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Interplay between Microbes and the Circadian Clock

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Circadian rhythms influence virtually all life forms on our planet, a notion that opens the question on how the circadian cycles of individual organisms may interplay with each other. In mammals, a potentially dangerous environmental stress is represented by encounters with infectious agents. Microbial attack is a major risk for organismal homeostasis and therefore needs to be efficiently counteracted by mechanisms implemented by the host immune system. Accumulating evidence shows that the immune system may anticipate an emerging pathogenic exposure through an enhanced inflammatory state. Notably, the circadian clock orchestrates these anticipatory responses to fluctuating conditions in the external world. In this article, we review the current knowledge about the relationship between the circadian clock and pathogenic infections. We discuss the role of the circadian clock against infection and specific pathogens, the core clock proteins involved in the defense mechanisms, and the specific tissue or cell type in which they function to counteract the infection. Finally, circadian oscillations in the gut microbiome composition and its possible role in protecting against foodborne pathogen colonization are presented.

Circadian clocks are intrinsic, time-tracking systems that allow organisms to adapt their behavior and physiology to the appropriate time of day. Circadian rhythms have evolved to enable organisms to anticipate environmental changes such as food availability and predatory pressure, and are indeed conserved among virtually all forms of life on Earth (Peek et al. 2015). A wide array of biological processes function under circadian control and contribute to whole-body physiology and homeostasis (Schibler and Sassone-Corsi 2002; Abbott et al. 2015; Gerber et al. 2015; McLoughlin et al. 2015; Tsang et al. 2016).

In mammals, the master circadian timekeeper is localized in the suprachiasmatic nucleus (SCN) of the hypothalamus and is mainly entrained by light signals and transduced by specialized photoreceptors present in the retina (Doyle and Menaker 2007). In addition, different regions of the brain or peripheral organs, such as the liver, intestine, and heart, have local clocks (Kyriacou and Hastings 2010) whose function contributes to regulate homeostasis and physiological responses (Mohawk et al. 2012). Through signaling mechanisms that remain uncharacterized, the SCN appears to operate as an orchestra director to keep peripheral

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clocks in synchrony, ensuring that physiology across the entire organism is temporally integrated and thus maximally adaptive (Hastings et al. 2003).

The clock molecular machinery is characterized by a complex transcriptional–translational feedback loop that ensures 24 h oscillation in gene expression. The positive arm of the mammalian clock machinery is comprised of CLOCK and BMAL1, two transcriptional activators that heterodimerize and induce the expression of clock-controlled genes (CCGs) by binding to their promoters at E-box consensus sites. Cryptochromes (*Cry1, Cry2*) and Period genes (*Per1*, *Per2*, *Per3*) are CCGs encoding proteins that form the negative arm of the circadian machinery. PER and CRY proteins are classically thought to translocate into the nucleus to inhibit CLOCK:BMAL1-mediated transcription, thereby closing the negative feedback loop. Moreover, the nuclear related orphan receptors (RORs) and REV-ERB-α/β represent additional layers of circadian regulation through the control of *Bmal1* rhythmicity (Guillaumond et al. 2005). An important aspect of the clock system is that a delay between positive element transcription and negative element expression is required to keep the clock in an appropriate phase. Post-translational modifications, such as phosphorylation and acetylation, play an essential role in generating this delay, thus being involved in fine-tuning the function and accuracy of the molecular clock system (Aguilar-Arnal and Sassone-Corsi 2013).

An additional, important layer of control relates to chromatin remodeling of cyclic expressed genes. Notably, the core circadian protein CLOCK has an acetyltransferase activity counterbalanced by the deacetylase activity of SIRT1 (Asher et al. 2008; Nakahata et al. 2008), a protein showing direct interaction with CLOCK. SIRT1 activity and the levels of NAD^+, its cofactor necessary for acetyl group removal, oscillate following a 24 h cycle (Nakahata et al. 2009; Ramsey et al. 2009), revealing a link between protein acetylation, cyclic rhythms, and cell energy metabolism. Indeed, both NAD^+ and the CLOCK cofactor acetyl-CoA can be considered as indicators of the cellular energy status. Acetyl-CoA is at the metabolic crossroads of glycolysis, fatty acid oxidation, ketogenesis, amino acid metabolism, and tricarboxylic acid cycle, making its synthesis and usage an ideal parameter to sense metabolic network functions. Similarly, the intracellular NAD^+/NADH ratio is a central determinant of nutritional status, regulating the activity of NAD^+-dependent deacetylases, such as SIRT1 and SIRT6, which in turn might regulate the global acetylation processes (Masri and Sassone-Corsi 2014).

