HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2022 June 09.

Published in final edited form as:

Semin Cell Dev Biol. 2022 June ; 126: 37–44. doi:10.1016/j.semcdb.2021.09.004.

Circadian rhythms in infectious diseases and symbiosis

Filipa Rijo-Ferreira[1,*], **Joseph S. Takahashi**[1,2,*]

[1]Department of Neuroscience, Peter O'Donnell Jr. Brain Institute, University of Texas Southwestern Medical Center, Dallas, TX, United States

[2]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX, United States

Abstract

Timing is everything. Many organisms across the tree of life have evolved timekeeping mechanisms that regulate numerous of their cellular functions to optimize timing by anticipating changes in the environment. The specific environmental changes that are sensed depends on the organism. For animals, plants, and free-living microbes, environmental cues include light/dark cycles, daily temperature fluctuations, among others. In contrast, for a microbe that is never free-living, its rhythmic environment is its host's rhythmic biology. Here, we describe recent research on the interactions between hosts and microbes, from the perspective both of symbiosis as well as infections. In addition to describing the biology of the microbes, we focus specifically on how circadian clocks modulate these host-microbe interactions.

Introduction

Our rhythmic environment shaped us. It created evolutionary pressures that proved advantageous to have an intrinsic clock that allowed us to anticipate rhythmic changes in the environment. In recent years it has become clearer than ever just how much the circadian clock regulates many additional aspects of mammalian physiology (1). The clock in mammals is controlled at the molecular level by core transcriptional activators (CLOCK and BMAL) and repressors (CRY and PER), with two additional interconnected loops, chromatin interactions, as well as many additional layers of post-transcriptional and post-translational regulation, which together regulate the expression of genes involved in myriad cellular functions (2). Thus, the clock is a master regulator of multiple systems in the body, including sleep, metabolism, and the immune system. Immune responses show temporal changes in antibody levels and abundance and sensitivity of immune cells. Yet, it is not known how these fluctuations impact host-microbe interactions in health and disease. Additionally, microbes themselves may have their own intrinsic clocks that regulate their own biology and many of the microbes that are pathogenic have intrinsic rhythms, as well. Here we will review recent advances in this active area of research.

* Filipa.ferreira@utsouthwestern.edu, joseph.takahashi@utsouthwestern.edu.

1. Circadian rhythms in host-microbe interactions

It has long been known that the sensitivity to endotoxins varies between morning and evening (3). Although we were aware of the increased survival of mice to a challenge of lipopolysaccharide (LPS) at dawn versus dusk, only in the past decade have we begun to understand the clock control over the immune system. The immune response is under circadian control in many ways, from the trafficking of immune cells, to the activation of innate and adaptive immunity, to host–microbe interactions (4).

Circadian rhythms in infections

Similar to endotoxin challenges, many studies have shown that the outcome of an infection (whether bacterial, viral, or parasitic) depends on the time of day at which the infection is initiated (5-8).

Infection with bacteria.—Several types of bacterial infections have been connected to circadian rhythms. For example, clock disrupted mutant flies (per^{01} and tim^{01}) infected with two Gram-positive pathogens, *Streptococcus pneumoniae* and *Listeria monocytogenes* are sensitive to infection and die significantly sooner than wild-type flies (9). This increased sensitivity to the bacterial infection may be a consequence of the circadian regulation of phagocytosis that is disrupted in these fly mutants (10). In mice, infection with Listeria has also been shown to have a circadian component. When infections are initiated at the beginning of the rest phase (*Zeitgeber time* 0, ZT0) mice have higher colonization in spleen, liver and peritoneum, compared with infections initiated at the end of the resting phase (ZT8) (11). Similarly, infections with *Streptococcus pneumoniae* also show different immune responses depending on the time of the day (12). *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. typhimurium*) infection also causes a higher bacterial load when infection is initiated during the rest phase than when infection is initiated in the middle of the active phase in mice. This difference is dependent on a functional copy of CLOCK (7). *Chlamydia*, one of the most common vaginal bacterial infections, also causes more detrimental infections depending on the time of day (13). When mice are infected with *C. muridarum* during their rest period, it leads to higher numbers of bacteria being shed and lower fertility (Fig. 1). Thus, there is a circadian rhythm in the timing at which the host is more efficient in managing an infection. Most of the bacterial infections studied so far show poorer management by the host when initiated during the rest phase (at a time at when infection might not normally occur in the wild). It would be interesting going forward to understand if these circadian differences in infection outcome are a consequence of evolutionary pressures to the circadian rhythms in immune response; or whether evolutionary pressures caused the bacterium *itself* to become more efficient establishing the infection depending on the time of the day (more discussed in section B).

Infection with parasites.—The outcome of parasitic infection also depends on the timing of the host circadian cycle. Timing of host rhythms impact malaria parasite replication success (14). *Leishmania amazonensis* parasite burden shows a circadian profile (15), and *Bmal1* in monocytes is responsible for modulating the magnitude of *Leishmania major* infection variation throughout the day (6, 16). Similar findings have been described for the

intestinal parasitic helminth *Trichuris muris*, with mice infected at the start of the active phase showing delayed clearance. Ablation of *Bmal1* in antigen-presenting dendritic cells (DCs) leads to a partial loss of time-of-day dependency of helminth expulsion from the body (5). Therefore, the circadian clock (with focus to date mainly on the core transcriptional activator BMAL1) can regulate cellular immunity against bacteria and parasites (Fig. 1, and see viral section).

Behavioral changes of the host by parasitic infections: Infection with *Trypanosoma brucei* leads to advances in the timing of locomotor behavior of mice and humans by disrupting the timing at which they sleep (17-19). This distinct disruption gave rise to the name of the disease 'sleeping sickness'. We recently showed that alterations to the circadian clock underlies these behavioral changes, and, so far, this is the only infection known to shorten the period of the host's oscillations (20-22). Upon *T. brucei* infection we observed that, while the overall activity of mice decreased, they become more active than healthy animals during the rest phase, similar to the behavioral changes described for human sleeping sickness patients. Moreover, when in constant darkness, the infected mice revealed an increasingly shorter period as the infection progressed. Molecularly, we also observed that the expression of a clock repressor PERIOD2 protein rhythm had a shorter period in infected mice than in healthy mice, highlighting how sleeping sickness infection affects the circadian clock (20).

Circadian behavioral changes by parasitic infections have been also described for insects (cricket and ant) and snails (hairworm and fungus) (23-25). In the case of the circadian behavioral manipulation of carpenter ant workers by the fungus *Ophiocordyceps unilateralis* s.l. infected ants wander out of the nest earlier in the day than healthy ants. This behavioral disruption assists the fungus in completing its life cycle (24). Expression of the clock genes, *period* and *cycle*, have been shown to be upregulated in infected ants at 10 am, in comparison with healthy time-matched control ants (24). This upregulation is complex to interpret, as these clock elements belong to both the negative and positive arm of the clock. Higher resolution of sampling covering the entire circadian cycle would be needed to understand the circadian disruption. It would be interesting to know whether these examples of infections, such as with the fungus, also lead to a circadian dysregulation by accelerating and thus shortening the circadian period of the ant's clock.

