscillator) is based on a heterodimer formed by the transcription factors (TFs), White Collar 1 (WC-1), and White Collar 2 (WC-2). These GATA Zn-finger proteins interact through PAS domains to form the White Collar Complex (WCC), which controls the expression of the *frequency* (*frq*) gene. When FRQ (the negative element) is produced, it dimerizes to then associate with another core-clock component: FRH (FRQ-RNA-Helicase). This FRQ-FRH complex interacts with kinases, causing progressive phosphorylation of FRQ and promoting inactivation of WCC (by phosphorylation) with the concomitant drop in *frq* mRNA levels. The existing FRQ protein continues to be progressively phosphorylated in over 100 Ser/Thr sites by several kinases including CK1, CK2, and PKA, (Guo and Liu 2010; Montenegro-Montero et al. 2015). As this happens, the interaction between FRQ/FRH and the WCC decreases and therefore WCC recovers activity, as the hyperphosphorylated FRQ is degraded by the proteasome (He and Liu 2005). As FRQ is degraded new FRQ is produced and clear oscillations in *frq* and FRQ levels can be observed daily, every 22.5 h under free-running conditions (constant darkness). Recent results have highlighted posttranslational modifications of FRQ as a major mechanism regulating the speed of the clock, revealing also that degradation of hyperphosphorylated FRQ is not a critical event for the TTFL to reinitiate a new clock cycle (Larrondo et al. 2015; Dunlap and Loros 2018).

WC-1 is not only a TF, but also a photoreceptor, as it contains a light oxygen and voltage (LOV)-sensing domain. Under free-running conditions, such as constant darkness (DD), the WCC binds to a specific sequence in the *frq* promoter known as the clock-box (*c-box*), which is both necessary and sufficient to sustain *frq* rhythmic expression (Froehlich et al. 2003). In the presence of light, WCC suffers a conformational change and, driven by LOV-LOV interactions, forms a multimer with other light-activated WCCs. This leads to changes in DNA binding specificity, and therefore light-activated WCC recognizes a different sequence in the *frq* promoter, the proximal light regulatory element (*pLRE*), allowing a massive increase in *frq* expression upon exposure to light. These changes in *frq* levels are critical for the entrainment of the clock by environmental light (Froehlich et al. 2002; Montenegro-Montero et al. 2015).

In addition to the action of WCC on both the *pLRE* and *c-box*, several chromatin-remodeling events have been shown to control proper *frq* expression at both sites (Proietto et al. 2015; Wang et al. 2014). Nevertheless, although some co-repressors such as RCO-1 have been reported as core-clock component necessary for *frq* rhythmic levels by controlling *frq* chromatin status (Zhou et al. 2013),

recent data has challenged the clock-essential role originally assigned to RCO-1 (Olivares-Yanez et al. 2016).

In general, these rhythmic core mechanisms occurring at the circadian oscillator can be visualized as daily cycles in *frq* and FRQ levels. Importantly, through the input and output signaling pathways, respectively, this oscillator can be synchronized to the environment while it temporally controls a series of processes, such as conidiation (Montenegro-Montero et al. 2015).

Light and temperature are environmental cues that allow synchronizing and entraining the circadian oscillator, through the input pathways. In the case of light, *frq* expression is acutely induced through the *pLRE*, which leads to phase advances or delays, or to a complete resetting of the clock, depending on the intensity of the light stimuli (Crosthwaite and Heintzen 2010). While temperature pulses can also lead to changes in phase, probably the most remarkable—but poorly understood—characteristic of clocks is their emergent property of temperature compensation (period remains constant over a range of physiological temperatures). In the case of Neurospora, casein kinase 2 (CK2) has been shown to mediate such property by phosphorylation reactions that balance stabilization and destabilization of FRQ (Mehra et al. 2009).

The central oscillator in return controls a series of the cellular process through the output pathways. One of the key mechanisms by which the core oscillator provides rhythmic expression of *clock-controlled genes* (*ccg*s) relays on oscillatory transcription. This is in part mediated by a hierarchical arrangement of rhythmically expressed TFs [reviewed in (Montenegro-Montero et al. 2015)]. RNA-seq data indicates that up to 40% of the Neurospora genome may be rhythmically expressed, through mechanisms that also involve posttranscriptional control (Hurley et al. 2014). Nevertheless, the contribution of transcriptional versus posttranscriptional mechanisms in *ccg*s expression is a matter of debate in Neurospora, as well as in other models (Montenegro-Montero and Larrondo 2016). For simplicity, this section will just focus on the former. Under circadian conditions, the WCC directly recognizes promoters of hundreds of genes among which several encode for TFs, such as CSP-1, ADV-1, and SUB-1 (which are referred as *first tier* TFs). Not surprisingly, mRNAs for these TFs exhibit rhythmic expression, as well as their target genes (Smith et al. 2010; Sancar et al. 2015). Thus, WCC not only rhythmically controls *frq* in the TTFL, but also is a pivotal link between the core oscillator and the output pathways, acting as the executor of a hierarchical cascade of rhythmic transcription.

In Neurospora, conidiation provides a strong phenotype for clock output which can be easily visualized through the race-tube assay: hollow glass tubes, filled with agar, in which Neurospora grows from one end to the other producing asexual spores (conidia) once a day. This assay permits calculating clock properties such as period and phase, the latter defined as the time of appearance of a clock event (i.e., band of conidia) in relation with a reference point (i.e., transition between light and dark) (Montenegro-Montero et al. 2015).

Although luciferase (*luc*) has been a popular real-time reporter in the study of all circadian systems, such as Arabidopsis, cyanobacteria, and mammals (Welsh et al.

2005), its use in Neurospora started rather late (Gooch et al. 2008). Thus, codon optimized firefly luciferase controlled by the *frq* promoter helped visualizing *frq* expression dynamics under several physiological conditions, allowing to interrogate clock mechanisms in strains with altered output (Shi et al. 2007), or exhibiting growth defects (Olivares-Yanez et al. 2016). The design of modular circadian transcriptional reporters by putting luciferase under the control of a *c-box + minimal promoter* also allows to easily examine, simultaneously, multiple strains and genotypes in 96-well plates, revealing unexpected details of the circadian oscillator (Olivares-Yanez et al. 2016; Larrondo et al. 2015; Gooch et al. 2014; Hurley et al. 2013). Likewise, fusing *luc* with *ccg* promoters allows for the study of circadian output (Sancar et al. 2011; Hurley et al. 2014). In addition, another useful strategy has been to directly fuse, by homologous recombination (Larrondo et al. 2009), the *luc* sequence to the *frq* gene at its endogenous locus, generating a FRQ-LUC translational fusion, allowing real-time monitoring of FRQ levels (Larrondo et al. 2012).

Readers interested in other aspects of Neurospora core-clock mechanisms, including the role of kinases, period determination, and a detailed analysis of input and output pathways, can consult other specialized reviews elsewhere (Montenegro-Montero et al. 2015; Guo and Liu 2010; Hurley et al. 2015).

## 3 Rhythms in Non-pathogenic Fungi

Compared to the previous section that contained abundant data on circadian mechanisms in Neurospora, the next paragraphs may seem disappointing, as there is scarce information on clock mechanisms in other fungi.

There have been several reports describing rhythmic fungal phenomena, some dating over 60 years ago (Uebelmesser 1954; Schmidle 1951), yet most of those circadian systems have not been molecularly dissected (reviewed in (Bell-Pedersen et al. 1996). In the case of *Sordaria fimicola*, a Sordariomycete like Neurospora, the existence of rhythms in spore discharge, had also been reported (Austin 1968). Later on, it was shown that its *frq* gene was able to complement a *N. crassa frq* mutant strain, rescuing rhythmic conidial banding (Merrow and Dunlap 1994). One of the important things that this paper came to confirm was that *frq* was actually a gene involved in clock regulation and not just a regulator of asexual reproduction (conidiation).

Other genus belonging to the class of the Sordariomycetes is Trichoderma, where genomic analyses have confirmed the presence of *frq*, *wc*-1, and *wc*-2 homologs in *T. atroviride*, *T. reesei, T. virens*, and *T. pleuroticola* (Steyaert et al. 2010). In the latter, circumstantial data suggests the production of conidial rings under DD with a period near 24 h, although no rhythmic conidial phenotype has been described for any of the other mentioned Trichoderma species (Steyaert et al. 2010). Importantly, the absence of evidence is not evidence of absence: In this case, the lack of a visible overt rhythm is, by no means, evidence of the absence of

circadian regulation. Nevertheless, the *wc*-1 and *wc*-2 homologs (*blr*-1 and *blr*-2) are known to mediate blue light-induced conidiation in *T. atroviride*, as one would expect based on the available *N. crassa* model (Casas-Flores et al. 2004).

The fungus *Pyronema confluens* belongs to the early diverging Pezizomycetes group of filamentous ascomycetes. The sequencing of its genome revealed the presence of *frq, wc*-1, *wc*-2, and *frh* homologs (Traeger et al. 2013), whereas experiments confirmed that *P. confluens frq* expression is induced by light. Moreover, its mRNA oscillates under free-running conditions (DD), peaking in the subjective morning with a circadian period that is temperature compensated. Nevertheless, no overt circadian output phenotype was observed, although from a handful of analyzed genes only two behaved as *ccg*s displaying an oscillatory expression pattern (as seen by RT-qPCR) with profiles of morning-specific genes (Traeger et al. 2013; Traeger and Nowrousian 2015). Although this work shows that this *frq* homolog oscillates, strictly speaking it does not prove that the oscillations mechanism depends on *frq*, as the latter could be just a *ccg* in *P. confluens*. Therefore, additional work is needed to confirm a central role for this gene in clock regulation in this fungus.

The presence of a functional *frq* homolog, exhibiting rhythmic expression in *P. confluens* suggests that *frq* was present in the common ancestor of the filamentous ascomycete fungi (Pezizomycotina) (Traeger and Nowrousian 2015). In silico analysis appear to suggest that *frq* has been lost several times during evolution. For example, *frq* or *frq*-like sequences appear to be absent in most members of the Eurotiomycetes, which contains genus such as Aspergillus or Penicillium (Montenegro-Montero et al. 2015; Traeger and Nowrousian 2015). In addition, a FRQ-like sequence is present in the genome of *Saitoella complicata,* a fungus in the early diverging Taphrinomycotina subphylum of the Ascomycota. Yet, there is no vestige of *frq-like* sequences in *Saccharomyces cerevisiae* or other members of the Saccharomycotina subphylum (Montenegro-Montero et al. 2015). Moreover, our additional in silico studies identified a FRQ-like protein in *Rhizophagus irregularis*, whereas recently its mRNA expression has been confirmed (Lee et al. 2018). *R. irregularis* is an early diverging mycorrhizal fungus that belongs to the Glomeromycota phylum, which is located before the divergence of Basidiomycota and Ascomycota (Montenegro-Montero et al. 2015). This argues that the idea of FRQ being the result of a recent innovation restricted to a certain group of fungi followed by subsequent losses is unlikely. Indeed, distant FRQ homologs were also identified in some basidiomycetes (Montenegro-Montero et al. 2015) mainly restricted to the Pucciniomycotina subdivision. As an example, the *Sporobolomyces roseus* genome encodes for a FRQ-like sequence that exhibits a 28% identity with *N. crassa* FRQ. While these in silico analyses (Montenegro-Montero et al. 2015; Hevia et al. 2016) further expand the extent and depth of previous studies of *frq* distribution (Salichos and Rokas 2010), additional robust phylogeny studies are required to identify the evolutionary origin of FRQ and other core-clock components, as well as the co-evolution of ancillary co-opted components.

And what happens underground, under conditions in which light information may be restricted? That is a typical scenario in which one can encounter arbuscular

mycorrhizal fungi (AMF) interacting with plant roots, yet there is evidence of WCC homologs in such organisms and confirmation that in some, these homologs are actively expressed as seen in *Rhizoglomus irregularis*, an AMF belonging to the Glomeromycota (see above). Thus, a recent report not only describes that both the *wc*-1 and *wc*-2 homologs are expressed, but that there is also a *frq* homolog which expression is increased after a light pulse (Lee et al. 2018). Nevertheless, while discussing their results, the authors erroneously state that "so far, all the fungal species with conserved *frq* and *WC* proteins have a circadian clock" (Lee et al. 2018). Such statement is inaccurate, as the mere presence of *frq* and *wcc* encoding genes is not enough to assure that a clock will be functional, as this needs to be confirmed following some of the criteria stated above and found elsewhere (Montenegro-Montero et al. 2015). However, the fact that a *frq* homolog is present and expressed in a Glomeromycota member is quite remarkable. Our own analysis of the *R. irregularis* genome indicates that the encoded FRQ sequence exhibits a 45.5% of identity to the *N. crassa* one, while two other Rhizophagus genomes (*R. cerebriforme* and *R. diaphanous*) have sequences with 45.6 and 47.6% of identity to Neurospora FRQ. It is also worth mentioning the description, based on a sophisticated ecological observatory setup, of rhythms in Arbuscular mycorrhizal hyphal growth during 24 h, which appeared to parallel circadian oscillations occurring in the plants (Hernandez and Allen 2013). At the depths at which these fungi are normally interacting with the roots, light levels are rather negligible while temperature tends to display little daily variation (Hernandez and Allen 2013). Therefore, both light and temperature may not be acting as entraining or synchronizing cues for those fungal rhythms, which raises intriguing questions regarding their circadian nature as well as their dependence and/or interrelationship with the plant clock.