ANTICIPATION OF ENVIRONMENTAL CHALLENGES BY THE ENDOGENOUS CLOCK

A remarkable feature of the endogenous clock is to anticipate a variety of fluctuations in the environment allowing an appropriate physiological adaptation. An imminent threat in everyday life is the potential encounter with pathogens. The vulnerability of the susceptible host and the virulence of the pathogen may vary both with developmental stage and the time of the day, thus affecting the outcome of the interaction. Obviously, during the active phase and feeding, the probability of infection with food-borne pathogens is higher, requiring anticipatory mechanisms that entrain the magnitude of the immune response to the time of maximal risk. Circadian variations in immune function and disease susceptibility have been noted in animals as well as in humans (Roberts 2000). Lymphocyte proliferation and trafficking (Hiemke et al. 1995; Fortier et al. 2011; Druzd et al. 2017), T-cell lineage specification (Yu et al. 2013), cytokine and chemokine expression (Hayashi et al. 2007; Rahman et al. 2015), response to antigen presentation (Fortier et al. 2011), and leukocyte tissue recruitment (Scheiermann et al. 2012) are significant examples of diurnal immune functions. In the human blood, higher counts of lymphocytes, T lymphocytes, and B lymphocytes have been consistently observed in the nighttime (Dimitrov et al. 2009; Mazzoccoli et al. 2010; Ackermann et al. 2012; Cermakian et al. 2013). Moreover, cytokines and other effector molecules present daily changes in their levels. Serum concentration and in vitro production of inter-

feron (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-6, and IL-12 are oscillating in humans with a zenith at night or in the early morning (Born et al. 1997; Dimitrov et al. 2006; Cermakian et al. 2013).

Not only immune system components, but also the manifestation of many human diseases display diurnal characteristics. Chronic obstructive pulmonary disease and asthma show more severe exacerbation during night and early morning (Sundar et al. 2015), whereas patients with rheumatoid arthritis experience the most painful joint stiffness in the morning, which is often ascribed to joint fluid viscosity, but is also associated with a peak in proinflammatory cytokine blood level at that time of the day (Gibbs and Ray 2013). On the other hand, ischemic and hemorrhagic stroke (Elliott 1998; Gupta and Shetty 2005), myocardial infarction (Cohen et al. 1997; Muller et al. 1997), and vaso-occlusive crisis (Auvil-Novak et al. 1996) are more frequent at dawn.

The clock within immune cells (Arjona and Sarkar 2005; Keller et al. 2009; Curtis et al. 2014), together with circadian neuronal and endocrine signals (Logan and Sarkar 2012), work in concert to regulate the host immune response. Recently, several studies have highlighted the importance of a functional clock system to cope with a variety of bacterial and viral pathogens (Tognini et al. 2017). In this article, we will review the mechanisms underlying the interaction between the circadian clock and specific infectious agents. We will discuss how diurnal variations in the host immune response are generated and how the clock and the infectious state reciprocally influence each other. Additionally, we will give some evidence on the importance of a healthy gut microbiota for normal development of the immune system and its responses.

CIRCADIAN CLOCK GATING OF INFLAMMATORY RESPONSES

More than 50 years ago, it was noted that the severity of *Escherichia coli* endotoxin administration in mouse models depended on the daily time of treatment (Halberg et al. 1960). In particular, the immune system responded in a more efficient manner during or immediately before the host phase of activity. It is conceivable that, to optimize the host fitness, control exerted by the circadian clock on the immune system might boost the inflammatory response when potential pathogen attacks are most likely.