Infection with viruses.—Similar to what has been shown for bacterial infections, time-of-day of infection influences virus progression both in mice and in individual cells. Viral infections are enhanced when host circadian rhythms are abolished by disrupting the *Bmal1* gene, in infections with herpes, influenza A, and respiratory viruses of the Paramyxoviridae family (8, 26) (Fig. 1). *Bmal1*$^{-/-}$ mice intranasally infected with the respiratory syncytial virus (RSV) have a higher viral load than wild-type mice (26). However, these observations may be in part related with the anti-inflammatory effect of BMAL1 protein (4). Nonetheless, the clock itself plays a role since misalignment of circadian rhythms through chronic jet lag exacerbates acute viral bronchiolitis caused by Sendai virus (SeV) or influenza A virus in mice (27). In addition, when activating the expression of the clock protein, REV-ERB, with an agonist (SR9009) both Dengue virus (DENV) and Zika (ZIKV) infection (28), as well as

human immunodeficiency virus 1 (HIV) (29), and hepatitis B virus (30), are all inhibited. These findings suggest that the circadian clock regulates host-viral interactions in these infections. However, since the clock agonists used in these studies have been shown to have off target effects, namely effects on cell proliferation and metabolism that are independent of REV-ERB (31), further studies are required.

Interestingly, even the survival of the host has been observed to fluctuate depending on the time the viral infection is initiated. Encephalitis caused by the vesicular stomatitis virus (VSV) infection leads to a higher mortality rate if the intranasal infection of mice occurs at the beginning of the rest phase (32). Similarly, time of day influences outcome of infection by influenza A virus (IAV). However, in this case, when infection is initiated in the morning mortality is three-fold lower than when mice are infected in the evening, closer to their active phase (33). Interestingly, hyperoxia early after birth disrupts the development of normal circadian rhythms in mice, and, despite only leading to subtle effects on the clock later in adulthood, this disruption abolishes the daily differences in response to influenza A infection (34) (Fig. 1).

SAR-CoV2 pandemic and the clock.: While living in a pandemic caused by SARS-CoV2, it is impossible to neglect the potential impact of circadian rhythms in managing the COVID-19 infection (35-38), as well as more broadly how the Stay-at-Home orders affect our circadian rhythms and sleep patterns. Overall, early on in the pandemic sleep behavior improved in adults. Prior to the pandemic, ~60% of individuals slept less than the recommended 7h per night, whereas only ~37% of participants in a study in Argentina failed to reach 7h of sleep during the lockdown (39). Increases in total sleep were also seen in the United States and Canada where university students increased their sleep duration by ~30 min, bringing the total time slept closer to public health recommendations (40, 41). Similar sleep duration increases were observed in participants across 40 countries (42) and on different continents (43). Taken together, these studies highlight a chronic sleep deficit under pre-pandemic social time pressures (42). However, as the pandemic progressed, sleep disruption generally increased and was associated with an increase in mental health problems and anxiety (44-47).

Vaccine development and distribution have been a *tour de force* during the SARS-CoV2 pandemic and were achieved at an unprecedented speed. Considering the rhythms in immune response, it will be interesting to assess in metadata studies whether time of vaccination with anti-SARS-CoV2 had differential impacts in building immunity (more discussed in section C). Is time of day, i.e., morning vs. afternoon vaccination a co-variate of becoming infected in vaccinated individuals? (Fig. 2) It may be of particular interest since there are recent reports of ‘breakthrough’ infections (48).

Inflammation and the downregulation of the clock.

The inflammation caused by infections can disrupt the circadian clock by dramatically decreasing the amplitude of circadian rhythms, both at the behavioral and molecular level. Proinflammatory cytokines added to cultures are sufficient to decrease the amplitude of molecular rhythms. Moreover, cytokine injection leads to ‘sickness-like behavior’ (49).

Similarly, parasitic infections decrease the amplitude of circadian behavioral rhythms. The reduced active-phase activity was observe in multiple parasitic infections by the causative agents of sleeping sickness (*Trypanosoma brucei*) (20), malaria (*Plasmodium chabaudi*) (20, 50), and Chagas disease (*Trypanosoma cruzi*) (51). The decrease in circadian amplitude of activity rhythms is also accompanied by the downregulation circadian gene expression, of both positive elements (*Bmal1*) and negative elements (*Per1*) of the clock (20). Of these parasitic infections, only *T. brucei* infection changes the period of the host clock both behaviorally and molecularly, in addition to the decrease in amplitude that is common to other infections (20). Bacterial infection by *Salmonella*, has also been shown to downregulate the expression of the clock gene *Per2* in mice (7). In plants, the downregulation of clock gene expression by bacterial infections is also observed (52, 53), suggesting once again that the amplitude reduction may be a consequence of host inflammation when fighting an infection. Notably, in humans, respiratory tract expression of most clock genes (*BMAL1, NPAS2, PER2, DBP* and *NR1D1* [*REV-ERB α*]) is reduced in adult asthma patients (27). Thus, infections, possibly through the inflammatory response they induce, appear to generally downregulate clock gene expression. How does the clock become downregulated? Is cytokine signaling reducing expression of both positive and negative elements of the clock? It has been proposed that the non-canonical pathway of NF-κb may interact directly with clock proteins (54). In addition to understanding this process mechanistically, it would be interesting to investigate the consequences that downregulation of expression has on the clock regulation of sickness-like behavior, metabolism, and the immune response itself.

Circadian rhythms in symbiosis

Circadian rhythms in symbiotic host-microbe interactions have also been described. An interesting example is the squid-bacteria interaction. There is a daily rhythm in light production when the symbiont *Vibrio* bacteria are brightest in the evening and the squid is out foraging. Moreover, every dawn ~90% of the symbiont bacteria population is expelled from the squid microvilli (55). Lately, other examples of how mammalian host-microbiome interactions vary through the day have come to light. This includes cross-kingdom interactions in both the mammalian intestinal setting as well as in the saliva (56, 57).

Microbiome.—The metabolic interactions between the gut tissue and its microbiome have been a major research focus in the past decade, and both the host and microbiota rhythms seem to affect one another. The intestinal microbiota in mice undergoes rhythmic fluctuations in its composition (58), biogeography, and metabolome patterns (59). Disruption of clock genes in the host abolishes rhythms in the abundance of certain microbiota (58), which seem to be restored upon time-restricted feeding (58, 59). On the other hand, the absence of gut microbes perturbs the expression of circadian clock genes in the mouse liver (60).