In the case of the ascomycete *Aureobasidium pullulans*, belonging to the Dothideomycetes class, daily formation of concentric rings, under particular media and light conditions, was known to exist, yet it was not clear if they were a manifestation of a *bona fide* clock (Slepecky and Starmer 2009). Analysis of an *A. pullulans* strain, isolated from the Argentinian Patagonia, confirmed that the formation of such daily rings was under circadian control, as they persisted (with a period of ~24.5 h) under constant conditions (DD) and could also entrain to different light:dark (LD) cycles (Franco et al. 2017). Moreover, these rhythms were also shown to be temperature compensated, as determined by growing *A. pullulans* at temperatures ranging from 10° to 20 °C. Remarkably, this appears to be the first experimental evidence of a circadian clock running at such low temperatures, something that may not be so surprising if one considers the natural cold ecological niche of this Patagonian *A. pullulans* isolate. At the molecular level, *A. pullulans* bears homologs of *wc*-1, *wc*-2, and *frq*, and while *frq* levels did not exhibit any clear oscillations under the tested conditions, it did show a strong response to light (Franco et al. 2017).

In *S. cerevisiae,* despite the absence of a *frq* homologue, or WCC encoding sequences, interesting phenomena have been described: rhythms in amino acid uptake, cell division, and metabolism (Edmunds et al. 1979). Interestingly, careful

examination of these metabolic oscillations has revealed that they share several mechanistic similarities to circadian rhythms, including the importance of post-translational modifications mediated by CK1 (Causton et al. 2015). In addition, these yeast metabolic rhythms have been described to exhibit circadian entrainment, responding to cycle length as well as the strength of environmental signals. Nevertheless, such oscillations display a weak free-running rhythm, consistent with a weak damped oscillator (Eelderink-Chen et al. 2010). Another member of saccharomycotina, *Schizosaccharomyces pombe*, does not appear to have *bona fide* circadian rhythms, although infradian temperature compensated oscillations in heat tolerance and ultradian rhythms in cell division have been observed [reviewed in (Montenegro-Montero et al. 2015)].

Previous genomic studies had concluded that ascomycetes genomes in the Eurotiomycetes class did not have *frq* homologs (Salichos and Rokas 2010). Nevertheless, subsequent analysis by our group identified FRQ-like sequences encoded in the genomes of *Talaromyces aculeatus* and *Talaromyces stipitatus.* Interestingly, such sequences appear shorter than the one in Neurospora, presenting identities of 19.5 and 20.6%, respectively (Montenegro-Montero et al. 2015). The Eurotiomycetes class also hosts the genus Aspergilli, with iconic fungi such as *Aspergillus nidulans*. As for most Eurotiomycetes, bioinformatic analyses of the *A. nidulans* genome failed to identify a *frq* homolog, although there are genes encoding for *wc*-1 and *wc*-2 homologs, which are involved in photobiology (Fuller et al. 2015). Aspergillus lacks any distinguishable circadian rhythm in conidiation, although rhythms in sclerotia formation have been reported in *Aspergillus flavus* (Greene et al. 2003), a plant and human pathogen (see below). On the other hand, when growing *A. nidulans* under DD conditions, infradian rhythms (28–32 h) in *glyceraldehyde*-3-*phosphate dehydrogenase* (*gpdA*) mRNA levels have been described. Importantly, when analyzing *gpdA* oscillations under temperature cycles, it presents entrainment with a period of 24 h (Greene et al. 2003).

As hinted earlier, the absence of an overt circadian phenotype, such as conidiation, does not necessarily imply the absence of an underling functional circadian clock. A clear example is Neurospora. Indeed, observing rhythmic conidiation in Neurospora isolates, under laboratory conditions, may not always be trivial, and actually, the *Neurospora* strain utilized since the 60's as the "WT strain" for circadian studies is a mutant isolate, called *band* (Sargent et al. 1966). This strain exhibited a robust circadian banding phenotype (spores observed as a defined daily band of conidia), and therefore, the *band* (*bd*) mutation has been systematically crossed into strains or isolates that are subjected to circadian studies. Subsequent studies revealed that *bd* corresponds to a point mutation in the *ras*-1 gene ($ras\text{-}1^{bd}$), which enhances circadian conidiation, allowing clear visualization of this phenotype in the race-tube assay, under laboratory conditions (Belden et al. 2007).

An exciting observation, with interesting implications in organismal dynamics, was recently described for the mushroom *Neonothopanus gardneri*, a fungus present in the Brazilian forest (Oliveira et al. 2015). This basidiomycete was shown to exhibit rhythms in bioluminescence that would persist even under DD conditions, with a period of $\sim$22 h, and a peak in the subjective night. One of the hallmarks of

this study is that it provided a nice example of adaptive advantage conferred by a biological clock to a fungus: Bioluminescence emitted at night helps attracting insects, which would then facilitate spore dispersion. Nevertheless, the molecular basis of the clock that regulates this rhythmic bioluminescence in *N. gardneri* remains unknown. Still, in silico analyses have revealed that weak FRQ homologs are present in a few members of the Basidiomycota phylum, further supporting the idea that FRQ was gained and lost several times during fungal evolution (Montenegro-Montero et al. 2015; Salichos and Rokas 2010).

## 4 Rhythms in Plant-Pathogenic Fungi

As indicated earlier, the presence and influence of clocks in pathogenic organisms have been a poorly explored area in general, with only a handful of publications reporting on this topic for fungal pathogens. Nevertheless, in the context of plant–pathogen interactions, the insect *Trichoplusia ni* stands as a nice example of circadian modulation of a pathogen's dynamics (Goodspeed et al. 2012). Rhythmic feeding behavior has been observed for *T. ni*, with a peak at midday under a light: dark cycle (LD), that persist even under DD conditions.

Overall, there is scarce, but interesting data regarding the presence and effect of circadian rhythms in pathogenic fungi, as we narrate now.

The soybean pathogen *Cercospora kikuchii*, which produces the phytotoxin cercosporin, is an ascomycete that belongs to the Dothideomycetes class (Daub and Ehrenshaft 2000). This fungal plant pathogen displays circadian rhythm of hyphal melanization under LD, a behavior that persists for many days under DD conditions, with a period close to 24 h. Importantly, this rhythmic melanization also persists at different temperatures between 20 and 30 °C (Bluhm et al. 2010). This same publication indicated the absence of a *frq* homolog in *C. kikuchii*, or from the closely related fungus *Cercospora zeae-maydis*, as tested by PCR or EST examination, respectively. Nevertheless, in silico genomic analysis has subsequently confirmed the presence of a *frq* homolog not only in *C. zeae-maydis,* but also in other members of the Capnodiales order, to which Cercospora belongs, including the closest related fungus *Mycosphaerella graminicola* (Montenegro-Montero et al. 2015). In addition, although the hemibiotrophic fungal maize pathogen *C. zeae-maydis* does not exhibit rhythmic hyphal melanization (Bluhm et al. 2010), it presents CRP1, a homolog of the *N. crassa* WC-1 (Kim et al. 2011a). A Δ*crp*1 *C. zeae-maydis* is impaired in virulence, exhibiting loss of stomatal tropism, defects in appressoria formation, conidiation as well as in the production of cercosporin. Importantly, it has been indicated that under field conditions *C. zeae-maydis* displays an alternating lesion pattern resulting from bands of conidiophores interspersed by areas of vegetative growth, which allows for the untested hypothesis that a circadian clock is governing such phenotype (Kim et al. 2011a). If such a clock actually actively impacts its pathogenic potential, depending on the time at which inoculation occurs, remains to be assessed.

As mentioned earlier, although no *frq* homologs are present in Aspergilli (Salichos and Rokas 2010; Montenegro-Montero et al. 2015), rhythms in sclerotia formation have been documented in the human and plant pathogen *Aspergillus flavus*. These developmental rhythms are dependent on the media composition, can be entrained by temperature and light cycles, and persist even under DD, with a period that is temperature compensated between 30 and 40 °C. This period of sclerotia formation nevertheless is of 33 h, closer to an infradian rhythm, compared to the ~22.5 h seen in Neurospora (Greene et al. 2003). Recently, a study that focused on the transcriptional control of genes needed for sclerotia development, including ones participating in cellular fusion, found some parallels with events occurring in *N. crassa*, raising interesting hypothesis of potential *ccg*s controlling this rhythmic phenotype in *A. flavus* (Zhao et al. 2017).

*Magnaporthe oryzae* has been ranked as probably the most important phytopathogen worldwide based both in its agronomic and scientific relevance (Dean et al. 2012). This ascomycete, a great model to study plant–pathogen interactions, infects important crops including rice, causing the well-known rice blast disease, a major problem as approximately one half of the world relays on rice as a primary food source (Dean et al. 2012). The genome of this Sordariomycetes exhibits homologs of *frq*, *wc*-1, and *wc*-2 (Salichos and Rokas 2010). And while there has not been yet a molecular characterization of a functional circadian oscillator in *M. oryzae*, diverse observations have led to propose, although not yet prove, that conidiation and virulence in this fungus are under circadian control (Deng et al. 2015). Some of the data that has led to this idea is that under LD cycles *M. oryzae* presents a conidial banding pattern or that the release of conidia occurs at night time (Lee et al. 2006). It is also interesting the fact that *M. oryzae frq* mRNA oscillates under a 12:12 LD cycle, although its behavior has not been reported under constant dark (Deng et al. 2015). Probably, the most compelling data regarding the presence of *bona fide* rhythms in this fungus relates to the expression of the *twilight* (*twl*) gene, which is induced by light, and displays rhythmic oscillations under LD and DD conditions, peaking at dawn (Deng et al. 2015). In LD *frq* mRNA continues to oscillate in the absence of TWL, which indicates that TWL is not a core-clock component, although it is necessary for full virulence (Deng et al. 2015). On the other hand, MGWC-1, the homolog of *N. crassa* WC-1, although not necessary for spore production, is needed for spore release (Lee et al. 2006) and it is also required to suppress disease development in LL (constant light) (Kim et al. 2011b).

There are also reports describing mycelial banding in the plant pathogen *Sclerotinia fructigena,* from the Leotiomycetes class (Jensen and Lysek 1983). This ascomycete, also known as *Monilinia fructigena*, shows mycelial banding that is temperature compensated and that persists even under free-running conditions (Jensen and Lysek 1983). Interestingly, it was reported that out of 181 examined isolates of this fungus that exhibited rhythmic growth pattern under LD cycles, only 30 of them would still present such rhythm in DD (Jensen and Lysek 1983). Our in silico analyses confirmed the presence of *frq, wc*-1, and *wc*-2 homologs in its genome, as it has also been described for *Sclerotinia sclerotiorum*, a close relative of *M. fructigena* (Salichos and Rokas 2010).

Further general information of clock phenomena in other fungi can be found elsewhere (Ramsdale 2008; Montenegro-Montero et al. 2015).

## 4.1 Molecular Characterization of the Circadian Clock of the Plant-Pathogenic Fungus Botrytis cinerea

*B. cinerea* is an aggressive necrotrophic fungal plant pathogen, causing the killing and rotting of plant material, also known as gray mold disease (Tudzynski and Siewers 2004). The genus Botrytis includes over 20 species, of which *B. cinerea* is the most infamous one, ranking second after *M. oryzae* as the most important phytopathogen, in terms of its relevance (Dean et al. 2012). *B. cinerea*, along with the necrotrophs *S. sclerotiorum* and *M. fructigena*, belongs to the Leotiomycetes class.

*B. cinerea* has been reported to infect over 1000 different plant species, including several relevant commercial crops, positioning this fungus as a major agronomical problem (Veloso and van Kan 2018). This versatile necrotroph is a professional assassin, capable of killing the host cells through the controlled production of reactive oxygen species, toxins, plant degrading enzymes, and an oxidative burst produced by the plant in response to *B. cinerea*. As a result, plant cells die and serve as a substrate for fungal growth and spreading of the infection (Veloso and van Kan 2018).

Among the many primary hosts of *B. cinerea* are dicotyledonous plants such as grape vine, strawberry, tomato, ornamental flowers, as well as monocots, although the latter are considered poor hosts. Botrytis infection is not limited to only one type of tissue, and, indeed, it can infect leaves, stems, flowers, and fruits, whether they are ripe or unripe (Schumacher and Tudzynski 2012). In addition, *B. cinerea* can remain quiescent in infected flowers for long periods, until the fruit ripens. Moreover, this fungus can be active at low temperatures (i.e., as low as 0 °C), with the obvious complications as post-harvest disease can occur as products are stored or in transit to international markets (Elad et al. 2007). Although the infection process can equally occur from 0 to 30 °C (Elad et al. 2007), optimal temperature for germination and infection is 20 °C, while adequate levels of humidity are also important for infection under field conditions (Latorre and Rioja 2002).