Intriguingly, the peaks of circadian oscillation of various immune system features are unique and time-of-day specific. For the mouse they are blood and splenic Ly6C^{hi} monocytes (Zeitgeber time [ZT]8) (Nguyen et al. 2013), leukocyte recruitment to tissues (ZT13) and blood (ZT5) (Scheiermann et al. 2012), lung cytokines (ZT6) and chemokines (ZT0-ZT18) after lipopolysaccharide (LPS) treatment, *Cxcl5* expression in bronchiolar epithelial cells (ZT0) (Gibbs et al. 2014), Toll-like receptor 9 (*Tlr9*) expression in splenic tissue (ZT19), macrophages (ZT11) and B cells (ZT15-ZT19) (Silver et al. 2012), and lymphocytes (ZT5 in blood, ZT13 in lymph nodes) (Druzd et al. 2017). Moreover, the achrophase of core clock genes involved in the inflammatory response does not overlap, suggesting that the asynchronicity in the diurnal profile of the diverse immune molecules and effectors might work as a defense in case of an overwhelming immune reaction such as during sepsis (Man et al. 2016). Sepsis is a serious illness caused by an abnormal activation of the immune system against an infection, leading to tissue and organ injuries, and possibly death. Interestingly, splenic *Tlr9* expression, a highly conserved pattern recognition receptor (PRR) that recognizes pathogen-associated molecular patterns (PAMPs) from both viruses and bacteria, displays circadian fluctuation that correlates with severity in sepsis outcome in mice. Indeed, animals subjected to cecal ligation and puncture (CLP) at ZT19, when TLR9 is elevated, experience exacerbated sepsis and earlier mortality as compared to mice receiving puncture at ZT7 (Silver et al. 2012). In keeping with an involvement of the circadian system, *Clock*-deficient mice, a model characterized by lack of rhythmicity in peripheral oscillators (DeBruyne et al. 2007), had a higher survival rate, lower serum levels of inflammatory cytokines, and, finally, a better outcome after polymicrobial sepsis regardless of the CLP circadian time (Wang et al.

2016). While the molecular mechanisms have not been fully elucidated, CLOCK directly binds the promoter of *Tlr9* gene, activates its expression (Silver et al. 2012), and positively controls the expression of nuclear factor (NF)-κB, a key transcription factor involved in innate immune response (Spengler et al. 2012), indicating a possible dampening of the excessive inflammatory reaction driven by sepsis with a consequently less severe result on clock disruption.

THE CIRCADIAN CLOCK PRIMES THE IMMUNE SYSTEM TO FIGHT A VARIETY OF PATHOGEN ATTACKS

A tight circadian regulation of the host immune system is emerging as a crucial strategy to fight different infectious agents, and distinct immune cells and clock proteins have been implicated in the response to specific pathogens. In this section, we show how the clock system in different tissues contributes to fight infection agents of various origins, in particular, airborne and foodborne pathogens.

Airborne Pathogens

During the active period of the circadian cycle, mammals may be in contact with environmental microorganisms. The clock appears to coordinate the immune response to intranasal attacks in a diurnal fashion, as highlighted by the studies discussed below.

Herpes and Influenza Viruses

Murid herpesvirus 4 (MuHV-4) and Herpes simplex virus 1 (HSV-1) showed higher virulence in mice infected at ZT0 compared to mice infected at ZT12 (Edgar et al. 2016), confirming the higher susceptibility of the host during the resting phase. Disruption of the clock in mice ablated of the *Bmal1* gene results in complete abolishing of the infection diurnal gating. Indeed, high levels of replications were observed when the *Bmal1*-null animals were inoculated at any time of the day. It is worth noting that the clock is primarily involved in regulating and anticipating the first encounter with the pathogen (acute phase). Indeed, circadian disruption by deletion of *Bmal1* did not influence the latent infection.

A remarkable twist in the relationship between the clock system and herpes viruses was the demonstration of physical and functional interaction between the regulatory protein ICP0 of HSV-1 and BMAL1 in vitro, promoting the expression of specific transcripts (Kawaguchi et al. 2001). Whereas the precise mechanism responsible for this transcriptional alteration has not been explored, another in vitro study has shown that CLOCK is a component of the HSV-1 transcriptional machinery and its acetyltransferase activity might contribute to chromatin remodeling and viral gene expression (Doi et al. 2006; Kalamvoki and Roizman 2011). The diurnal behavior of these interactions, however, needs further investigation.