Gut microbiome daily rhythms are clearly linked with food intake and metabolism (61). In mice, body composition is regulated by the gut microbiota via the transcription factor NFIL3 (62), part of one of the three interconnected transcription-translation circadian clock

loops. In addition, it has been suggested that the microbiota also imposes rhythms in host metabolism through histone deacetylase 3 (57). There is a complex equilibrium between host and microbiota that impacts the homeostasis of the gut. Diet timing and microbiota rhythmicity modulate the small intestine epithelial cells major histocompatibility complex (MHC) class II expression rhythm, linking its daily rhythms with immune balance (63).

Multiple studies have shown that an alignment of internal rhythms to the environment is beneficial for healthspan. Time of day of feeding plays an important role (64, 65). Human saliva also displays changes in the diversity and relative bacterial abundance throughout the day (66) and this microbiota is altered by the timing of meals (67). Similar to what has been observed in mice, a study with German participants showed that arrhythmic gut microbiome signatures in humans predict risk of type 2 diabetes (56).

2. The microbe's own clock

In this section we will mostly focus on clocks of microbial pathogenic organisms and some commensal microbes. Even though not all the pathogens we will mention here are microbes, such as the *Schistosoma* or filaria parasites, they do cause massive health burdens and have a parasitic life cycle, thus they have been included in the parasite section.

Bacteria clock.

From prokaryotes, the most well-known clock is found in cyanobacteria. In particular, we have a good understanding of fine tuning of the molecular clock machinery of the species Synechococcus *elongatus* (68-70). Nonetheless, for most prokaryotes we still have poor knowledge regarding the presence or regulation of circadian rhythms (71). Although they are not necessarily exposed to external environmental cues, bacteria that colonize or cause infections are exposed to the hosts' rhythmic biology. Prokaryotes are incredibly diverse and studies have shown that there is little conservation in circadian components across phylogenetic groups. Some bacterial pathogens have the Kai proteins (the central components of the cyanobacteria clock), such as *Legionella pneumophila*. However, despite increasing the fitness of the bacterium, it remains unclear whether it regulates a circadian clock (72).

Symbiotic gut bacterium could gain an advantage in the competition for resources by having a clock that anticipates when nutrients are entering the digestive tract. However, so far, it is unclear whether intestinal microbiome bacteria have a self-sustained rhythm *in vivo*. The rhythmic fluctuation in intestinal microbiome abundance is dramatically dampened in circadian mutant arrhythmic hosts (58), which may suggest the microbiota rhythms rely solely on host's rhythms instead of an intrinsic clock of bacteria. Nonetheless, *Klebsiella aerogenes*, a gastrointestinal commensal bacterium, has a circadian rhythm in swarming and motility *in vitro*. This swarming rhythm is enhanced by melatonin (73). Moreover, its rhythms can be entrained by temperature cycles and self-sustained under constant conditions (74), suggesting at least some of the gastrointestinal bacteria may have intrinsic rhythms *in vivo*. To highlight how complex host-bacteria circadian interactions are, some rhythms in the host gut require the presence of bacteria. As examples, the microbiota is required

for diurnal metabolic rhythms in nutrient uptake in the mouse small intestine and rhythmic clock expression in the liver (57, 60).

Bacillus subtilis a non-photosynthetic bacterium has recently been shown to have an intrinsic clock (75). *B. subtilis* is found as a commensal in the gastrointestinal microbiomes of many animals, including humans (76). Interestingly this bacterium does not have any of the cyanobacteria clock machinery, *i.e.*, it lacks KaiA, KaiB and KaiC, but its rhythmicity is entrained by both light and temperature cycles and persists in constant conditions. Notably, its circadian rhythmicity may be linked with biofilm formation (75) (Fig. 1).

Hourglass versus self-sustained clock.—The existence of clocks to anticipate the rhythmic changes in the environment may not be fully universal (77). Cyanobacteria such as *Prochlorococcus marinus* do not have circadian rhythms of physiological properties that are under clock control in other cyanobacteria, and instead rely on an hourglass based on environmental cues (78-80). In other words, the rhythmic physiological properties rapidly become arrhythmic when under constant conditions. It remains unclear why these bacteria do not have traditional cyanobacteria clocks. Are they perhaps too divergent and rely on another clock mechanism? They do lack one of the clock components KaiA (80). Could it be that they have a clock but their clock controls other physiological properties that have not been measured? An unbiased approach such as transcriptome or phosphoproteome study could help clarify this.

Fungal clock.

The most well-studied fungal circadian clock is from the filamentous *Neurospora crassa*, from which some of the first clock genes were discovered (81). However, not much is known about the circadian mechanism of other fungal species, including the circadian regulation of human pathogenic fungi (82). Nonetheless, the necrotrophic fungal plant pathogen *Botrytis cinerea* has a functional clock that shares similar components and circuitry with the *Neurospora* circadian system. This fungus produces smaller lesions on *Arabidopsis* leaves when their interaction occurs at dawn. Importantly, disruption of *B. cinerea* rhythms (mutation or overexpression of the frequency gene) abrogates circadian regulation of fungal virulence, highlighting the contribution of the pathogen clock to this phenomenon (83). While there is circadian regulation of the plant-fungal pathogen interaction, it would be interesting to investigate if this also happens in mammalian fungal infections.

Parasite clock.

Many parasitic infections show rhythmic daily patterns. The human infectious stage of the *Schistosoma mansoni* parasite (known as cercariae forms) emerges from snails and swims in fresh water to infect humans by penetrating through the skin. Interestingly, the emergence of this infectious stage is rhythmic and matches the behavior of its final host: occurring during the daytime in parasites that infect humans and in the early evening in parasites that infect nocturnal rats (84). Filarial parasite's transmissible forms are rhythmically present in the blood, with their higher numbers matching the vector feeding pattern (85). A fluctuating number of parasites in the blood is common to many other parasite species, such as *Trypanosoma rotatorium* in the blood of the frog (86), and *Trypanosoma congolense* and

Trypanosoma lewisi in the blood of rodents (87) whose numbers fluctuate throughout the day. Despite these and many other examples of rhythmic patterns in parasitic infections, until recently we did not know if these behaviors were intrinsic to the parasite or whether parasites were simply responding to rhythmic environmental cues, either external or from their host (88). We showed that *Trypanosoma brucei* parasites, unicellular parasites that cause the sleeping sickness in Africa has intrinsic rhythms in their gene expression and metabolism (89). This study served as a proof of principle that obligatory single-celled protist parasites (never free living during their life cycle) have evolved intrinsic circadian time-keeping mechanisms to regulate many cellular functions.