Conidiation in Botrytis, at least for the B05.10 wild-type strain, is dependent on the presence of light (Canessa et al. 2013). In the absence of light, microconidia are first produced, while after some weeks (3–4), sclerotia, dark melanin covered structures, are formed (Schumacher and Tudzynski 2012). While *B. cinerea* macroconidia are key in the infection process, microconidia fail to successfully infect as they generate a short germinal tube not compatible with the process (Jarvis 1977).

*B. cinerea* exhibits high genetic variability, which impacts aggressiveness, production of secondary metabolites, virulence factors, as well as the mode of

reproduction displayed by the different isolates (Schumacher and Tudzynski 2012). For example, regarding the latter point, while in the dark the B05.10 strain forms sexual structures (microconidia and sclerotia), the T4 strain (also a popular laboratory isolate) presents an "always conidia phenotype," which means that even in the dark it produces macroconidia, failing to form sexual structures (Canessa et al. 2013). Therefore, these different behaviors to a defined stimulus serve as cautionary tale: how critical is to choose a defined and well-characterized strain to conduct phenotypic and molecular assays. In the case of *B. cinerea*, due to its genetic stability (and easy genetic manipulation), the aggressive isolate B05.10 has become the standard recipient strain for genetic modifications and virulence studies (Buttner et al. 1994; Tuzdynski and Kokkelink 2009).

Although for a long time evidence indicated that *B. cinerea* responded to light at the phenotypic level, there was no molecular data on how this was occurring, yet a likely candidate to be mediating some of the light responses was the homolog of Neurospora WC-1 (Schumacher and Tudzynski 2012). As mentioned earlier for *N. crassa*, the TF and blue light receptor WC-1 along with WC-2 form the WCC, commanding responses to light and also being a core-clock component (Chen et al. 2010). In silico analysis of the Botrytis genome not only confirmed the presence of both WC-1 and WC-2, but also of a *frq* homolog, allowing their further molecular characterization. Thus, it was possible to confirm how light, through the action of *B. cinerea* WC-1 (BcWCL1), mediates some phenotypical responses and also activates changes in gene expression in this fungus (Canessa et al. 2013). As expected, several of the tested genes that respond to light in *B. cinerea* stop responding in a BcWCL1 knockout strain. Nevertheless, and in opposition to *N. crassa* where all light responses depend on WC-1, some light-responsive genes kept on being activated by this stimulus in a Δ*bcwcl*1 genetic background (Canessa et al. 2013), exemplifying the complex and fascinating photobiology of this organism (Schumacher 2017). Importantly, among the BcWCL1 light-activated genes, was *B. cinerea frq* homolog (*bcfrq*1), suggesting that at least in the context of light-induction its regulation was comparable to the Neurospora paradigm.

Despite the existence of core-clock components in Botrytis, and their regulation by light, this fungus fails to display rhythmic spore development in DD. Nevertheless, the *B. cinerea* B05.10 strain exhibits bands under LD cycles; although they are not made in a daily fashion and surprisingly, they are enhanced in the absence of BcWCL1 (Canessa et al. 2013). This lack of an overt circadian phenotype was not necessarily evidence of the absence of a molecular clock, and, indeed, molecular exploration of Botrytis *bcfrq*1 showed that its mRNA oscillates under LD (Hevia et al. 2015), being high during the lights-on period, to then decrease during the lights-off phase. Most importantly, it was possible to verify that *bcfrq*1 expression was anticipating the dark-to-light transition, as its levels would increase before lights on, a key feature of a circadian behavior (Dunlap 1999). Another classic circadian feature exhibited by *bcfrq*1 expression was that *bcfrq*1 mRNA levels displayed a robust oscillatory pattern under DD (as seen in *N. crassa* (Aronson et al. 1994)). This rhythm is lost in LL, since expression of *bcfrq*1 remains high and insensitive to the negative feedback mechanism, consistent with

the fact that in *N. crassa*, the circadian clock is dysfunctional under constant light (Crosthwaite et al. 1995). And, just as *bcfrq*1 requires BcWCL1 to respond to light (Canessa et al. 2013), it also depends on this TF to oscillate in DD, as seen in Neurospora (Lee et al. 2003). Additional experiments demonstrated that BcFRQ1 was a core-clock component, acting as the negative element, and not just an oscillatory gene. Thus, when an additional copy of *bcfrq*1 is expressed under the control of a strong actin promoter (overexpressing strain, OE::*bcfrq*1), the levels of the endogenous *bcfrq*1 mRNA remain low, as the transcripts coming from de $actin_{prom}$-*bcfrq*1 locus are high. (Hevia et al. 2015). Therefore, the BcFRQ1 generated from the $actin_{prom}$-*bcfrq*1 locus constantly closes the negative feedback loop on the native *bcfrq*1 (controlled by its endogenous promoter) by persistently inhibiting the WCC, recapitulating the original demonstration of the Neurospora circadian negative feedback loop (Aronson et al. 1994). Clock oscillations in *bcfrq*1 mRNA levels are expected to produce daily rhythms in BcFRQ1 protein levels. A method that allows easy tracking of FRQ levels in vivo, confirming the latter, is to generate a FRQ-LUCIFERASE translational fusion, obtained by fusing the *luc* sequence to *frq* (at its endogenous locus), as already tested in Neurospora (Larrondo et al. 2012). Thus, by quantifying bioluminescence, it was possible to confirm that BcFRQ1-LUC levels oscillate under DD and also under temperature cycles. In addition, using this BcFRQ1-LUC reporter, it was possible to confirm that Botrytis entrains to temperature cycles of different period length (T), which would not occur in the absence of a functional circadian oscillator (Pregueiro et al. 2005). The relevance of all these different results is that they provided, for the first time, molecular evidence of the existence of a functional circadian oscillator in a fungus other than *Neurospora* in general and in particular in any pathogenic organism (Hevia et al. 2015).

In Neurospora, FRQ plays a key role in controlling its circadian clock but, other than that it does not seem to serve any further function in additional cellular processes (Montenegro-Montero et al. 2015). Intriguingly, FRQ in Botrytis plays extra-circadian roles as inferred from the analysis of *B. cinerea* Δ*bcfrq*1, as this mutant produces (in rich undefined media) microconidia and sclerotia (sexual development) in the presence of light (Hevia et al. 2015). Nonetheless, Δ*bcfrq*1 recovers its normal developmental program in light (production of macroconidia), by simply changing media composition, which suggests an existing connection between nutrient signaling pathways and the processes controlled by BcFRQ1, different from the ones associated to the circadian clock (Hevia et al. 2015). Indeed, the phenotypes for Δ*bcfrq*1 are observed in constant light, a condition where—as mentioned earlier—the clock is not running. These BcFRQ1 non-circadian roles may imply an active repression of sexual development in the light, under particular nutritional conditions, and current efforts in our laboratory are devoted to explore such ideas.

Considering that non-extra-circadian functions have never been described for FRQ in Neurospora makes this unexpected extra-circadian function for BcFRQ1 all the more intriguing. Whether these extra functions of clock proteins are particular of pathogenic or necrotrophic fungi are, still, an open question.

## 5 Circadian Modulation of Plant–Pathogen Interactions

There is compelling evidence indicating that *Arabidopsis thaliana* immunity is modulated by a circadian clock, which allows anticipating and responding to the attack of certain pathogens (Sharma and Bhatt 2014; Ingle et al. 2015). Nevertheless, most of these reports have focused on the response of Arabidopsis to microbial pathogens in which there is no molecular evidence of endogenous circadian regulation, such as the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*), the bacteria *Pseudomonas syringae,* or the insect *T. ni.* Nonetheless, the characterization of a clock in *B. cinerea* B05.10 allowed analyzing the contribution of each circadian system in the outcome of a bipartite plant–pathogen interaction (Hevia et al. 2015).

### *5.1* The *B. cinerea- A. thaliana* Interaction*:* A Question of Time

Having simultaneous access to both *A. thaliana* and *B. cinerea* clock mutants allowed, for the first time, to analyze the contribution of a pathogen's clock to the outcome of plant–fungal organismal dynamics. The data revealed that the circadian machinery of this necrotrophic fungus is necessary to achieve a maximal relative virulence at dusk and that therefore the efficiency of the interaction between the pathogen and its host differs with the time of the day (Hevia et al. 2015). Importantly, the same conclusion was obtained when confronting the wild-type fungus to arrhythmic *A. thaliana* plants, since the leaves showed bigger lesions when inoculating the plants with Botrytis at dusk (night) than at dawn (morning), further indicating that the fungal clock is controlling the temporal aspect of the process (Hevia et al. 2015). Interestingly, the arrhythmic Arabidopsis mutants, corresponding to CCA1ox (constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1) and *Δ*975 (triple mutant in PSEUDORESPONSE REGULATORS PRR9 PRR7 PRR5), appeared to be overall more susceptible to infection than WT Arabidopsis, yet displaying bigger lesions in dusk-inoculated leaves. On the other hand, when eliminating the *B. cinerea* clock by tampering with its core oscillator components (Δ*bcwcl*1, Δ*bcfrq*1, or by OE::*bcfrq*1), it became clear that the *B. cinerea* circadian oscillator was responsible for the time-of-the-day difference in lesion size, either under DD or LD conditions. Thus, the data indicates that the fungal clock exerts control on Botrytis physiology such that it displays its maximal pathogenic potential at nighttime and that this effect disappears if the plant is infected with a *B. cinerea* clock mutant. Since it has been reported that one of the principal plant defense mechanism against necrotrophic fungi, jasmonic acid (JA), displays rhythmic levels with lows at night time (Goodspeed et al. 2012), it was plausible that such difference could explain the Botrytis-night increased effectiveness. Nevertheless, experiments conducted with WT Botrytis and Arabidopsis, utilizing environmental perturbations showed otherwise. By growing both organisms in

inverted LD cycles (e.g., daytime for Arabidopsis and nighttime for the fungus, and vice versa), it was observed that bigger lesions were obtained infecting dawn plants if the utilized fungal inoculum had dusk time. On the other hand, plants that had night (dusk) time yielded smaller lesions as they had been inoculated with fungus having morning (dawn) time. These data further confirms the weight of the fungal clock in regulating this organismal interaction, while downplaying a potential role of rhythmic JA levels in the interaction between *B. cinerea* B05.10– *A. thaliana* Col-0, at least under the tested experimental conditions (Hevia et al. 2015).

While both Δ*bcfrq*1 and OE::*bcfrq*1 arrhythmic mutant lose the ability to produce bigger lesions at dusk, they exhibit interesting differences in their overall virulence. Δ*bcfrq*1 shows increased aggressiveness at all times compared to the wild-type strain, while overexpression of *bcfrq*1 leads to equally reduced virulence at dawn and dusk, marked by very small lesions (Hevia et al. 2015). Thus, it would appear as if during parts of the day the clock is serving as a break for virulence (high *bcfrq*1 expression), which could be conceptualized as investing energy, efforts, and resources to other processes at certain hours, while concentrating on virulence at some other times. In other words, it becomes too expensive to be the best at everything 24/7, and therefore, the fungus needs to compromise and organize resources and strategies around the clock.