Interestingly, in contrast to herpes virus, influenza A virus (IAV) does not exploit the host-cell transcriptional machinery, although it still displays an increase in viral protein production in arrhythmic cells, which is linked to modification in the levels of specific enzymes implicated in protein biosynthesis (Edgar et al. 2016). Therefore, different types of viruses appears to directly influence the molecular clock via a subset of distinct interactions with core clock proteins (Kawaguchi et al. 2001; Kalamvoki and Roizman 2011) and their gene expression (Edgar et al. 2016), or indirectly through secondary mechanisms of cell physiology governed by the circadian clock. Whereas the exact mechanisms have not been elucidated to date, the cited examples clearly highlight that the host immune response and, in particular, the cellular reaction to viral attack are profoundly associated with diurnal oscillations by a functional clock.

Streptococcus Pneumoniae

It has been recently shown that the endogenous clock in bronchiolar epithelial cells regulates the magnitude of pulmonary inflammation and the circadian oscillation in its manifestation (Gibbs et al. 2014). *S. pneumoniae* was more aggressive when the mice were infected at ZT0 (light ON, beginning of the sleeping phase) instead of at

ZT12 (light OFF, beginning of the active/feeding phase), displaying a higher bacterial burden in lung and blood 48 h after treatment. The mechanism responsible for the daily inflammatory response operates through the lung oscillation of the chemokine CXCL5, a powerful neutrophil chemoattractant. CXCL5 was shown to be under circadian regulation through rhythmic glucocorticoid receptor (GR)-driven repression. Importantly, diurnal GR binding to the *Cxcl5* promoter was disrupted by bronchiolar-specific ablation of *Bmal1*, causing an abnormal CXCL5 production and an enhancement in neutrophil recruitment on *S. pneumoniae* infection. Finally, bronchiolar epithelial cell–specific *Bmal1*-null mice displayed a lack of circadian gating in their immune response to bacterial infection (Gibbs et al. 2014). This finding indicates that the local clock cross talking with systemic glucocorticoid signals plays a crucial role in the time-of-day-dependent synchronization of pulmonary innate immunity, making the immune response more efficient when the pathogenic attack is most likely to happen. Thus, the results of this study highlight the tight link between lung clock function, glucocorticoid signals, and innate immunity. In humans, several inflammatory pulmonary diseases, such as chronic obstructive pulmonary disorder and asthma, show circadian fluctuations in the manifestation of the symptomatology (Sundar et al. 2015). Local pharmacological targeting of the endogenous clock in the lung might be a promising treatment to alleviate the severity of these pathologies. On the other hand, the data suggests that variability among patients in the efficacy of steroid-based medicaments might be caused by disruption of circadian rhythms.

Vesicular Stomatitis Virus (VSV)

Intranasal VSV administration is a classic model of encephalitis in mice. Importantly, it has been shown that VSV infection displays a strong diurnal rhythmicity. Mice infected at the beginning of the resting phase (ZT0) had a lower survival rate than animals infected at the start of the active phase (ZT12). This finding was associated with an increase in blood CCL2 and higher levels of inflammatory monocytes in the olfactory bulb. To dissect the molecular mechanisms behind the diurnal control of the mortality on VSV infection, the authors investigated changes in core clock genes along the circadian cycle in the olfactory bulb. The nuclear receptor *Rev-erb*-α was differentially expressed between ZT0 and ZT12, identifying this transcriptional repressor as a possible regulator of the rhythmic VSV effects in vivo. Interestingly, the peak of *Rev-erb*-α gene expression (ZT12) coincided with the time of greater survival rate after VSV treatment, and REV-ERB-α-driven repression of CCL2 was identified as the mechanism underlying this specific neuroinflammatory state and mediating the diurnal effect on the survival rate (Gagnidze et al. 2016). Therefore, REV-ERB-α appears to operate as a modulator of the expression of important inflammatory mediators during virus-dependent encephalitis.