One of the most famous examples of daily rhythms in parasites are the rhythmic fevers in malaria infections. Those fevers are a consequence of the blood-stage parasites having a synchronous asexual cycle, with a coordinated cycle from the moment of invasion of the red blood cells until their bursting. This cycle lasts 24 hours or multiples of 24 hours, depending on the *Plasmodium* species (90). Malaria is also caused by a unicellular protist parasite, although not closely related to *T. brucei*. It was clearly demonstrated almost 100 years ago that this parasite rhythm is intimately linked with the circadian rhythm of the host (91) and its interpretation was that the parasite rhythms were a consequence of the host rhythmicity, a perception that has been carried over to these days. However, parasite rhythms persist independent of host rhythms (92-94) in both mouse models and human parasites in culture, strongly suggesting that he malaria parasite has its own rhythms. Interestingly, the interaction with the host is important for the parasite population to be synchronized with each other, although the periodicity is intrinsically generated by the parasite (92). One other key observation is that in parasites whose asexual cycle rhythm is over 24h, such as *Plasmodium falciparum*, there appears to be an underlying 24h gene expression rhythmicity within the 48h developmental cycle of the human parasite (93, 94) (Fig. 1).

3. Circadian medicine for infectious diseases

Circadian rhythms in host-microbe interactions and in microbes themselves may provide an opportunity to optimize current medical interventions. Excitingly, there are two major, yet simple and actionable, points that arise from these studies: potential optimization of 1) treatment against infectious agents by time-of-day drug administration, and 2) of boosting immunity by time of day of vaccination (Fig. 2).

Optimization of treatment by time-of-day drug administration

Circadian medicine is a concept that considers time of day of drug administration to optimize treatment and potentially reduce treatment toxicity (65, 95-97). This concept is currently factored into the management of cholesterol levels and high blood pressure whereby administering the drug at night is more effective (98, 99). It is also being considered for glioblastoma cancer treatment, where initial results have shown there is an increase in 6-month survival rate when certain treatments are administered in the morning (100).

Although circadian medicine has not been widely considered to treat infectious diseases, we argue that the same concepts apply. As a proof of concept, it is worth mentioning our

studies on *T. brucei* rhythms. We found that *T. brucei* has a circadian clock and its rhythmic biology leads to time-of-day difference in susceptibility of the parasite to suramin treatment. *T. brucei* shows a circadian IC50 profile in response to the drug, in which there is over 2.5x higher sensitivity to suramin drug treatment when comparing two time-points that are just 4h apart (89).

Overall, we believe that circadian medicine for infectious diseases is worth exploring. The time-of-day administration may be useful when the drug targets are more highly expressed due to microbe's rhythmic biology, but also because mammalian physiology is so dynamic that there may be inherent rhythms in absorption and metabolization of the drugs.

Enhancement of immunity by time of day of vaccination

In humans, influenza antiviral vaccine administration in the morning led to higher antibody response over afternoon vaccination response in participants over 65 years old (101) (Fig. 2). In mice, time of day of vaccination against *Listeria monocytogens* led to differences in bacterial load upon infection. This effect is dependent on the clock of CD8 T cells which are more responsive to vaccination in the mid-rest phase than during the mid-active phase (102). Overall, the body's immune response is under circadian control and thus the time of day of vaccination may boost immunity against pathogens. Further studies are needed to fully identify the molecular and cellular players responsible for a potential higher protection to infections by vaccination and whether those can be boosted by defining the time or enhancing its circadian rhythms. This is an area of research that should be studied further, particularly in light of the global pandemic, but also when considering vaccination for influenza and other pathogens.

Final remarks

Interactions between hosts and microbes are complex, from symbiotic relationships where both parties benefit, to infections. Most of the studies of these relationships are at a particular moment in time or at a resolution of weeks and months for chronic infections. Reanalyzing these interactions at a higher resolution within a day is providing novel information about the daily dance between the biology of both entities: the host and microbe. Uncovering insights into the biology of pathogens will open up new possibilities to explore how to optimize current efforts to limit infections.

Acknowledgments

We thank Fernando Augusto (made-for.studio) for the design of the figures and Kimberly Cox (Efferent Manuscript Services). Apologies to those whose work was not cited because of content and length constraints. This work was supported by the Howard Hughes Medical Institute and NIH/NIGMS 1K99GM132557 (F.R.-F.). J.S.T. is an Investigator in the Howard Hughes Medical Institute.

References

1. Rijo-Ferreira F, Takahashi JS. Genomics of circadian rhythms in health and disease. Genome Med. 2019;11(1):82. [PubMed: 31847894]
2. Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017.

3. Halberg F, Johnson EA, Brown BW, Bittner JJ. Susceptibility rhythm to E. coli endotoxin and bioassay. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1960;103:142–4.

4. Scheiermann C, Gibbs J, Ince L, Loudon A. Clocking in to immunity. Nature reviews Immunology. 2018;18(7):423–37.

5. Hopwood TW, Hall S, Begley N, Forman R, Brown S, Vonslow R, et al. The circadian regulator BMAL1 programmes responses to parasitic worm infection via a dendritic cell clock. Scientific reports. 2018;8(1):3782. [PubMed: 29491349]

6. Kiessling S, Dubeau-Laramee G, Ohm H, Labrecque N, Olivier M, Cermakian N. The circadian clock in immune cells controls the magnitude of Leishmania parasite infection. Scientific reports. 2017;7(1):10892. [PubMed: 28883509]

7. Bellet MM, Deriu E, Liu JZ, Grimaldi B, Blaschitz C, Zeller M, et al. Circadian clock regulates the host response to Salmonella. Proc Natl Acad Sci U S A. 2013;110(24):9897–902. [PubMed: 23716692]

8. Edgar RS, Stangherlin A, Nagy AD, Nicoll MP, Efstathiou S, O'Neill JS, et al. Cell autonomous regulation of herpes and influenza virus infection by the circadian clock. Proc Natl Acad Sci U S A. 2016.

9. Shirasu-Hiza MM, Dionne MS, Pham LN, Ayres JS, Schneider DS. Interactions between circadian rhythm and immunity in Drosophila melanogaster. Curr Biol. 2007;17(10):R353–5. [PubMed: 17502084]

10. Stone EF, Fulton BO, Ayres JS, Pham LN, Ziauddin J, Shirasu-Hiza MM. The circadian clock protein timeless regulates phagocytosis of bacteria in Drosophila. PLoS Pathog. 2012;8(1):e1002445. [PubMed: 22253593]

11. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian Gene Bmal1 Regulates Diurnal Oscillations of Ly6Chi Inflammatory Monocytes. Science. 2013.