As it was previously indicated (see above), in the presence of constant light (LL) *B. cinerea* presents a non-functional circadian clock, since *bcfrq*1 expression is flat and low (Hevia et al. 2015), whereas the *A. thaliana* clock maintains its functionality under constant light conditions (McClung 2001). Remarkably, when WT plants are challenged with WT Botrytis in LL, differences in the size of lesions between infections carried out at dawn and dusk time were still observed. Nevertheless, such differences are no longer observed if CCA1ox arrhythmic plants are challenged with the wild-type fungus. These results demonstrate that under this particular environmental condition of constant light, the only clock that is functional, the one in Arabidopsis, has a preeminent role in determining the evolution and outcome of this organismal interaction. Likewise, a recent study also highlights the importance of the *A. thaliana* clock in the defense response against a *B. cinerea* (Ingle et al. 2015). The data, obtained with a *B. cinerea* pepper isolate, different from the B05.10 strain, also indicates that the outcome between Botrytis and Arabidopsis changes depending on the time of inoculation, such that bigger lesions are seen when the plant is inoculated at subjective night time, in concordance with previous reports (Hevia et al. 2015). Interestingly, this phenotype analyzed under LL conditions disappears in arrhythmic *elf*3-1 (EARLY FLOWERING 3) and *cca*1-*lhy* (MYB TFs CCA1 and LHY double mutant) plants. Transcriptomic data indicates that hundreds of Arabidopsis genes are differentially expressed at dawn versus dusk in response to Botrytis infection; among them, some key genes related to defense responses, which could partially explain the lower susceptibility of *A. thaliana* at dawn (Ingle et al. 2015). Importantly, several of these genes of interest correspond to TFs, targets of core-clock components, that exhibit rhythmic expression patterns, coincident with the idea that the defense responses are under strong circadian control in constant light. While differences in lesion size between

dawn and dusk were also observed in experiments conducted in LD between WT plants and the WT Botrytis isolate, the arrhythmic *elf*3-1 or *cca*1-*lhy* mutants were not tested under LD or DD (Ingle et al. 2015). Indeed, both LD and DD are conditions under which it has been reported that other arrhythmic plants challenged with WT *B. cinerea* (B05.10 strain) are still more susceptible at night which supports the fundamental role of the fungal clock in determining the outcome of the interaction (Hevia et al. 2015). Ingle and cols. also reported that under their experimental conditions, temporal changes in JA signaling appear to participate in this temporal differential response to the pathogen. JAZ transcriptional repressors participate in the activation of defense genes in response to JA and, when plant single or triple *jaz* mutants were infected, under LD or LL conditions, the plant appeared overall more resistant, and there was no difference in the size of the lesions at night compared to morning. Such results would indicate that the increased susceptibility displayed by Arabidopsis to Botrytis at dusk depends on changes in JA signaling. Interestingly, as mentioned earlier, the Botrytis strain utilized by Ingle and cols. is not the standard *B. cinerea* B05.10 strain and instead is a less characterized strain: a pepper isolate, where no circadian characterization has been conducted, and more importantly, a strain that produces conidia both in the light and in the dark (Ingle et al. 2015). As previously commented, it has been observed that the genetic diversity of *B. cinerea* sometimes translates in anomalous responses to the presence and absence of light: While some strains behave like B05.10 (conidiation in response to light, sclerotia in the dark, referred as "light-responsive strains"), different isolates exhibit diverse phenotypes such as "always sclerotia," "always conidia," and some "always fluffly," meaning that instead of changing their developmental program in light vs dark, they exhibit the above-mentioned phenotypes irrespective of the light conditions (Canessa et al. 2013). These differences, that remain to be molecularly dissected, also reflect divergence in the way light is sensed (or on how light-dependent genetic programs are implemented). Yet, this diversity of light-responsive phenotypes could also imply that the circadian clocks in some of these strains may be altered. This idea once again highlights the importance of strain selection when it comes to evaluating a particular biological process.

## 5.2 *Other Circadian Examples of Microbial Pathogen—A. thaliana Interactions*

Different aspects of the circadian modulation of plant immunity have been provided in the context of infections caused by oomycetes, insects, and bacteria (Sharma and Bhatt 2014; Korneli et al. 2014). It has been reported that the plant circadian clock, through its central component CCA1, modulates resistance against the oomycete *Hpa*, activating the expression of defense genes near the morning (dawn), when CCA1 expression is high (Wang et al. 2011). Not surprisingly *cca*1 clock-less

mutants display reduced resistance, whereas an arrhythmic mutant based on CCA1-overexpression displays increased overall defense response. This opposing resistance phenotype, by manipulating a central plant clock component, draws an interesting parallel to what happens when the fungal *B. cinerea* clock is turned arrhythmic by altering *bcfrq*1 levels by deletion of the gene or its overexpression (see above).

When *A. thaliana* plants are infected with *Pseudomonas syringae* pv *tomato* DC3000 and maintained under LL conditions, they exhibit decreased susceptibility when inoculated, by infiltration, at dawn compared to dusk (Bhardwaj et al. 2011). In addition, Arabidopsis arrhythmic plants (CCA1ox and *elf*-3) exhibit enhanced susceptibility at all times, as they show loss of temporal regulation in the defense response against this pathogen. Interestingly, *A. thaliana* plants display increased susceptibility at dawn to *P. syringae* pv *maculicola* DG3, if the bacteria are sprayed on the leaves, instead of infiltrated (Zhang et al. 2013). This enhanced susceptibility at dawn disappears in CCA1ox arrhythmic plants, mutants that also show overall enhanced susceptibility in both LL and LD. These studies along with other reports (Korneli et al. 2014) highlight the circadian modulation of plant immunity responses and the different outcomes depending on the time and mode of inoculation and infection. Interestingly, recent data has provided new insights, although this time from the pathogen's perspective. Extensive analyses of the ability of *P. syringae* pv. tomato DC3000 (*PsPto)* to sense different light wavelengths have shown that quality of light, and the time at which is administered, can impact virulence (Santamaria-Hernando et al. 2018). Thus, experiments carried by inoculating tomato plants with Pseudomonas, by immersion, at dusk or dawn, reported higher virulence at dawn whether the bacterial cells had been kept in the dark or given a 10-min light pulse of blue or white light. Nevertheless, when Pseudomonas cells were treated with a 10-min red light pulse previous to dusk or dawn inoculation, no time-of-the-day changes in virulence was observed and moreover, virulence appeared overall reduced. Similarly, loss of dawn-preferential virulence was also registered when analyzing different Pseudomonas photoreceptor mutants subjected to particular light treatments (Santamaria-Hernando et al. 2018). Although no circadian clock has been described for Pseudomonas, these results highlight how environmental manipulations of a pathogen can modulate key aspects of the plant–pathogen interaction, apparently overriding circadian changes in the plant. Thus, one could conceive that misleading environmental cues (red light in the night) may alter the way *PsPto* senses the environment, compromising its ability to take advantage of time-of-the-day opportunities, such as stomata opening at dawn.

## 6 Clock Regulation of Entomopathogenic Fungi

Several fungal entomopathogens have developed sophisticated mechanisms that allow them to manipulate host behavior, in such a way that they can increase the chances of spores discharge to infect new hosts. In this context, the term "zombie"

has been utilized to describe insects infected by a fungal or non-fungal entomopathogen that alters insect behavior and morphology, benefiting the pathogen. Interestingly, studies focused on such fungal–insect interactions have reported fascinating temporal aspects in the development of stereotypical phenotypes, or in key steps of the infectious cycle as, for example, the timing of fungal conidiation from insect cadavers.

Early field studies have reported daily rhythms in air-spore counts of several fungal species, including insect parasitic zygomycetes from the genus Entomophthora (Hamilton 1959; Lacey 1962). Thus, measurements have revealed a relative increase in Entomophthora spore levels in the last hours of the night (05:00-07:00 h), which could be interpreted as a response to environmental cues, such as increased humidity, or light from sunrise. Subsequent studies have confirmed these daily rhythms in spore release, with a peak around dawn, in different Entomophthora species such as *E. aphidis* and *E. muscae* (Wilding 1970). Part of this response could be directly modulated by light, be the product of entrained environmental conditions or overt circadian output. A report by Callaghan (Callaghan 1969) in *Conidiobolus coronatus* (*Entomophthora coronate*) indicates that a diurnal rhythm of spore discharge can be induced by alternating light:dark cycles, but it fails to persist under constant darkness and, therefore, appears to be conditioned by light at sunrise. But overall, these consistent daily rhythms of Entomophthora conidia, observed in the field (Wilding 1970), pose interesting questions regarding the underlying mechanisms. Importantly, such periodicity echoes the observations of diurnal patterns of aphids deaths, when infected with Entomophthora fungi (Milner et al. 1983). Indeed, under LD cycles, infected aphids tend to die at specific times (during the light phase), while differences in timing of death appeared to be dictated by the species of the parasitic fungus. Thus, aphids infected with the zygomycetes *Entomophthora planchoniana* and *Ervnia neoaphidis* (*Pandora neoaphidis*) preferentially died in a 4-h period window, with mortality peaks at 8 or 14 h post-dawn, respectively (Milner et al. 1983). These types of observations have led to postulate that such timing relates to the fact that entomophthoran fungi are adapted to transmit preferentially at night, as it has been postulated in studies involving the fungus *Entomophthora gammae* and the soybean looper *Pseudaletia includens*, insects that when infected die between 18:00 and 22:00 h, while *E. gammae* air conidia are reported to reach peaks after midnight (Newman and Carner 1974).

Other studies have measured *Neozygites fresenii* conidia in the air over cotton fields. This zygomycete when infecting the cotton aphid *Aphis gossypii* exhibited a clear daily rhythm of spore discharge reaching a peak at night, between 01:00 and 05:00 h (Steinkraus et al. 1996). As discussed by the authors, such timing of spore discharge puts conidia in the air at a time of the day (night) at which temperatures are rather low and humidity levels high, conditions that could favor survival and germination.

Interestingly, other reports contain similar observations for different fungal-insect systems. Thus, conidia of *Entomophaga maimaiga* appear to be preferentially discharged from dead gypsy moth larvae (*Lymantria dispar*) between

02:00 and 08:00 h (Hajek and Soper 1992). Importantly, the data suggests that the fungus also manipulates the larvae to die mainly in the afternoon, such that sporulation can occur at night, although not of the same day (Nielsen and Hajek 2006). Nevertheless, the evidence also appears to indicate that light cues and not merely a circadian clock may be responsible for these specific timings in the *E. maimaiga*-gypsy moth system (Nielsen and Hajek 2006).

Some recent studies have also focused on the timing of stereotypical behavior and death postures adopted by infected insects. The soldier beetle, *Chauliognathus pensylvanicus*, adopts a peculiar final posture attached to flowers, with its mandibles in a final "grim death grip" when infected by the zygomecete *Empusa lampyridarum*. Lock in place by its mandibles, the soldier beetle body is lean upward at a 45° angle with wings raised as in a flight position, which clears space for conidiophores to emerge from the abdomen of the dead insect. Remarkably, preliminary data indicates that this "grim death grip" tends to occur in the early morning of one day, while the wing-opening and conidiation process does not occur until the dark hours of the following morning, about 15–22 h after the death grip (Steinkraus et al. 2017).

The fungus *E. muscae* infects house flies (*Musca domestica*), killing them, using the insect tissue as food source and as a launching platform for abundant spore dissemination. To maximize that, the fungus manipulates the fly behavior such that in its last hours of life, the fly climbs up, affixes itself in place, and leaves its wings in a final up position, providing plenty of space for spores to be launched from reproductive structures emerging from the fly's abdomen. Interestingly, it has been reported that the time of the day at which flies stop moving, to then die, is not random and that instead it tends to occur around sunset (Krasnoff et al. 1995; Bellini et al. 1992). Recent studies, utilizing a *E. muscae* "Berkeley" isolate, capable of infecting *Drosophila melanogaster*, have verified a time preference for the fly last locomotory movements (Elya et al. 2018). Thus, in their very last day of life (about 4 days after being inoculated with the fungus), infected flies stop moving between 0-5 h before sunset (Elya et al. 2018). Remarkably, such behavior, both in regular house flies, or in *E. muscae* "Berkeley" infected *D. melanogaster*, it is only observed under LD cycles, since if flies are maintained in constant darkness (DD) they die at random hours throughout the day, instead of in a gated fashion (Elya et al. 2018; Krasnoff et al. 1995). As flies can sustain clear circadian rhythms in DD, these results could suggest that rhythmic light cues—processed by the fungus or by the fly—may be needed to confer a time-of-the-day preference to timing of death. Nevertheless, a relevant piece of information is presented in the study of Krasnoff and cols., as they report that the absence of time-of-the-day preference exhibited for infected flies housed under DD, can be overcome by entraining them under three 12:12 LD cycles before releasing them into DD (Krasnoff et al. 1995). This could be taken as that the Drosophila clock needs strong entrainment (before DD), to see such a phenotype. Nevertheless, the fact that the timing of last locomotor activity in the LD-to-DD protocol exhibited a period closer to 21 h, instead of a 24 h rhythm, was interpreted by the authors as evidence of a free-running fungal clock controlling this phenotype (Krasnoff et al. 1995). Thus,

such attractive hypothesis suggests that after 3 days of LD entrainment, the fungal clock would free run in the absence of external cues, manifesting its endogenous period, controlling the time of last movement of the fly. On the other hand, under LD conditions, external cues would synchronize the fungal clock to 24 h, and therefore, the phenomena would manifest itself with that periodicity, around the time of sunset (Krasnoff et al. 1995). Interestingly, transcriptomic analysis of *E. muscae* "Berkeley" as it infects *D. melanogaster*, confirmed the expression of the blue light-sensing WC-1 homolog in *E. muscae* (Elya et al. 2018), and although this fungus does not seem to harbor a *frq*-like gene, the existence of a functional circadian clock is plausible. Since it is now possible to study this entomopathogenic fungus in a Drosophila model, classic circadian experiments can be conducted to test if the fungal or the fly clock (or a cross-talk between them) is responsible for this temporal phenotype. Indeed, different Drosophila clock mutants, in combination with environmental perturbations, can be utilized to evaluate the contribution of the fly clock to this phenomenon, as it has been done in the past to assess the relevance of the fly clock in the circadian modulation of the response to bacterial infections (Lee and Edery 2008).