Sendai Virus (SeV)

SeV is naturally found in mice, rats, guinea pigs, hamsters, and pigs. Its infection principally affects the respiratory system, causing acute bronchiolitis followed by chronic airway changes that resemble the manifestation of asthma in humans. Wild-type (WT) mice were compared to *Bmal1*-null mice and tamoxifen-inducible *Bmal1*-null mice after being inoculated with SeV (Ehlers et al. 2017). Both *Bmal1* mutant animals displayed greater susceptibility to infection characterized by higher mortality rate, viral RNA expression, and viral load. Moreover, the altered antiviral response caused by *Bmal1* ablation exacerbated chronic lung inflammation. Similar effects were observed in jet-lagged mice after SeV inoculation. Intriguingly, decreased levels of *Bmal1* and some CCGs were found in airway cells of patients with asthma, suggesting an important connection between circadian disruption and lung pathology (Ehlers et al. 2017). Although the diurnal character of the sensitivity to infection was not analyzed, these data nonetheless strengthen the necessity of a functional clock and a correct circadian rhythmicity for an efficient host immune reaction to pathogenic insults.

Foodborne Pathogens

Feeding is an essential aspect of organismal everyday life. Feeding-time corresponds to the phase of activity, which, as already mentioned, is distinguished by a higher probability to be attacked by bacteria or viruses present in the external milieu. Food is essential for survival; however, it might be an easy source of infectious agents. As in the case of airborne pathogens, the internal clock positions the immune system for a possible foodborne pathogen assault, by anticipating the imminent risk. Here, the last animal model studies in the field are discussed.

Listeria Monocytogenes

Ly6C^{hi} monocytes represent fundamental defenses against *L. monocytogenes* infection, which is responsible for a specific infection called "listeriosis." Mice infected at the starting of their resting phase (ZT0) displayed a higher number of bacteria in their spleen, liver, and peritoneum than animals treated at ZT8 (4 h before the start of their active phase). Furthermore, Ly6C^{hi} monocyte recruitment to the peritoneum features daily rhythmicity, with monocyte numbers being higher at ZT8 than at ZT0. The investigators found that in myeloid-specific *Bmal1* knockout (KO) mice characterized by a cell-specific disruption of the clock system, the diurnal tissue trafficking of Ly6C^{hi} monocytes was totally abolished. The chemokine *Ccl2* gene circadian fluctuation in bone marrow–derived macrophages was BMAL1-mediated through the recruitment of the polycomb repressive complex 2 (PRC2) to the promoter of its gene, indicating that functional interactions between the clock and epigenetic modifications are responsible to sustain diurnal variation in cytokine levels and, thus, in Ly6C^{hi} monocytes (Nguyen et al. 2013). This study highlights the important role of myeloid BMAL1 as an "epigenetic" anti-inflammatory mediator through its rhythmic binding to the *Ccl2* promoter and silencing via histone methylation via the PRC2 complex recruitment. Although far from being a therapeutic application, further investigations might elucidate the molecular events that underline the phenotype observed in this study, hopefully opening novel avenues for clinical applications.

Salmonella Typhimurium

Salmonella Typhimurium is a pathogenic Gram-negative bacterium generally found in the intestinal lumen. The outer membrane consisting largely of LPSs is principally responsible for its toxicity. In a mouse model of infectious colitis, animals showed specific reactions to acute *Salmonella enterica* infection in a time-of-day-dependent fashion. Bacterial colonization in the colon was higher in mice treated at ZT4 (daytime, resting phase) than in mice treated at ZT16 (nighttime, active phase), as was the level of cecum inflammation. Remarkably, *Clock* mutant mice, which display profound impairment in circadian rhythmicity, showed no difference in colonization between daytime and nighttime infection. Moreover a significant decrease in overall proinflammatory gene expression was present, indicating that a functional clock is indispensable to coordinate the host immune response to *Salmonella* (Bellet et al. 2013). Outstanding questions remain unsolved: which cell-specific clocks are involved in the timing regulation of the immune response to *Salmonella*? Are components of the core clock directly interacting with the pathogens? Is the intestinal clock cross talking with other peripheral clocks during the infection? Their elucidation could unveil novel approaches to treat infectious colitis in humans.