12. Gibbs J, Ince L, Matthews L, Mei J, Bell T, Yang N, et al. An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. Nat Med. 2014;20(8):919–26. [PubMed: 25064128]

13. Lundy SR, Ahmad T, Simoneaux T, Benyeogor I, Robinson Y, George Z, et al. Effect of Time of Day of Infection on Chlamydia Infectivity and Pathogenesis. Scientific reports. 2019;9(1):11405. [PubMed: 31388084]

14. O'Donnell AJ, Schneider P, McWatters HG, Reece SE. Fitness costs of disrupting circadian rhythms in malaria parasites. Proc Biol Sci. 2011;278(1717):2429–36. [PubMed: 21208950]

15. Laranjeira-Silva MF, Zampieri RA, Muxel SM, Floeter-Winter LM, Markus RP. Melatonin attenuates Leishmania (L.) amazonensis infection by modulating arginine metabolism. J Pineal Res. 2015;59(4):478–87. [PubMed: 26383232]

16. Carvalho Cabral P, Olivier M, Cermakian N. The Complex Interplay of Parasites, Their Hosts, and Circadian Clocks. Front Cell Infect Microbiol. 2019;9:425. [PubMed: 31921702]

17. Rijo-Ferreira F, Takahashi JS. Sleeping Sickness: A Tale of Two Clocks. Frontiers in Cellular and Infection Microbiology. 2020;10(551).

18. Njamnshi AK, Seke Etet PF, Ngarka L, Perrig S, Olivera GC, Nfor LN, et al. The Actigraphy Sleep Score: A New Biomarker for Diagnosis, Disease Staging, and Monitoring in Human African Trypanosomiasis. Am J Trop Med Hyg. 2020;103(6):2244–52. [PubMed: 33078699]

19. Buguet A, Tapie P, Bert J. Reversal of the sleep/wake cycle disorder of sleeping sickness after trypanosomicide treatment. J Sleep Res. 1999;8(3):225–35. [PubMed: 10476010]

20. Rijo-Ferreira F, Carvalho T, Afonso C, Sanches-Vaz M, Costa RM, Figueiredo LM, et al. Sleeping sickness is a circadian disorder. Nat Commun. 2018;9(1):62. [PubMed: 29302035]

21. Rijo-Ferreira F, Bjorness TE, Cox KH, Sonneborn A, Greene RW, Takahashi JS. Sleeping Sickness Disrupts the Sleep-Regulating Adenosine System. J Neurosci. 2020;40(48):9306–16. [PubMed: 33097636]

22. Lundkvist GB, Sellix MT, Nygard M, Davis E, Straume M, Kristensson K, et al. Clock gene expression during chronic inflammation induced by infection with Trypanosoma brucei brucei in rats. J Biol Rhythms. 2010;25(2):92–102. [PubMed: 20348460]

23. Thomas F SR A Martin G Manu C Durand P Renaud F. Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? J Evol Biol. 2002;15(3).
24. de Bekker C, Ohm RA, Loreto RG, Sebastian A, Albert I, Merrow M, et al. Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. BMC genomics. 2015;16:620. [PubMed: 26285697]
25. Levri EP LCM. The effects of size, reproductive condition, and parasitism on foraging behaviour in a freshwater snail,Potamopyrgus antipodarum. Animal Behaviour. 1996;51(4):891–901.
26. Majumdar T, Dhar J, Patel S, Kondratov R, Barik S. Circadian transcription factor BMAL1 regulates innate immunity against select RNA viruses. Innate Immun. 2017;23(2):147–54. [PubMed: 27913791]
27. Ehlers A, Xie W, Agapov E, Brown S, Steinberg D, Tidwell R, et al. BMAL1 links the circadian clock to viral airway pathology and asthma phenotypes. Mucosal Immunol. 2018;11(1):97–111. [PubMed: 28401936]
28. Zhuang X, Magri A, Hill M, Lai AG, Kumar A, Rambhatla SB, et al. The circadian clock components BMAL1 and REV-ERBalpha regulate flavivirus replication. Nat Commun. 2019;10(1):377. [PubMed: 30670689]
29. Borrmann H, Davies R, Dickinson M, Pedroza-Pacheco I, Schilling M, Vaughan-Jackson A, et al. Pharmacological activation of the circadian component REV-ERB inhibits HIV-1 replication. Scientific reports. 2020;10(1):13271. [PubMed: 32764708]
30. Zhuang X, Forde D, Tsukuda S, D'Arienzo V, Mailly L, Harris JM, et al. Circadian control of hepatitis B virus replication. Nat Commun. 2021;12(1):1658. [PubMed: 33712578]
31. Dierickx P, Emmett MJ, Jiang C, Uehara K, Liu M, Adlanmerini M, et al. SR9009 has REV-ERB-independent effects on cell proliferation and metabolism. Proc Natl Acad Sci U S A. 2019;116(25):12147–52. [PubMed: 31127047]
32. Gagnidze K, Hajdarovic KH, Moskalenko M, Karatsoreos IN, McEwen BS, Bulloch K. Nuclear receptor REV-ERBalpha mediates circadian sensitivity to mortality in murine vesicular stomatitis virus-induced encephalitis. Proc Natl Acad Sci U S A. 2016;113(20):5730–5. [PubMed: 27143721]
33. Sengupta S, Tang SY, Devine JC, Anderson ST, Nayak S, Zhang SL, et al. Circadian control of lung inflammation in influenza infection. Nat Commun. 2019;10(1):4107. [PubMed: 31511530]
34. Issah Y, Naik A, Tang SY, Forrest K, Brooks TG, Lahens N, et al. Loss of circadian protection against influenza infection in adult mice exposed to hyperoxia as neonates. eLife. 2021;10.
35. Haspel J, Kim M, Zee P, Schwarzmeier T, Montagnese S, Panda S, et al. A Timely Call to Arms: COVID-19, the Circadian Clock, and Critical Care. J Biol Rhythms. 2021;36(1):55–70. [PubMed: 33573430]
36. Kronfeld-Schor N, Stevenson TJ, Nickbakhsh S, Schernhammer ES, Dopico XC, Dayan T, et al. Drivers of Infectious Disease Seasonality: Potential Implications for COVID-19. J Biol Rhythms. 2021;36(1):35–54. [PubMed: 33491541]
37. Sengupta S, Ince L, Sartor F, Borrmann H, Zhuang X, Naik A, et al. Clocks, Viruses, and Immunity: Lessons for the COVID-19 Pandemic. J Biol Rhythms. 2021;36(1):23–34. [PubMed: 33480287]
38. Ray S, Reddy AB. COVID-19 management in light of the circadian clock. Nat Rev Mol Cell Biol. 2020;21(9):494–5. [PubMed: 32699357]
39. Leone MJ, Sigman M, Golombek DA. Effects of lockdown on human sleep and chronotype during the COVID-19 pandemic. Curr Biol. 2020;30(16):R930–R1. [PubMed: 32810450]
40. Wright KP Jr., Linton SK, Withrow D, Casiraghi L, Lanza SM, Iglesia H, et al. Sleep in university students prior to and during COVID-19 Stay-at-Home orders. Curr Biol. 2020;30(14):R797–R8. [PubMed: 32693068]
41. Smit AN, Juda M, Livingstone A, U SR, Mistlberger RE. Impact of COVID-19 social-distancing on sleep timing and duration during a university semester. PLoS One. 2021;16(4):e0250793. [PubMed: 33901264]
42. Korman M, Tkachev V, Reis C, Komada Y, Kitamura S, Gubin D, et al. COVID-19-mandated social restrictions unveil the impact of social time pressure on sleep and body clock. Scientific reports. 2020;10(1):22225. [PubMed: 33335241]