There is no doubt that zombie ants have captivated the attention of general public due to the clear change in behavior experimented by the ants, and the dramatic death and post-mortem phenotypes caused by fungal growth. Interestingly, a timing component is distinguishable in the infection process (de Bekker et al. 2014). Thus, field studies in a Thai rain forest have looked into the kinetics of the interaction between the fungus *Ophiocordyceps unilateralis s.l.* and its host ant *Camponotus leonardi*. Ants infected with this Sordariomycetes showed locomotor activity restricted to certain part of the day, followed by a clear preference for leave biting (Hughes et al. 2011). Before they die, these ants show stereotypical behavior that finalizes in a death grip, as they bite plant leaves to then be killed by the fungus. Interestingly, in the analyzed population, the timing of this final biting is synchronized around noon. It is also noticeable the fact that the ants exhibit a marked preference for abaxial leaf veins (Hughes et al. 2011). Thus, these observations allow speculating that such specific behavior could be controlled by a fungal clock. Moreover, the preference of abaxial versus adaxial side of the leaf could also be somehow controlled by the fungus. Indeed, it is known that light-sensing components in general not only allow responding to light or darkness, but also tuning responses depending on light-spectrum composition. Thus, the red/far-red light ratio is different on top versus the bottom side of the leaf, something that could be sensed by the fungus, eliciting changes that allow further controlling the ant behavior.

A laboratory-experimental setup to study the effect of an American *O. unilateralis s.l.* species isolate, on its natural host *Camponotus castaneus*, has been recently achieved. This setup that was kept under strict light:dark 24 h cycles showed that infected ants adopted the biting position at 09:00 h (3 h after lights on), to then die around 14:00 h (de Bekker et al. 2015). Such timing appears phase advanced compared to what was observed in the wild, in Thailand (Hughes et al. 2011), which is biting around noon followed by death 6 h later. These phase

differences could be associated with species-specific determinants or with particular characteristics of the experimental setup.

The new American isolated species of the *O. unilateralis* complex (de Bekker et al. 2015), now named *Ophiocordyceps kimflemingiae*, has served as a substrate for genomic and transcriptomic studies which have opened the door to explore the circadian biology of this ant fungal parasite (de Bekker et al. 2017). Thus, analysis of the *O. kimflemingiae* genome confirmed the presence of the Neurospora clock homologs *frq*, *wc*-1, and *wc*-2, as well as associated clock component. De Bekker and cols. where also able to identify laboratory conditions in which to grow *O. kimflemingiae* such that its morphological growth would resemble the one adopted when growing it in the *C. castaneus* ant. Moreover, they were able to analyze *C. castaneus* gene expression over 48 h by RNA-seq, with 4 h interval resolution. The analysis of the times course, one under LD and the other under DD conditions, revealed a different number of oscillating transcripts. In LD, over 300 genes showed oscillatory expression, whereas in DD the number was only 154. In LD, the *frq* homolog showed a rhythmic pattern, although in DD it did not pass the threshold to be classified as such. Interestingly, among the oscillating genes in LD, there were several predicted to be involved in parasite–host interactions, and 14 genes encoding for TFs, which suggests a potential hierarchical transcriptional cascade leading to ample *ccg* expression. Also, among the rhythmic transcripts, there was overrepresentation of genes encoding for 41 small-secreted and 15 secreted proteins, including five enterotoxins and six proteases in the latter category. Moreover, among the secreted enzymes, there were a tyrosinase and tyrosine phosphatase (de Bekker et al. 2017), which had been already identified as genes of interest in a previous study (de Bekker et al. 2015). And, although the overlap between the total number of rhythmic genes under LD and DD conditions was small (only 26 candidates), the above-mentioned type of genes encoding for small-secreted and secreted proteins was overrepresented (5 and 6, respectively). The temporal pattern of these 11 genes is also interesting, as nine peaks in the dark phase in LD, or during the subjective night in DD. And, among these night-expressed genes, there were ones encoding for chloroperoxidase, exo-beta-D-glucosaminidase, and enterotoxin, amid other attractive ones. Of interest is also the day-peak expression of two rhythmic genes encoding for TFs and for a histidine phosphotransferase.

In general, the analysis of the time courses revealed that in LD 52% of the rhythmic genes were up-regulated during the light period. On the other hand, analysis of the DD time course indicated that the majority of the rhythmic genes (64%) had peaks of expression during the subjective night. Based on diverse analyses of their data, the authors conclude that the majority of the day-active genes observed in LD are probably only light-driven and not necessarily clock regulated, whereas among the night-active rhythmic genes, clock-control is more likely to be occurring. Thus, while an important number of TFs reach maximal expression in light, several rhythmic genes that encode for secreted components get expressed during the night and may play important roles in the interaction with the ant.

The fact that ant foraging behavior (which is under tight clock regulation) becomes disrupted when the fungal infection is in place suggests that somehow the fungus can alter the host circadian regulation or the temporal control of the ant behavior. Interestingly, limited transcriptomic data of infected ants revealed changes in the expression of two clock components of infected versus not infected ants, which is commented by the authors as a circumstantial, but provoking piece of information that could help explaining their altered behavior (de Bekker et al. 2014).

## 7 Circadian Regulation of Human Pathogenic Fungi: An Area Awaiting for Systematic Exploration

While there is scarce information regarding the regulation of virulence of fungal phytopathogens, there is even less data reporting the existence of clock phenomena, mechanisms, or daily regulation of virulence of human pathogenic fungi.

Nevertheless, there is an increasing number of reports describing rhythmic modulation of the mammalian immune system and how the ability to respond to different immune challenges varies throughout the day (Labrecque and Cermakian 2015; Scheiermann et al. 2018). And, although there are several nice examples of circadian modulation of the innate immunity in response to bacteria, as well as viruses (Curtis et al. 2014; Scheiermann et al. 2018), this has not been systematically addressed for fungal pathogens.

And while, as mentioned earlier, there is no description of clock regulation of human pathogenic fungi, this section will revise some reports that are setting the pace for future exploration in this area. Thus, there have been studies on the effect of light on the physiology of important human fungal pathogens. One of the earlier reports focused on *Cryptococcus neoformans*, a fungus that represents a serious threat for immunocompromised individuals, showing that it was capable of sensing blue light through a WC-1 ortholog. Deletion of this gene led to attenuated virulence in a mouse model (Idnurm and Heitman 2005). Similarly, a WC-1 homolog was also identified in the plant and human pathogen *Fusarium oxysporum*. While deletion of the *F. oxysporum wc*-1 does not affect virulence in tomato, it decreases virulence when tested in mice, indicating that this gene is important to achieve full virulence (Ruiz-Roldan et al. 2008).

Until today, there is not enough evidence to interpret the phenotypes of these particular *wc*-1 mutants to defects in circadian regulation. Instead, these defects may originate on altered genetic programs due to the absence of White Collar TFs and the inability to mount some responses to light. Indeed, functional WC-1 signaling appears important to cope with stress, and some of these mutants, for example, appear more sensitive to oxidative stress (Fuller et al. 2015). In the case of *Aspergillus fumigatus*, oxidative stress sensitivity only becomes evident when another light-sensing component, the phytochrome *fphA*, is simultaneously deleted

(Fuller et al. 2013). Nevertheless, the existence of a functional clock in this organism remains an open question.

In the interphase of the host–pathogen dynamics, there are plenty of examples of how time of the day may influence the ability of a mammalian host to overcome bacterial and viral insults. And although some of these examples go almost 50 years back (Feigin et al. 1969), the molecular understanding of how clock regulation modulates immunity is still work in progress. Thus, for example, it is known that in macrophages about 8% the transcriptome oscillates daily, which includes different components involved in pathogen recognition (Keller et al. 2009). Recently, this circadian paradigm was tested in the context of *A. fumigatus* infection, particularly assessing if macrophage activity against this pathogen, or its clearance from the lungs was under circadian regulation (Chen et al. 2018). Thus, the authors measured Dectin-1 expression in macrophages, as this receptor can play a key role in recognizing fungal components. Nevertheless, they did not observe circadian expression of Dectin-1, nor clock expression of other receptors such as Dectin-2 or TLR4. Likewise, they failed to observe rhythmic phagocytosis of labeled *A. fumigatus* spores by macrophages. Nevertheless, when testing if clearance of *A. fumigatus* from the lungs changed throughout the day, they observed significant differences if inoculation had occurred at ZT0 vs ZT12, a mechanism that could depend on other macrophage receptors and/or some other immune cells, such as neutrophils. Interestingly, this twofold enhanced clearance of the fungus, when animals had been inoculated at night, was only evident when smaller fungal inocula ($10^5$ spores vs $10^7$ spores) were utilized (Chen et al. 2018).

In toto, while these observations indicate that light is an environmental variable capable of modulating virulence, little is known on how circadian variables may modulate fungal pathogenic potential or the interaction dynamics between pathogenic fungi and a mammalian host. It would be interesting to envision strategies, based on concepts such as chronotherapy, being applied in the efficient treatment of fungal pathogens, either by defining the best times and doses for antifungal administration or by perturbation of the clock of the pathogen (i.e., by distinct disruptive photoperiods).

Additionally, it will be informative to witness what emerges out of comprehensive microbiome studies not only focused on bacterial components, but also addressing the fungal composition and temporal variation of different mammalian associated microbiota. Indeed, recent studies have shown that the intestinal microbiota, in both mice and humans, exhibits diurnal compositional and functional oscillations (Thaiss et al. 2014). Such studies, focused on the bacterial aspects of the gut microbiota, have revealed unexpected effects of this microbial rhythm on the host circadian oscillations (Thaiss et al. 2016). Therefore, it would be interested not to only unveil if, for example, the natural skin or mucous fungal microbiota may exhibit daily changes, but to also understand whether such hypothetical oscillations could modulate the growth of opportunistic or pathogenic fungi.

## 8 Evidence of Clock Regulation in Other Pathogenic Organisms

Several reports describe the existence of daily rhythms of parasitic infections affecting mammalian hosts, although none of those examples include fungi [reviewed in (Rijo-Ferreira et al. 2017b)]. The manipulation of time variables in organismal interaction dynamics appears as a smart way to play around defense/susceptibility daily fluctuations of the host, as well as maximizing the chances of encountering new fresh victims. Thus, some of these rhythms seem to have adapted to the final host, as seen for the trematodes *Schistosoma mansoni* (Mouahid et al. 2012). Indeed, this parasite presents a specific developmental stage as it emerges from snails to then swim in fresh water, where it infects its hosts. While the emergence of this infectious stage is rhythmic and its chronotype appears to match its diurnal host (humans), a new night chronotype was described to be able to infect rats, a nocturnal animal (Mouahid et al. 2012). Such adaptation could be interpreted as the ability of the parasite to use endogenous circadian regulation to adapt to a time at which a new suitable host is present.

Herein, we will mention two different examples for which molecular data can explain, at least in part, reported rhythms in parasites.

### *8.1 Trypanosoma*

Daily rhythms in the amount of blood parasites have been described for different organisms, including various Trypanosoma species such as *T. rotatorium* in the blood of frogs, or *T. congolense* and *T. lewisi* in the blood of rodents (Rijo-Ferreira et al. 2017b).

In the case of *T. brucei,* the causal agent of sleeping sickness in humans, no daily rhythmic levels of this parasite have been detected in bloodstream. Nevertheless, a hallmark of this disease is the disruption of the sleep/wake cycle, along with alteration of core body temperature and the timing of endocrine secretion, which strongly suggest a circadian alteration of the host caused by the parasite. Indeed, studies have shown that mice infected with *T. brucei* suffer circadian alteration that manifests as phase advance of the host clock (associated with a period shortening), and abnormal activity during the rest phase. Notably, the period shortening is not only occurring at the organismal level, but also at the cellular level, both in vivo and in vitro (Rijo-Ferreira et al. 2018).

In vitro studies have revealed that *T. brucei* has an intrinsic circadian clock that modulates its metabolism as well as gene expression. Time courses of cultivated *T. brucei,* in its bloodstream form, showed that $\sim$10% of its genes exhibit rhythmic expression, as measured by RNA-seq. Since light appeared as a weak entraining signal, temperature was assessed as an entraining cue. A time course of temperature-entrained (32/37 °C) bloodstream *T. brucei,* yielded $\sim$1490 genes

(∼15% of the genome) oscillating, while a time course collected under constant temperature (after the parasites had been temperature-entrained for 3 days), revealed 1092 rhythmic transcripts (∼11 of the genome). Interestingly, a similar experiment was conducted with *T. brucei* procyclic forms, adapted to the tsetse fly gut environment, with a protocol involving a 23/28 °C temperature entrainment, or under constant temperature after this entrainment, yielding 1123 and 854 rhythmic genes, respectively. What is remarkable is that comparing the circadian gene sets obtained from the bloodstream or the procyclic (insect) forms reveals only 127 genes in common (Rijo-Ferreira et al. 2017a). Thus, the *T. brucei* clock appears to be able to sense the environment (fly vs bloodstream) and temporally control different gene sets, to better face the evolutionary constrains and challenges that each microenvironment imposes.