CIRCADIAN RHYTHMS AND NUTRITION: HOW A HEALTHY LIFESTYLE CONTRIBUTES TO A STRONGER IMMUNE SYSTEM

The studies discussed show an intimate relationship between clock function and the host immune response to various infectious agents in multiple tissues. The endogenous clock has also been involved in other important aspects related to organismal homeostasis, and in particular to metabolic regulation (Eckel-Mahan and Sassone-Corsi 2013). Metabolically active organs, such as the liver, pancreas, and intestine,

all contain clocks, whose oscillation is orchestrated by the central SCN clock and, importantly, by other external cues such as food (Richards and Gumz 2012). Indeed, feeding and food accessibility are fundamental ZTs for peripheral tissue clocks (Asher and Sassone-Corsi 2015). On the other hand, rhythmic feeding behavior is controlled by the endogenous clock system, and both master clock and peripheral clocks participate in the maintenance of diurnal feeding patterns and energy metabolism (Bechtold and Loudon 2013). The importance of the circadian cycle for a correct metabolic function has been elucidated both by genetic animal models and by human studies. In particular, clock Δ19 mutant mice displayed altered feeding rhythms, hyperphagia, and obesity and developed metabolic syndrome (Turek et al. 2005). Clock KO-(deficient) mouse model showed a disruption in both the diurnal transcriptome and metabolome in the liver and altered energy expenditure (Eckel-Mahan et al. 2012). Furthermore, tissue-specific deletion of distinct core clock components has unveiled how clock proteins are tied to distinct metabolic processes ranging from glucose transport to gluconeogenesis, lipolysis, adipogenesis, mitochondrial oxidative phosphorylation, etc. (Asher and Schibler 2011).

These are just a few examples highlighting the importance of a functional clock to preserve metabolic homeostasis. Even more impressive are epidemiological studies showing the adverse effects of circadian misalignment on metabolism and other aspects of organismal physiology such as immunity and mental health (Johnston et al. 2016). Accumulating evidence has consistently shown that some modern lifestyle habits contribute to disrupt the endogenous body rhythm through alteration in sleep-activity cycles and food intake. Shift work, frequent transoceanic flights, or simply spending late nights awake in front of the laptop or smartphone profoundly influence our body timing with possible detrimental consequences. Indeed, shift workers show a higher incidence of sleep and mood disorders, metabolic, and cardiovascular diseases (Baron and Reid 2014). Moreover, shift-work-associated disorders include increased risk of infections, autoimmune diseases, and cancer, suggesting the importance of a correct body rhythm synchrony for immune system function. In humans, sleep and, consequently, circadian rhythms appear to be a regulator of immunity (Labrecque and Cermakian 2015). Sleep deprivation or other modifications of the sleep regimen seem to influence immune functions, for example, by partially shifting the phase of cytokine secretion (Cuesta et al. 2016). As previously mentioned, many parameters belonging both to innate or adaptive immunity are characterized by circadian oscillations in human blood. This fluctuation originates by a synergistic action of the circadian system and sleep (Lange et al. 2010), the latter being a consequence of the body diurnal rhythm. The daily changes in the immune system components are reached through a tight coordination of endocrine and electrical signals (cortisol, epinephrine), which depends on the sleep–wake cycle (Lange et al. 2010). Thus, it becomes clear how important it is to maintain a correct daily cycle, via the alignment of the internal clock with the 24-h environmental rhythm, for optimizing the organismal immune response.

Intracellular metabolic reprogramming in immune cells modulates their function (O'Neill et al. 2016). Therefore, nutrition and calorie intake are not only involved in sustaining metabolic homeostasis, but are also critical determinants of immune responses. Food composition and, in particular, the role of macronutrients and micronutrients on immune system function has been deeply explored, showing that both malnutrition and overnutrition (i.e., overweight and obesity) can actually affect immunity (Cunningham-Rundles et al. 2005). One of the common nutrients, which shape both innate and adaptive immune responses, are amino acids. Indeed, polarization of macrophages, T-cell proliferation, and differentiation are governed by the amino acid metabolic pathway, in which mechanistic target of rapamycin (mTOR) signaling plays the central role (Newton et al. 2016; O'Neill et al. 2016). On the other hand, activation of AMP kinase, which turns on intracellular catabolic processes and inhibits mTOR, limits inflammation (O'Neill and Hardie 2013). Commensal bacteria are one of the unique crit-