43. Robbins R, Affouf M, Weaver MD, Czeisler ME, Barger LK, Quan SF, et al. Estimated Sleep Duration Before and During the COVID-19 Pandemic in Major Metropolitan Areas on Different Continents: Observational Study of Smartphone App Data. J Med Internet Res. 2021;23(2):e20546. [PubMed: 33493126]
44. Coiro MJ, Asraf K, Tzischinsky O, Hadar-Shoval D, Tannous-Haddad L, Wolfson AR. Sleep quality and COVID-19-related stress in relation to mental health symptoms among Israeli and U.S. adults. Sleep Health. 2021;7(2):127–33. [PubMed: 33691986]
45. Gualano MR, Lo Moro G, Voglino G, Bert F, Siliquini R. Effects of Covid-19 Lockdown on Mental Health and Sleep Disturbances in Italy. Int J Environ Res Public Health. 2020;17(13).
46. Hyun S, Hahm HC, Wong GTF, Zhang E, Liu CH. Psychological correlates of poor sleep quality among U.S. young adults during the COVID-19 pandemic. Sleep Med. 2021;78:51–6. [PubMed: 33385779]
47. CDC. Coping with Stress. 2021.
48. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. The New England journal of medicine. 2021.
49. Cavadini G, Petrzilka S, Kohler P, Jud C, Tobler I, Birchler T, et al. TNF-alpha suppresses the expression of clock genes by interfering with E-box-mediated transcription. Proc Natl Acad Sci U S A. 2007;104(31):12843–8. [PubMed: 17646651]
50. Prior KF, O'Donnell AJ, Rund SSC, Savill NJ, van der Veen DR, Reece SE. Host circadian rhythms are disrupted during malaria infection in parasite genotype-specific manners. Scientific reports. 2019;9(1):10905. [PubMed: 31358780]
51. Fernandez Alfonso T, Celentano AM, Gonzalez Cappa SM, Golombek DA. The circadian system of Trypanosoma cruzi-infected mice. Chronobiol Int. 2003;20(1):49–64. [PubMed: 12638690]
52. de Leone MJ, Hernando CE, Romanowski A, Careno DA, Soverna AF, Sun H, et al. Bacterial Infection Disrupts Clock Gene Expression to Attenuate Immune Responses. Curr Biol. 2020;30(9):1740–7 e6. [PubMed: 32220315]
53. Li Z, Bonaldi K, Uribe F, Pruneda-Paz JL. A Localized Pseudomonas syringae Infection Triggers Systemic Clock Responses in Arabidopsis. Curr Biol. 2018.
54. Bellet MM, Zocchi L, Sassone-Corsi P. The RelB subunit of NFkappaB acts as a negative regulator of circadian gene expression. Cell Cycle. 2012;11(17):3304–11. [PubMed: 22894897]
55. McFall-Ngai M Divining the essence of symbiosis: insights from the squid-vibrio model. PLoS Biol. 2014;12(2):e1001783. [PubMed: 24504482]
56. Reitmeier S, Kiessling S, Clavel T, List M, Almeida EL, Ghosh TS, et al. Arrhythmic Gut Microbiome Signatures Predict Risk of Type 2 Diabetes. Cell Host Microbe. 2020;28(2):258–72 e6. [PubMed: 32619440]
57. Kuang Z, Wang Y, Li Y, Ye C, Ruhn KA, Behrendt CL, et al. The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. Science. 2019;365(6460):1428–34. [PubMed: 31604271]
58. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell. 2014;159(3):514–29. [PubMed: 25417104]
59. Thaiss CA, Levy M, Korem T, Dohnalova L, Shapiro H, Jaitin DA, et al. Microbiota Diurnal Rhythmicity Programs Host Transcriptome Oscillations. Cell. 2016;167(6):1495–510 e12. [PubMed: 27912059]
60. Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host Microbe. 2015;17(5):681–9. [PubMed: 25891358]
61. Zarrinpar A, Chaix A, Yooseph S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metab. 2014;20(6):1006–17. [PubMed: 25470548]
62. Wang Y, Kuang Z, Yu X, Ruhn KA, Kubo M, Hooper LV. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. Science. 2017;357(6354):912–6. [PubMed: 28860383]