The *T. brucei* clock was shown to be temperature compensated, and also to be running in vivo, as tested in a mouse model. Many of these cycling genes impact metabolic pathways, ATP levels, and even resistance of the parasite to the commonly used drug suramin. Therefore, the parasite is not always the same throughout the day, in terms of its expression pattern and also on the way it interacts with its local environment. Analysis of the *T. brucei* genome does not allow explaining the underlying clock mechanisms driving these rhythms. Yet, it has been postulated that posttranscriptional mechanisms may be playing an important role, in accordance with the observed behavior of the asynchronous expression of polycistronic units in this parasite (Rijo-Ferreira et al. 2017a).

## *8.2 Plasmodium*

An interesting and puzzling observation is that replication of malaria parasites occur in synchrony with the circadian rhythm of the host (Mideo et al. 2013). In its blood stage, Plasmodium, the causing agent of Malaria, exhibits a synchronous asexual cycle, from the invasion of the red blood cells until their timely bursting. Such cycles, which are associated with cyclic fevers in the host, last 24 h or multiples of 24 h, depending on the species (Hawking et al. 1968). Recent studies conducted with *Plasmodium chabaudi*, in a mouse model, have shown that inflammation-induced hypoglycemia negatively affects *P. chabaudi* replication, while active proliferation of the parasite occurs during food intake, in synchrony with the host circadian cycle. Thus, circadian fluctuations of TNFα and food intake play a key role in synchronizing the parasite with the host rhythm such that if time of food availability is inverted, then the cycle of *P. chabaudi* is also inverted, whereas no rhythm is observed in diabetic mice (Hirako et al. 2018). Thus, rhythms associated with the time of feeding and metabolism, and not light-entrained rhythms at the level of the mouse suprachiasmatic nucleus, appear key in determining the phase of *P. chabaudi* oscillations (Prior et al. 2018).

## 9 Conclusions

Despite the overwhelming diversity of existing fungal species, almost everything that we know about circadian mechanisms in fungi has been deciphered in *N. crassa*. Nevertheless, there are a large number of observations that indicate that rhythmic phenomena are common in different fungi, pathogenic, and non-pathogenic ones. Only in recent years, molecular data has emerged depicting the clockworks in other fungi, providing the tools to directly assess the role of circadian regulation in diverse processes, such as fungal virulence. The implementation of real-time reporters such as luciferase has boosted the speed by which fundamental aspects of circadian mechanisms can be addressed. As this type of research expands to different fungi, it will be possible to understand what is transversal and what is particular to different species. Moreover, the abundance of fungal genomes can help in providing new insights into the evolution of fungal clock components, with lessons that can be also extrapolated to metazoans. As time passes, our knowledge about how hours are measured within cells keeps on advancing.

**Acknowledgements** Work in our laboratories is funded by iBio, Iniciativa Científica Milenio-MINECON (L.F.L and P.C.), and CONICYT/FONDECYT 1171151 and International Research Scholar of the Howard Hughes Medical Institute, both to L.F.L.

## References

Aronson BD, Johnson KA, Loros JJ, Dunlap JC (1994) Negative feedback defining a circadian clock—autoregulation of the clock gene-frequency. Science 263(5153):1578–1584. https://doi.org/10.1126/science.8128244

Austin B (1968) An endogenous rhythm of spore discharge in *Sordaria fimicola*. Ann Bot 32 (2):262–278

Belden WJ, Larrondo LF, Froehlich AC, Shi M, Chen CH, Loros JJ, Dunlap JC (2007) The band mutation in *Neurospora crassa* is a dominant allele of ras-1 implicating RAS signaling in circadian output. Genes Dev 21(12):1494–1505. https://doi.org/10.1101/gad.1551707

Bell-Pedersen D, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, Zoran MJ (2005) Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet 6 (7):544–556. https://doi.org/10.1038/nrg1633

Bell-Pedersen D, Garceau N, Loros JJ (1996) Circadian rhythms in fungi. J Genet 75(3):387–401

Bellini R, Mullens BA, Jespersen JB (1992) Infectivity of two members of the *Entomophthora muscae* complex [Zygomycetes: Entomophthorales] for *Musca domestica* [Dipt.: Muscidae]. Entomophaga 37:11–19. https://doi.org/10.1007/bf02372969

Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC (2011) Defence responses of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* are regulated by the circadian clock. PLoS ONE 6(10):e26968. https://doi.org/10.1371/journal.pone.0026968 (PONE-D-11-15640 [pii])

Blackwell M (2011) The fungi: 1, 2, 3 … 5.1 million species? Am J Bot 98(3):426–438. https://doi.org/10.3732/ajb.1000298

Bluhm BH, Burnham AM, Dunkle LD (2010) A circadian rhythm regulating hyphal melanization in *Cercospora kikuchii*. Mycologia 102(6):1221–1228. https://doi.org/10.3852/09-041 (09-041 [pii])

Buttner P, Koch F, Voigt K, Quidde T, Risch S, Blaich R, Bruckner B, Tudzynski P (1994) Variations in ploidy among isolates of *Botrytis cinerea*: implications for genetic and molecular analyses. Curr Genet 25(5):445–450

Callaghan AA (1969) Light and spore discharge in Entomophthorales. Trans Br Mycol Soc 53:87–97. https://doi.org/10.1016/S0007-1536(69)80010-0

Canessa P, Schumacher J, Hevia MA, Tudzynski P, Larrondo LF (2013) Assessing the effects of light on differentiation and virulence of the plant pathogen *Botrytis cinerea*: characterization of the white collar complex. PLoS ONE 8(12):e84223. https://doi.org/10.1371/journal.pone.0084223

Casas-Flores S, Rios-Momberg M, Bibbins M, Ponce-Noyola P, Herrera-Estrella A (2004) BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. Microbiology 150(Pt 11):3561–3569. https://doi.org/10.1099/mic.0.27346-0 (150/11/3561 [pii])

Causton HC, Feeney KA, Ziegler CA, O'Neill JS (2015) Metabolic cycles in yeast share features conserved among circadian rhythms. Curr Biol 25(8):1056–1062. https://doi.org/10.1016/j.cub.2015.02.035

Chen CH, Dunlap JC, Loros JJ (2010) Neurospora illuminates fungal photoreception. Fungal Genet Biol 47(11):922–929. https://doi.org/10.1016/j.fgb.2010.07.005 (S1087-1845(10)00131-3 [pii])

Chen S, Fuller KK, Dunlap JC, Loros JJ (2018) Circadian clearance of a fungal pathogen from the lung is not based on cell-intrinsic macrophage rhythms. J Biol Rhythms 33(1):99–105. https://doi.org/10.1177/0748730417745178

Crosthwaite SK, Heintzen C (2010) Detection and Response of the *Neurospora crassa* circadian clock to light and temperature. Fungal Biol Rev 24:114–122

Crosthwaite SK, Loros JJ, Dunlap JC (1995) Light-induced resetting of a circadian clock is mediated by a rapid increase in frequency transcript. Cell 81(7):1003–1012

Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA (2014) Circadian clock proteins and immunity. Immunity 40(2):178–186. https://doi.org/10.1016/j.immuni.2014.02.002

Daub ME, Ehrenshaft M (2000) THE PHOTOACTIVATED CERCOSPORA TOXIN CERCOSPORIN: contributions to plant disease and fundamental biology. Annu Rev Phytopathol 38:461–490. https://doi.org/10.1146/annurev.phyto.38.1.461

de Bekker C, Merrow M, Hughes DP (2014) From behavior to mechanisms: an integrative approach to the manipulation by a parasitic fungus (*Ophiocordyceps unilateralis* s.l.) of its host ants (*Camponotus* spp.). Integr Comp Biol 54(2):166–176. https://doi.org/10.1093/icb/icu063

de Bekker C, Ohm RA, Loreto RG, Sebastian A, Albert I, Merrow M, Brachmann A, Hughes DP (2015) Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. BMC Genom 16:620. https://doi.org/10.1186/s12864-015-1812-x

de Bekker C, Will I, Hughes DP, Brachmann A, Merrow M (2017) Daily rhythms and enrichment patterns in the transcriptome of the behavior-manipulating parasite Ophiocordyceps kimflemingiae. PLoS ONE 12(11):e0187170. https://doi.org/10.1371/journal.pone.0187170

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13(4):414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Deng YZ, Qu Z, Naqvi NI (2015) Twilight, a novel circadian-regulated gene, integrates phototropism with nutrient and redox homeostasis during fungal development. PLoS Pathog 11(6):e1004972. https://doi.org/10.1371/journal.ppat.1004972

Dibner C, Schibler U (2017) Body clocks: time for the Nobel Prize. Acta Physiol (Oxf). https://doi.org/10.1111/apha.13024

Dodd AN, Salathia N, Hall A, Kevei E, Toth R, Nagy F, Hibberd JM, Millar AJ, Webb AA (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science 309(5734):630–633

Dunlap JC (1999) Molecular bases for circadian clocks. Cell 96(2):271–290

Dunlap JC (2008) Salad days in the rhythms trade. Genetics 178(1):1–13

Dunlap JC, Loros JJ (2018) Just-so stories and origin myths: phosphorylation and structural disorder in circadian clock proteins. Mol Cell 69(2):165–168. https://doi.org/10.1016/j.molcel.2017.11.028

Dunlap JC, Loros JJ, DeCoursey PJ (2004) Chronobiology: biological timekeeping. Sinauer Associates, Sunderland, Mass

Edgar RS, Green EW, Zhao Y, van Ooijen G, Olmedo M, Qin X, Xu Y, Pan M, Valekunja UK, Feeney KA, Maywood ES, Hastings MH, Baliga NS, Merrow M, Millar AJ, Johnson CH, Kyriacou CP, O'Neill NS, Reddy AB (2012) Peroxiredoxins are conserved markers of circadian rhythms. Nature 485(7399):459–464. https://doi.org/10.1038/nature11088 (nature11088 [pii])

Edmunds LN Jr, Apter RI, Rosenthal PJ, Shen WK, Woodward JR (1979) Light effects in yeast: persisting oscillations in cell division activity and amino acid transport in cultures of Saccharomyces cerevisiae entrained by light-dark cycles. Photochem Photobiol 30(5):595–601

Eelderink-Chen Z, Mazzotta G, Sturre M, Bosman J, Roenneberg T, Merrow M (2010) A circadian clock in Saccharomyces cerevisiae. Proc Natl Acad Sci USA 107 (5):2043–2047. https://doi.org/10.1073/pnas.0907902107

Elad Y, Williamson B, Tudzynski P, Delen N (2007) *Botrytis* spp. and diseases they cause in agricultural systems—an introduction. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds) Botrytis: biology, pathology and control. Springer Netherlands, pp 1–8. https://doi.org/10.1007/978-1-4020-2626-3_1

Elya C, Lok TC, Spencer QE, McCausland H, Martinez CC, Eisen M (2018) Robust manipulation of the behavior of *Drosophila melanogaster* by a fungal pathogen in the laboratory. Elife 7. https://doi.org/10.7554/elife.34414

Feeney KA, Hansen LL, Putker M, Olivares-Yanez C, Day J, Eades LJ, Larrondo LF, Hoyle NP, O'Neill JS, van Ooijen G (2016) Daily magnesium fluxes regulate cellular timekeeping and energy balance. Nature 532(7599):375–379. https://doi.org/10.1038/nature17407

Feigin RD, San Joaquin VH, Haymond MW, Wyatt RG (1969) Daily periodicity of susceptibility of mice to pneumococcal infection. Nature 224(5217):379–380

Franco DL, Canessa P, Bellora N, Risau-Gusman S, Olivares-Yanez C, Perez-Lara R, Libkind D, Larrondo LF, Marpegan L (2017) Spontaneous circadian rhythms in a cold-adapted natural isolate of *Aureobasidium pullulans*. Sci Rep 7(1):13837. https://doi.org/10.1038/s41598-017-14085-6

Froehlich AC, Liu Y, Loros JJ, Dunlap JC (2002) White Collar-1, a circadian blue light photoreceptor, binding to the frequency promoter. Science 297(5582):815–819. https://doi.org/10.1126/science.1073681 (1073681 [pii])

Froehlich AC, Loros JJ, Dunlap JC (2003) Rhythmic binding of a WHITE COLLAR-containing complex to the frequency promoter is inhibited by FREQUENCY. Proc Natl Acad Sci USA 100 (10):5914–5919. https://doi.org/10.1073/pnas.1030057100 1030057100 [pii]

Fuller KK, Loros JJ, Dunlap JC (2015) Fungal photobiology: visible light as a signal for stress, space and time. Curr Genet 61(3):275–288. https://doi.org/10.1007/s00294-014-0451-0

Fuller KK, Ringelberg CS, Loros JJ, Dunlap JC (2013) The fungal pathogen *Aspergillus fumigatus* regulates growth, metabolism, and stress resistance in response to light. MBio 4(2). doi: mBio.00142-13 [pii] https://doi.org/10.1128/mbio.00142-13