ical mediators linking nutrition to immune function. For instance, dietary fat perturbs host immune homeostasis in various peripheral organs through change in the gut microbiome, leading to detrimental effects on the host physiology (Devkota et al. 2012; Caesar et al. 2015). Little is known about the effect of the fasting/feeding cycle and the "timing" of food intake on the ability of the immune system to fight infections, reducing the risk of developing tumors or autoimmune and neurodegenerative diseases. The triple link between nutrition, circadian cycles, and immunity is emerging; however, further investigation is required to unveil the specific principles underlining this complex interaction and for future clinical applications.

DIURNAL CHANGES IN GUT MICROBIOME AND BODY RHYTHMS

Recent evidence has shown that the gut microbiota displays circadian fluctuation, which is mainly driven by diurnal food intake, leading to rhythmic abundance of microbial metabolites (Thaiss et al. 2014; Zarrinpar et al. 2014; Leone et al. 2015). The systemic oscillation of microbiota-derived metabolome reprograms the circadian transcriptome both locally and distally, thereby regulating host physiology such as metabolic function and drug detoxification (Leone et al. 2015; Montagner et al. 2016; Murakami et al. 2016; Thaiss et al. 2016). Furthermore, bacterial adherence to the epithelium shows temporal fluctuations, which also correlate to the host transcriptional oscillations. Thus, the disruption of microbiota oscillatory activity as a result of antibiotic treatment or specific dietary intake leads to disorganization of host rhythmicity (Thaiss et al. 2016), indicating that the gut microbes serve as a circadian organizer of peripheral clocks. This transcriptional reprogramming appears to function through nuclear receptors that occupy a pivotal position in the process of integrating microbiota-derived signals into the circadian network (Mukherji et al. 2013; Montagner et al. 2016; Murakami et al. 2016). The rhythmic expression of nuclear receptor PPARα in the gut epithelial cells, which is synchronized by microbial cues through TLR, keeps a proper corticosterone secretion for the temporal systemic demand (Mukherji et al. 2013). Although the core clock machinery robustly oscillates independently of microbial effect, the expression pattern of canonical clock genes is influenced by the presence and structure of the gut microbiota (Leone et al. 2015; Govindarajan et al. 2016). Altogether, the host–microbe interaction appears to be essential in keeping the host clock timed in an appropriate manner, which can be integrated with fluctuating environmental signals. In turn, a functional clock impacts the time-of-day oscillations of the microbial structure.

Because the commensal bacteria compete with the invading pathogens, the compositional oscillation of the microbiota contributes to the circadian variation of host defense against invading pathogens. Together with the circadian gating of host immunity, the host–microbe cross talk over the course of a day that will enhance the host defense system might be a promising target to fight against pathogens.

Despite accumulating evidence regarding the cross talk between microbiome and circadian gating of host physiological functions, many important questions remain unanswered. First, the cell-intrinsic roles for clock components in response to microbial exposure remain largely unsolved. It is also unclear how the various molecules with distinct circadian phase rhythmicity are coordinated so as to enable the host to effectively gain proper functions. This phase diversity might confer biological advantages to the organisms as to enhance flexibility in the response to the time-of-day-specific environmental stimuli. The same applies for the interaction between different organs, in which temporal coordination of tissue-specific functions may maximize their role in encountering external stress.

Finally, application of our basic research knowledge on the circadian clock to therapeutic approaches (chronotherapy) is gaining momentum among clock biologists and clinicians. The circadian disruptions induced by modern lifestyles lead to dysbiosis, which may predispose host-to-metabolic disorders and inflammation. Therefore, either timed feeding or probiotic therapies may be applied as the preventive ap-

proaches. Moreover, the optimal daily timing of antibiotic therapy for infectious diseases based on the scientific findings of temporal host immunity may minimize the side effects and maximize the effectiveness of the drugs. Further insights on clock-governed host response to the microbiome might pave the way for powerful therapeutic advances.

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