63. Tuganbaev T, Mor U, Bashiardes S, Liwinski T, Nobs SP, Leshem A, et al. Diet Diurnally Regulates Small Intestinal Microbiome-Epithelial-Immune Homeostasis and Enteritis. Cell. 2020;182(6):1441–59 e21. [PubMed: 32888430]
64. Manoogian ENC, Panda S. Circadian rhythms, time-restricted feeding, and healthy aging. Ageing Res Rev. 2017;39:59–67. [PubMed: 28017879]
65. Acosta-Rodríguez VA Filipa R-F; Green, Carla B; Takahashi, Joseph S Importance of circadian timing for aging and longevity. Nature Communications. 2021;12(2862).
66. Takayasu L, Suda W, Takanashi K, Iioka E, Kurokawa R, Shindo C, et al. Circadian oscillations of microbial and functional composition in the human salivary microbiome. DNA Res. 2017;24(3):261–70. [PubMed: 28338745]
67. Collado MC, Engen PA, Bandin C, Cabrera-Rubio R, Voigt RM, Green SJ, et al. Timing of food intake impacts daily rhythms of human salivary microbiota: a randomized, crossover study. FASEB J. 2018;32(4):2060–72. [PubMed: 29233857]
68. Tseng R, Goularte NF, Chavan A, Luu J, Cohen SE, Chang YG, et al. Structural basis of the day-night transition in a bacterial circadian clock. Science. 2017;355(6330):1174–80. [PubMed: 28302851]
69. Ito-Miwa K, Furuike Y, Akiyama S, Kondo T. Tuning the circadian period of cyanobacteria up to 6.6 days by the single amino acid substitutions in KaiC. Proc Natl Acad Sci U S A. 2020;117(34):20926–31. [PubMed: 32747571]
70. Swan JA, Golden SS, LiWang A, Partch CL. Structure, function, and mechanism of the core circadian clock in cyanobacteria. J Biol Chem. 2018;293(14):5026–34. [PubMed: 29440392]
71. Costantini C, Renga G, Sellitto F, Borghi M, Stincardini C, Pariano M, et al. Microbes in the Era of Circadian Medicine. Front Cell Infect Microbiol. 2020;10:30. [PubMed: 32117804]
72. Loza-Correa M, Sahr T, Rolando M, Daniels C, Petit P, Skarina T, et al. The Legionella pneumophila kai operon is implicated in stress response and confers fitness in competitive environments. Environ Microbiol. 2014;16(2):359–81. [PubMed: 23957615]
73. Paulose JK, Wright JM, Patel AG, Cassone VM. Human Gut Bacteria Are Sensitive to Melatonin and Express Endogenous Circadian Rhythmicity. PLoS One. 2016;11(1):e0146643. [PubMed: 26751389]
74. Paulose JK, Cassone CV, Graniczkowska KB, Cassone VM. Entrainment of the Circadian Clock of the Enteric Bacterium Klebsiella aerogenes by Temperature Cycles. iScience. 2019;19:1202–13. [PubMed: 31551197]
75. Eelderink-Chen Z, Bosman J, Sartor F, Dodd AN, Kovacs AT, Merrow M. A circadian clock in a nonphotosynthetic prokaryote. Sci Adv. 2021;7(2).
76. Hong HA, Khaneja R, Tam NM, Cazzato A, Tan S, Urdaci M, et al. Bacillus subtilis isolated from the human gastrointestinal tract. Res Microbiol. 2009;160(2):134–43. [PubMed: 19068230]
77. Johnson CH, Zhao C, Xu Y, Mori T. Timing the day: what makes bacterial clocks tick? Nat Rev Microbiol. 2017;15(4):232–42. [PubMed: 28216658]
78. Zinser ER, Lindell D, Johnson ZI, Futschik ME, Steglich C, Coleman ML, et al. Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, prochlorococcus. PLoS One. 2009;4(4):e5135. [PubMed: 19352512]
79. Chew J, Leypunskiy E, Lin J, Murugan A, Rust MJ. High protein copy number is required to suppress stochasticity in the cyanobacterial circadian clock. Nat Commun. 2018;9(1):3004. [PubMed: 30068980]
80. Holtzendorff J, Partensky F, Mella D, Lennon JF, Hess WR, Garczarek L. Genome streamlining results in loss of robustness of the circadian clock in the marine cyanobacterium Prochlorococcus marinus PCC 9511. J Biol Rhythms. 2008;23(3):187–99. [PubMed: 18487411]
81. Aronson BD, Johnson KA, Loros JJ, Dunlap JC. Negative feedback defining a circadian clock: autoregulation of the clock gene frequency. Science. 1994;263(5153):1578–84. [PubMed: 8128244]
82. Hevia MA, Canessa P, Larrondo LF. Circadian clocks and the regulation of virulence in fungi: Getting up to speed. Semin Cell Dev Biol. 2016;57:147–55. [PubMed: 27039027]

83. Hevia MA, Canessa P, Muller-Esparza H, Larrondo LF. A circadian oscillator in the fungus Botrytis cinerea regulates virulence when infecting Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2015;112(28):8744–9. [PubMed: 26124115]
84. Mouahid G, Nguema RM, Al Mashikhi KM, Al Yafae SA, Idris MA, Mone H. Host-parasite life-histories of the diurnal versus nocturnal chronotypes of Schistosoma mansoni: adaptive significance. Trop Med Int Health. 2019.
85. Paily KP, Hoti SL, Das PK. A review of the complexity of biology of lymphatic filarial parasites. Journal of parasitic diseases : official organ of the Indian Society for Parasitology. 2009;33(1-2):3–12. [PubMed: 23129882]
86. Southworth GC, Mason G, Seed JR. Studies on frog trypanosomiasis. I. A 24-hour cycle in the parasitemia level of Trypanosoma rotatorium in Rana clamitans from Louisiana. J Parasitol. 1968;54(2):255–8. [PubMed: 5647105]
87. Cornford EM, Freeman BJ, MacInnis AJ. Physiological relationships and circadian periodicities in rodent trypanosomes. Trans R Soc Trop Med Hyg. 1976;70(3):238–43. [PubMed: 982519]
88. Rijo-Ferreira F TJS, Figueiredo LM Circadian rhythms in parasites. PLoS Pathog. 2017;13(10): e1006590. [PubMed: 29023533]
89. Rijo-Ferreira F, Pinto-Neves D, Barbosa-Morais NL, Takahashi JS, Figueiredo LM. Trypanosoma brucei metabolism is under circadian control. Nat Microbiol. 2017;2:17032. [PubMed: 28288095]
90. Hawking F, Worms MJ, Gammage K. Host temperature and control of 24-hour and 48-hour cycles in malaria parasites. Lancet. 1968;1(7541):506–9. [PubMed: 4170834]
91. Taliaferro WH TL. Alteration in the time of sporulation of Plasmodium brasilianum in monkeys by reversal of light and dark. Am J Hyg. 1934;20:50–9.
92. Rijo-Ferreira F, Acosta-Rodriguez VA, Abel JH, Kornblum I, Bento I, Kilaru G, et al. The malaria parasite has an intrinsic clock. Science. 2020;368(6492):746–53. [PubMed: 32409471]
93. Smith LM, Motta FC, Chopra G, Moch JK, Nerem RR, Cummins B, et al. An intrinsic oscillator drives the blood stage cycle of the malaria parasite Plasmodium falciparum. Science. 2020;368(6492):754–9. [PubMed: 32409472]
94. Subudhi AK, O'Donnell AJ, Ramaprasad A, Abkallo HM, Kaushik A, Ansari HR, et al. Malaria parasites regulate intra-erythrocytic development duration via serpentine receptor 10 to coordinate with host rhythms. Nat Commun. 2020;11(1):2763. [PubMed: 32488076]
95. Ruben MD, Smith DF, FitzGerald GA, Hogenesch JB. Dosing time matters. Science. 2019;365(6453):547–9. [PubMed: 31395773]
96. Cederroth CR, Albrecht U, Bass J, Brown SA, Dyhrfjeld-Johnsen J, Gachon F, et al. Medicine in the Fourth Dimension. Cell Metab. 2019;30(2):238–50. [PubMed: 31390550]
97. Dallmann R, Okyar A, Levi F. Dosing-Time Makes the Poison: Circadian Regulation and Pharmacotherapy. Trends Mol Med. 2016;22(5):430–45. [PubMed: 27066876]
98. Montaigne D, Marechal X, Modine T, Coisne A, Mouton S, Fayad G, et al. Daytime variation of perioperative myocardial injury in cardiac surgery and its prevention by Rev-Erbalpha antagonism: a single-centre propensity-matched cohort study and a randomised study. Lancet. 2018;391(10115):59–69. [PubMed: 29107324]
99. Ruben MD, Wu G, Smith DF, Schmidt RE, Francey LJ, Lee YY, et al. A database of tissue-specific rhythmically expressed human genes has potential applications in circadian medicine. Sci Transl Med. 2018;10(458).
100. Damato AR LJ, Katumba RGN, Talcott GR, Rubin JB, Herzog ED, and Campian JL. Temozolomide chronotherapy in patients with glioblastoma: a retrospective single institute study. Neuro-Oncology Advances. 2021;vdab041.
101. Long JE, Drayson MT, Taylor AE, Toellner KM, Lord JM, Phillips AC. Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial. Vaccine. 2016;34(24):2679–85. [PubMed: 27129425]
102. Nobis CC, Dubeau Laramee G, Kervezee L, Maurice De Sousa D, Labrecque N, Cermakian N. The circadian clock of CD8 T cells modulates their early response to vaccination and the rhythmicity of related signaling pathways. Proc Natl Acad Sci U S A. 2019;116(40):20077–86. [PubMed: 31527231]