Gooch VD, Johnson AE, Bourne BJ, Nix BT, Maas JA, Fox JA, Loros JJ, Larrondo LF, Dunlap JC (2014) A kinetic study of the effects of light on circadian rhythmicity of the *frq* Promoter of *Neurospora crassa*. J Biol Rhythms 29(1):38–48. https://doi.org/10.1177/0748730413517981

Gooch VD, Mehra A, Larrondo LF, Fox J, Touroutoutoudis M, Loros JJ, Dunlap JC (2008) Fully codon-optimized luciferase uncovers novel temperature characteristics of the *Neurospora* clock. Eukaryot Cell 7(1):28–37. https://doi.org/10.1128/ec.00257-07

Goodspeed D, Chehab EW, Covington MF, Braam J (2013) Circadian control of jasmonates and salicylates: the clock role in plant defense. Plant Signal Behav 8(2):e23123. https://doi.org/10.4161/psb.23123

Goodspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF (2012) Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proc Natl Acad Sci USA 109(12):4674–4677. https://doi.org/10.1073/pnas.1116368109

Greene AV, Keller N, Haas H, Bell-Pedersen D (2003) A circadian oscillator in *Aspergillus* spp. Regulates daily development and gene expression. Eukaryot Cell 2(2):231–237. https://doi.org/10.1128/ec.2.2.231-237.2003

Guo J, Liu Y (2010) Molecular mechanism of the *Neurospora circadian* oscillator. Protein Cell 1 (4):331–341. https://doi.org/10.1007/s13238-010-0053-7

Hajek AE, Soper RS (1992) Temporal dynamics of *Entomophaga maimaiga* after death of gypsy moth (Lepidoptera: Lymantriidae) larval hosts. Environ Entomol 21(1):129–135. https://doi.org/10.1093/ee/21.1.129

Hamilton ED (1959) Studies on the air spora. Acta Allergol 13(2):143–173

Hawking F, Worms MJ, Gammage K (1968) Host temperature and control of 24-hour and 48-hour cycles in malaria parasites. Lancet 1(7541):506–509

He Q, Liu Y (2005) Degradation of the *Neurospora circadian* clock protein FREQUENCY through the ubiquitin-proteasome pathway. Biochem Soc Trans 33(Pt 5):953–956

Hernandez RR, Allen MF (2013) Diurnal patterns of productivity of arbuscular mycorrhizal fungi revealed with the soil ecosystem observatory. New Phytol 200(2):547–557. https://doi.org/10.1111/nph.12393

Hevia MA, Canessa P, Larrondo LF (2016) Circadian clocks and the regulation of virulence in fungi: getting up to speed. Semin Cell Dev Biol 57:147–155. https://doi.org/10.1016/j.semcdb.2016.03.021

Hevia MA, Canessa P, Muller-Esparza H, Larrondo LF (2015) A circadian oscillator in the fungus *Botrytis cinerea* regulates virulence when infecting Arabidopsis thaliana. Proc Natl Acad Sci USA 112(28):8744–8749. https://doi.org/10.1073/pnas.1508432112

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lucking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Koljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Moncalvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schussler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111(Pt 5):509–547. https://doi.org/10.1016/j.mycres.2007.03.004

Hirako IC, Assis PA, Hojo-Souza NS, Reed G, Nakaya H, Golenbock DT, Coimbra RS, Gazzinelli RT (2018) Daily rhythms of TNFalpha expression and food intake regulate synchrony of plasmodium stages with the host circadian cycle. Cell Host Microbe 23(6):796–808 e796. https://doi.org/10.1016/j.chom.2018.04.016

Hughes DP, Andersen SB, Hywel-Jones NL, Himaman W, Billen J, Boomsma JJ (2011) Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. BMC Ecol 11:13. https://doi.org/10.1186/1472-6785-11-13

Hurley J, Loros JJ, Dunlap JC (2015) Dissecting the mechanisms of the clock in *Neurospora*. Methods Enzymol 551:29–52. https://doi.org/10.1016/bs.mie.2014.10.009

Hurley JM, Dasgupta A, Emerson JM, Zhou X, Ringelberg CS, Knabe N, Lipzen AM, Lindquist EA, Daum CG, Barry KW, Grigoriev IV, Smith KM, Galagan JE, Bell-Pedersen D, Freitag M, Cheng C, Loros JJ, Dunlap JC (2014) Analysis of clock-regulated genes in

Neurospora reveals widespread posttranscriptional control of metabolic potential. Proc Natl Acad Sci USA 111 (48):16995–17002. https://doi.org/10.1073/pnas.1418963111

Hurley JM, Larrondo LF, Loros JJ, Dunlap JC (2013) Conserved RNA helicase FRH acts nonenzymatically to support the intrinsically disordered neurospora clock protein FRQ. Mol Cell 52(6):832–843. https://doi.org/10.1016/j.molcel.2013.11.005

Idnurm A, Heitman J (2005) Light controls growth and development via a conserved pathway in the fungal kingdom. PLoS Biol 3(4):e95. https://doi.org/10.1371/journal.pbio.0030095 (04-PLBI-RA-0453R3 [pii])

Ingle RA, Stoker C, Stone W, Adams N, Smith R, Grant M, Carre I, Roden LC, Denby KJ (2015) Jasmonate signalling drives time-of-day differences in susceptibility of *Arabidopsis* to the fungal pathogen Botrytis cinerea. Plant J 84(5):937–948. https://doi.org/10.1111/tpj.13050

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443(7113):818–822. https://doi.org/10.1038/nature05110

Jarvis WR (1977) Botryotinia and botrytis species. Taxonomy and pathogenicity. Agriculture and agri-food canada—agriculture et Agroalimentaire Canada 15

Jensen C, Lysek G (1983) Differences in the mycelial growth rhythms in a population of *Sclerotinia fructigena* Experientia

Keller M, Mazuch J, Abraham U, Eom GD, Herzog ED, Volk HD, Kramer A, Maier B (2009) A circadian clock in macrophages controls inflammatory immune responses. Proc Natl Acad Sci USA 106(50):21407–21412. https://doi.org/10.1073/pnas.0906361106

Kim H, Ridenour JB, Dunkle LD, Bluhm BH (2011a) Regulation of stomatal tropism and infection by light in Cercospora zeae-maydis: evidence for coordinated host/pathogen responses to photoperiod? PLoS Pathog 7(7):e1002113. https://doi.org/10.1371/journal.ppat.1002113 (PPATHOGENS-D-10-00513 [pii])

Kim S, Singh P, Park J, Park S, Friedman A, Zheng T, Lee YH, Lee K (2011b) Genetic and molecular characterization of a blue light photoreceptor MGWC-1 in *Magnaporth oryzae*. Fungal Genet Biol: FG & B 48(4):400–407. https://doi.org/10.1016/j.fgb.2011.01.004

Korneli C, Danisman S, Staiger D (2014) Differential control of pre-invasive and post-invasive antibacterial defense by the *Arabidopsis circadian* clock. Plant Cell Physiol 55(9):1613–1622. https://doi.org/10.1093/pcp/pcu092

Krasnoff SB, Watson DW, Gibson DM, Kwan EC (1995) Behavioral effects of the entomopathogenic fungus, *Entomophthora muscae* on its host *Musca domestica*: postural changes in dying hosts and gated pattern of mortality. J Insect Physiol 41:895–903. https://doi.org/10.1016/0022-1910(95)00026-Q

Labrecque N, Cermakian N (2015) Circadian clocks in the immune system. J Biol Rhythms 30 (4):277–290. https://doi.org/10.1177/0748730415577723

Lacey ME (1962) The summer air-spora of two contrasting adjacent rural sites. J Gen Microbiol 29:485–501. https://doi.org/10.1099/00221287-29-3-485

Larrondo LF, Colot HV, Baker CL, Loros JJ, Dunlap JC (2009) Fungal functional genomics: tunable knockout-knock-in expression and tagging strategies. Eukaryot Cell 8(5):800–804. https://doi.org/10.1128/ec.00072-09

Larrondo LF, Loros JJ, Dunlap JC (2012) High-resolution spatiotemporal analysis of gene expression in real time: in vivo analysis of circadian rhythms in *Neurospora crassa* using a

FREQUENCY-luciferase translational reporter. Fungal Genet Biol 49(9):681–683. https://doi.org/10.1016/j.fgb.2012.06.001

Larrondo LF, Olivares-Yanez C, Baker CL, Loros JJ, Dunlap JC (2015) Circadian rhythms. Decoupling circadian clock protein turnover from circadian period determination. Science 347 (6221):1257277. https://doi.org/10.1126/science.1257277

Latorre B, Rioja M (2002) Efecto de la Temperatura y de la Humedad Relativa Sobre la Germinación de Conidias de *Botrytis cinerea*. Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura 29(2):66–72

Lee JE, Edery I (2008) Circadian regulation in the ability of Drosophila to combat pathogenic infections. Curr Biol 18(3):195–199. https://doi.org/10.1016/j.cub.2007.12.054 (S0960-9822 (07)02486-4 [pii])

Lee K, Dunlap JC, Loros JJ (2003) Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. Genetics 163(1):103–114

Lee K, Singh P, Chung WC, Ash J, Kim TS, Hang L, Park S (2006) Light regulation of asexual development in the rice blast fungus, *Magnaporthe oryzae*. Fungal Genet Biol: FG & B 43 (10):694–706. https://doi.org/10.1016/j.fgb.2006.04.005

Lee SJ, Kong M, Morse D, Hijri M (2018) Expression of putative circadian clock components in the arbuscular mycorrhizal fungus Rhizoglomus irregulare. Mycorrhiza. https://doi.org/10.1007/s00572-018-0843-y

Lowrey PL, Takahashi JS (2011) Genetics of circadian rhythms in Mammalian model organisms. Adv Genet 74:175–230. https://doi.org/10.1016/b978-0-12-387690-4.00006-4

Mackey SR, Golden SS, Ditty JL (2011) The itty-bitty time machine genetics of the cyanobacterial circadian clock. Adv Genet 74:13–53. https://doi.org/10.1016/b978-0-12-387690-4.00002-7

McClung C (2006) Plant circadian rhythms. The Plant cell 18(4):792–803

McClung CR (2001) Circadian rhythms in plants. Annu Rev Plant Physiol Plant Mol Biol 52:139–162. https://doi.org/10.1146/annurev.arplant.52.1.139

Mehra A, Shi M, Baker CL, Colot HV, Loros JJ, Dunlap JC (2009) A role for casein kinase 2 in the mechanism underlying circadian temperature compensation. Cell 137(4):749–760

Merrow MW, Dunlap JC (1994) Intergeneric complementation of a circadian rhythmicity defect: phylogenetic conservation of structure and function of the clock gene frequency. EMBO J 13 (10):2257–2266

Mideo N, Reece SE, Smith AL, Metcalf CJ (2013) The Cinderella syndrome: why do malaria-infected cells burst at midnight? Trends Parasitol 29(1):10–16. https://doi.org/10.1016/j.pt.2012.10.006

Milner RJ, Holdom DG, Glare TR (1983) Diurnal patterns of mortality in aphids infected by entomophthoran fungi. Entomol Exp Appl 36:37–42. https://doi.org/10.1111/j.1570-7458.1984.tb03404.x

Montenegro-Montero A, Canessa P, Larrondo LF (2015) Around the fungal clock: recent advances in the molecular study of circadian clocks in neurospora and other fungi. Adv Genet 92:107–184. https://doi.org/10.1016/bs.adgen.2015.09.003

Montenegro-Montero A, Larrondo LF (2013) The neurospora circadian system: from genes to proteins and back in less than 24 hours. In: McCluskey K, Kasbekar D (eds) Neurospora. Genomics and molecular biology. Caister Academic Press, Norfolk, UK

Montenegro-Montero A, Larrondo LF (2016) In the driver's seat: the case for transcriptional regulation and coupling as relevant determinants of the circadian transcriptome and proteome in Eukaryotes. J Biol Rhythms 31(1):37–47. https://doi.org/10.1177/0748730415607321

Mouahid G, Idris MA, Verneau O, Theron A, Shaban MM, Mone H (2012) A new chronotype of *Schistosoma mansoni*: adaptive significance. Trop Med Int Health 17(6):727–732. https://doi.org/10.1111/j.1365-3156.2012.02988.x

Newman GG, Carner GR (1974) Diel periodicity of *Entomophthora gammae* in the soybean looper. Environ Entomol 3:888–890

Nielsen C, Hajek AE (2006) Diurnal pattern of death and sporulation in *Entomophaga maimaiga*-infected *Lymantria dispar*. Entomol Exp Appl 118(3):237–243. https://doi.org/10.1111/j.1570-7458.2006.00382.x

Olivares-Yanez C, Emerson J, Kettenbach A, Loros JJ, Dunlap JC, Larrondo LF (2016) Modulation of circadian gene expression and metabolic compensation by the RCO-1 co-repressor of *Neurospora crassa*. Genetics. https://doi.org/10.1534/genetics.116.191064