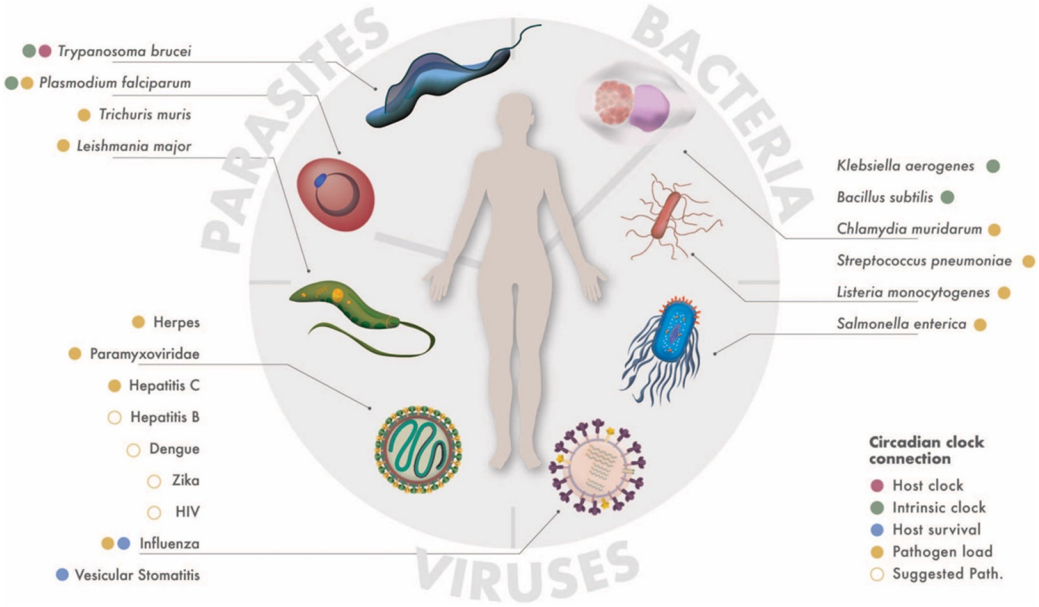

Figure 1:

Circadian rhythms underlying interactions between hosts and microbes. The circadian clock has been shown to impact many different mammalian infections. So far three main groups of mammalian pathogens and symbionts have been studied: viruses, bacteria, and parasites. We enumerate many of those that have been linked to the circadian system and highlight to which capacity: i) infection with the pathogen alters the circadian clock of the host (Host clock); ii) the microbe has an intrinsic clock independent of host or environmental signals (Intrinsic clock); iii) time of day of infection initiation impacts host survival (Host survival); and iv) time of day of infection initiation impacts pathogen load (Pathogen load). Some viral infections are also mentioned but are assigned to category v) Suggested Pathogen Load, because further studies are required (see section ‘Infection with viruses’).

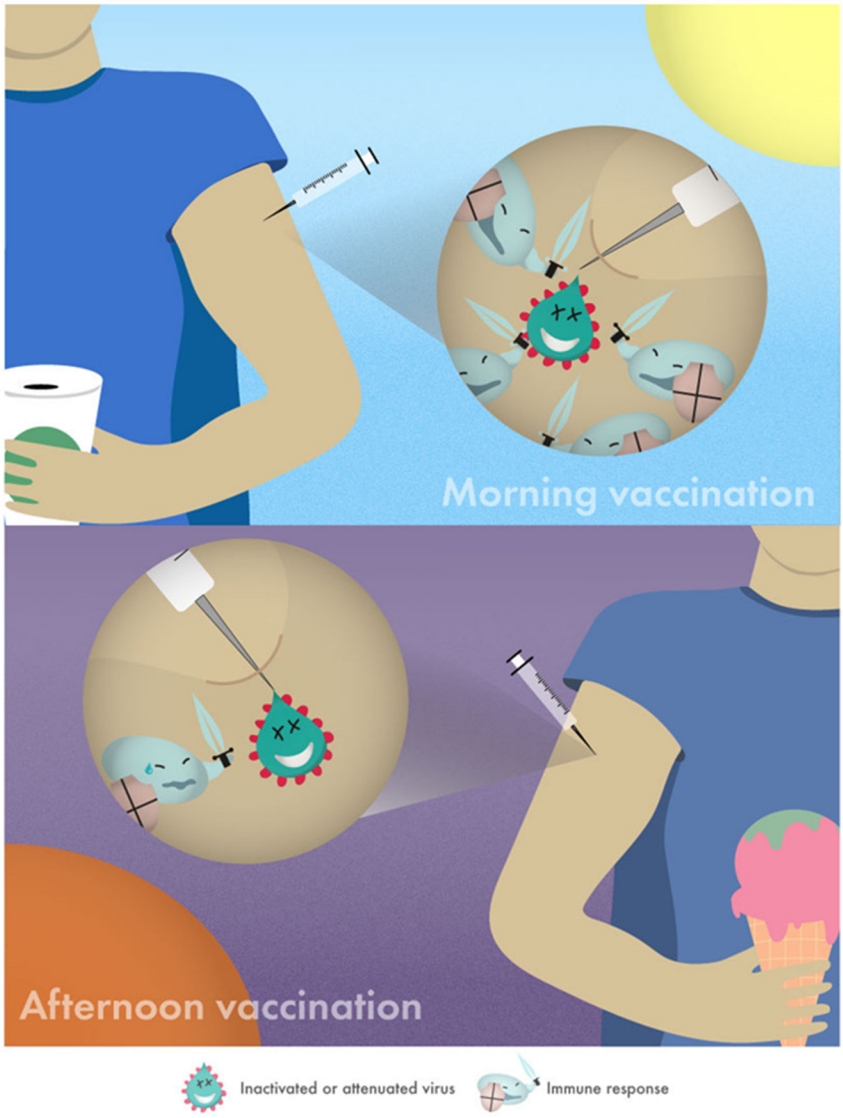

Figure 2:
Illustration of the potential for boosting immune response to infections by studying the effect of aligning the time of vaccination with internal biological rhythms.