Oliveira AG, Stevani CV, Waldenmaier HE, Viviani V, Emerson JM, Loros JJ, Dunlap JC (2015) Circadian control sheds light on fungal bioluminescence. Curr Biol 25(7):964–968. https://doi.org/10.1016/j.cub.2015.02.021

Ouyang Y, Andersson CR, Kondo T, Golden SS, Johnson CH (1998) Resonating circadian clocks enhance fitness in cyanobacteria. Proc Natl Acad Sci USA 95(15):8660–8664

Paschos GK, FitzGerald GA (2017) Circadian clocks and metabolism: implications for microbiome and aging. Trends Genet 33(10):760–769. https://doi.org/10.1016/j.tig.2017.07.010

Perelis M, Ramsey KM, Bass J (2015) The molecular clock as a metabolic rheostat. Diabetes Obes Metab 17(Suppl 1):99–105. https://doi.org/10.1111/dom.12521

Pregueiro AM, Price-Lloyd N, Bell-Pedersen D, Heintzen C, Loros JJ, Dunlap JC (2005) Assignment of an essential role for the Neurospora frequency gene in circadian entrainment to temperature cycles. Proc Natl Acad Sci USA 102(6):2210–2215

Prior KF, van der Veen DR, O'Donnell AJ, Cumnock K, Schneider D, Pain A, Subudhi A, Ramaprasad A, Rund SSC, Savill NJ, Reece SE (2018) Timing of host feeding drives rhythms in parasite replication. PLoS Pathog 14(2):e1006900. https://doi.org/10.1371/journal.ppat.1006900

Proietto M, Bianchi MM, Ballario P, Brenna A (2015) Epigenetic and posttranslational modifications in light signal transduction and the circadian clock in *Neurospora crassa*. Int J Mol Sci 16(7):15347–15383. https://doi.org/10.3390/ijms160715347

Ramsdale M (2008) Circadian rhythms in filamentous fungi. In: Gow NAR, Robson GD, Gadd GM (eds) The fungal colony, vol First digitally printed version 2008. British Mycological Society Symposia, pp 75–107

Rijo-Ferreira F, Carvalho T, Afonso C, Sanches-Vaz M, Costa RM, Figueiredo LM, Takahashi JS (2018) Sleeping sickness is a circadian disorder. Nat Commun 9(1):62. https://doi.org/10.1038/s41467-017-02484-2

Rijo-Ferreira F, Pinto-Neves D, Barbosa-Morais NL, Takahashi JS, Figueiredo LM (2017a) *Trypanosoma brucei* metabolism is under circadian control. Nat Microbiol 2:17032. https://doi.org/10.1038/nmicrobiol.2017.32

Rijo-Ferreira F, Takahashi JS, Figueiredo LM (2017b) Circadian rhythms in parasites. PLoS Pathog 13(10):e1006590. https://doi.org/10.1371/journal.ppat.1006590

Roden LC, Ingle RA (2009) Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant-pathogen interactions. Plant Cell 21(9):2546–2552. https://doi.org/10.1105/tpc.109.069922 (tpc.109.069922 [pii])

Romanowski A, Yanovsky MJ (2015) Circadian rhythms and post-transcriptional regulation in higher plants. Front Plant Sci 6:437. https://doi.org/10.3389/fpls.2015.00437

Rosbash M (2009) The implications of multiple circadian clock origins. PLoS Biol 7(3):e62. https://doi.org/10.1371/journal.pbio.1000062

Ruiz-Roldan MC, Garre V, Guarro J, Marine M, Roncero MI (2008) Role of the white collar 1 photoreceptor in carotenogenesis, UV resistance, hydrophobicity, and virulence of *Fusarium oxysporum*. Eukaryot Cell 7(7):1227–1230. https://doi.org/10.1128/ec.00072-08 (EC.00072-08 [pii])

Salichos L, Rokas A (2010) The diversity and evolution of circadian clock proteins in fungi. Mycologia 102(2):269–278

Sancar C, Sancar G, Ha N, Cesbron F, Brunner M (2015) Dawn- and dusk-phased circadian transcription rhythms coordinate anabolic and catabolic functions in *Neurospora*. BMC Biol 13(1):17. https://doi.org/10.1186/s12915-015-0126-4

Sancar G, Sancar C, Brugger B, Ha N, Sachsenheimer T, Gin E, Wdowik S, Lohmann I, Wieland F, Hofer T, Diernfellner A, Brunner M (2011) A global circadian repressor controls antiphasic expression of metabolic genes in *Neurospora*. Mol Cell 44(5):687–697. https://doi.org/10.1016/j.molcel.2011.10.019 (S1097-2765(11)00902-6 [pii])

Santamaria-Hernando S, Rodriguez-Herva JJ, Martinez-Garcia PM, Rio-Alvarez I, Gonzalez-Melendi P, Zamorano J, Tapia C, Rodriguez-Palenzuela P, Lopez-Solanilla E (2018) *Pseudomonas syringae* pv. tomato exploits light signals to optimize virulence and colonization of leaves. Environ Microbiol. https://doi.org/10.1111/1462-2920.14331

Sargent ML, Briggs WR, Woodward DO (1966) Circadian nature of a rhythm expressed by an invertaseless strain of *Neurospora crassa*. Plant Physiol 41(8):1343–1349

Scheiermann C, Gibbs J, Ince L, Loudon A (2018) Clocking into immunity. Nat Rev Immunol 18 (7):423–437. https://doi.org/10.1038/s41577-018-0008-4

Schmidle A (1951) Die Tagesperiodizität der asexuellen Reproduktion von Pilobolus spaerosporus. Arch Mikrobiol 16:80–100

Schumacher J (2017) How light affects the life of Botrytis. Fungal Genet Biol 106:26–41. https://doi.org/10.1016/j.fgb.2017.06.002

Schumacher J, Tudzynski P (2012) Morphogenesis and infection in *Botrytis cinerea*. In: Perez-Martın J, Di Pietro A (eds) Morphogenesis and pathogenicity in fungi. Topics in current genetics, vol 22. Springer-Verlag Berlin Heidelberg 2012, pp 225–241. https://doi.org/10.1007/978-3-642-22916-9_11

Sharma M, Bhatt D (2014) The circadian clock and defence signalling in plants. Molecular Plant Pathology. https://doi.org/10.1111/mpp.12178

Shi M, Larrondo LF, Loros JJ, Dunlap JC (2007) A developmental cycle masks output from the circadian oscillator under conditions of choline deficiency in *Neurospora*. Proc Natl Acad Sci USA 104(50):20102–20107. https://doi.org/10.1073/pnas.0706631104

Shin J, Heidrich K, Sanchez-Villarreal A, Parker JE, Davis SJ (2012) TIME FOR COFFEE represses accumulation of the MYC2 transcription factor to provide time-of-day regulation of jasmonate signaling in *Arabidopsis*. Plant Cell 24(6):2470–2482. https://doi.org/10.1105/tpc.111.095430

Slepecky RA, Starmer WT (2009) Phenotypic plasticity in fungi: a review with observations on *Aureobasidium pullulans*. Mycologia 101(6):823–832

Smith KM, Sancar G, Dekhang R, Sullivan CM, Li S, Tag AG, Sancar C, Bredeweg EL, Priest HD, McCormick RF, Thomas TL, Carrington JC, Stajich JE, Bell-Pedersen D, Brunner M, Freitag M (2010) Transcription factors in light and circadian clock signaling networks revealed by genomewide mapping of direct targets for *Neurospora* white collar complex. Eukaryot Cell 9(10):1549–1556. https://doi.org/10.1128/ec.00154-10 (EC.00154-10 [pii])

Steinkraus DC, Hajek AE, Liebherr JK (2017) Zombie soldier beetles: epizootics in the goldenrod soldier beetle, *Chauliognathus pensylvanicus* (Coleoptera: Cantharidae) caused by *Eryniopsis lampyridarum* (Entomophthoromycotina: Entomophthoraceae). J Invertebr Pathol 148:51–59. https://doi.org/10.1016/j.jip.2017.05.002

Steinkraus DC, Hollingsworth RG, Boys GO (1996) Aerial spores of *Neozygites fresenii* (EntoInophthorales: Neozygitaceae): density, periodicity, and potential role in CoUon Aphid (HoInoptera: Aphididae) Epizootics. Environ Entomol 25:48–57

Steyaert JM, Weld RJ, Loguercio LL, Stewart A (2010) Rhythmic conidiation in the blue-light fungus *Trichoderma pleuroticola*. Fungal Biol 114(2–3):219–223. https://doi.org/10.1016/j.funbio.2010.01.001 (S1878-6146(10)00003-6 [pii])

Thaiss CA, Levy M, Korem T, Dohnalova L, Shapiro H, Jaitin DA, David E, Winter DR, Gury-BenAri M, Tatirovsky E, Tuganbaev T, Federici S, Zmora N, Zeevi D, Dori-Bachash M, Pevsner-Fischer M, Kartvelishvily E, Brandis A, Harmelin A, Shibolet O, Halpern Z, Honda K, Amit I, Segal E, Elinav E (2016) Microbiota diurnal rhythmicity programs host transcriptome oscillations. Cell 167 (6):1495–1510 e1412. https://doi.org/10.1016/j.cell.2016.11.003

Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, Abramson L, Katz MN, Korem T, Zmora N, Kuperman Y, Biton I, Gilad S, Harmelin A, Shapiro H, Halpern Z, Segal E, Elinav E (2014) Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 159(3):514–529. https://doi.org/10.1016/j.cell.2014.09.048

Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar A, Marcet-Houben M, Poggeler S, Stajich JE, Nowrousian M (2013) The genome and development-dependent transcriptomes of Pyronema confluens: a window into fungal evolution. PLoS Genet 9(9): e1003820. https://doi.org/10.1371/journal.pgen.1003820

Traeger S, Nowrousian M (2015) Analysis of circadian rhythms in the basal filamentous Ascomycete Pyronema confluens. G3 (Bethesda). https://doi.org/10.1534/g3.115.020461

Tudzynski P, Siewers V (2004) Approaches to molecular genetics and genomics of Botrytis. In: Elad Y WB, Tudzynski P, Delen Neds (ed) Botrytis: biology, pathology and control, vol XIX. p 428

Tuzdynski P, Kokkelink L (2009) *Botrytis cinerea*: molecular aspects of a necrotrophic life style. In: H D (ed) The Mycota V: plant relationships, 2nd edn. Springer-Verlag, Berlin, Heidelberg, pp 29–50

Uebelmesser ER (1954) Uber den endonomen Tagesrhythmus der Sporangientragerbildung bon Pilobolus. Arch Mikrobiol 20:1–33

Veloso J, van Kan JAL (2018) Many shades of grey in botrytis-host plant interactions. Trends Plant Sci 23(7):613–622. https://doi.org/10.1016/j.tplants.2018.03.016

Wang B, Kettenbach AN, Gerber SA, Loros JJ, Dunlap JC (2014) Neurospora WC-1 recruits SWI/SNF to remodel frequency and initiate a circadian cycle. PLoS Genet 10(9):e1004599. https://doi.org/10.1371/journal.pgen.1004599

Wang W, Barnaby JY, Tada Y, Li H, Tor M, Caldelari D, Lee DU, Fu XD, Dong X (2011) Timing of plant immune responses by a central circadian regulator. Nature 470(7332):110–114

Welsh DK, Imaizumi T, Kay SA (2005) Real-time reporting of circadian-regulated gene expression by luciferase imaging in plants and mammalian cells. Methods Enzymol 393:269–288

West AC, Bechtold DA (2015) The cost of circadian desynchrony: evidence, insights and open questions. BioEssays 37(7):777–788. https://doi.org/10.1002/bies.201400173

Wilding N (1970) Entomophthora conidia in the air-spora. J Gen Microbiol 62(2):149–157. https://doi.org/10.1099/00221287-62-2-149

Yerushalmi S, Green RM (2009) Evidence for the adaptive significance of circadian rhythms. Ecol Lett 12(9):970–981. https://doi.org/10.1111/j.1461-0248.2009.01343.x (ELE1343 [pii])

Zhang C, Xie Q, Anderson RG, Ng G, Seitz NC, Peterson T, McClung CR, McDowell JM, Kong D, Kwak JM, Lu H (2013) Crosstalk between the circadian clock and innate immunity in *Arabidopsis*. PLoS Pathog 9(6):e1003370. https://doi.org/10.1371/journal.ppat.1003370

Zhao X, Spraker JE, Bok JW, Velk T, He ZM, Keller NP (2017) A cellular fusion cascade regulated by LaeA is required for sclerotial development in *Aspergillus flavus*. Front Microbiol 8:1925. https://doi.org/10.3389/fmicb.2017.01925

Zhou Z, Liu X, Hu Q, Zhang N, Sun G, Cha J, Wang Y, Liu Y, He Q (2013) Suppression of WC-independent frequency transcription by RCO-1 is essential for Neurospora circadian clock. Proc Natl Acad Sci USA 110(50):E4867-4874. https://doi.org/10.1073/pnas.1315133110