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Rhythm and bugs: Circadian clocks, gut microbiota, and enteric infections

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Abstract

Purpose of review—Highlight recent developments in understanding the dynamic relationship between circadian rhythms, the gut microbiome, and gastrointestinal (GI) infections.

Recent findings—In humans and mice, the composition and function of the intestinal microbiome displays diurnal rhythms orchestrated by feeding behaviors and host circadian gene expression. Jet lag, i.e., circadian disruption, perturbs these rhythms to produce gut dysbiosis. Furthermore, mice orally challenged with *Salmonella Typhimurium* show higher levels of colonization and inflammation when infected in the morning vs. other times of day. At the cellular level, recent studies highlight circadian regulation of innate and adaptive gut immunity in coordination with the microbiome, as well as intestinal stem cell growth and regeneration.

Summary—Taken together, these reports support a key role for circadian rhythms in regulating the gut microbiome and host responses to GI pathogens. Further research is needed to translate these findings to improving outcomes for patients with GI infections by guiding right interventions for the right patients at the right time.

Keywords

Circadian rhythms; microbiome; infection; gastrointestinal tract

INTRODUCTION

The gastrointestinal (GI) tract, like other organ systems, operates on a ~24-hour circadian schedule that anticipates—and prepares for—changes in the physical environment associated with day and night. Circadian rhythms regulate a number of GI functions, ranging from gastric acid production to small intestinal nutrient absorption to colonic motility (1–3). These rhythmic processes are controlled by the molecular mechanism of circadian rhythms that generate an autonomous molecular oscillator with a period of about 24 hours that directly influences diverse aspects of GI health including digestion, immunity, intestinal

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stem cell regeneration, and the risk for peptic ulcer disease and GI cancers (2,4–6) (Figure 1). Recently, it has been shown that the composition and function of gut microbiota also undergo diurnal changes (7,8), and that the circadian clock in the intestine works in close connection with gut microbiota (9). In this review, we summarize recent developments in understanding the dynamic relationship between circadian rhythms, the microbiome, and gut infections.

CIRCADIAN RHYTHMS AND THE GUT MICROBIOME

Circadian rhythms are entrained by external time cues aligning the phase of the internal clock to the outside environment (Figure 1). Light is not the only external time cue that controls the function of the circadian clock. There is growing appreciation for a more dynamic regulation of peripheral clocks, particularly in relation to feeding (2). Robust circadian rhythms have recently been described in explants of the mouse colon (10), as well the small intestine and 3D enteroids derived from mouse small intestine (11). Hence, physiological functions of GI system are determined by complex coordination between the master pacemaker in the suprachiasmatic nucleus (SCN) and the peripheral clocks in GI that process multiple inputs including light, nutrients, microbiota, and pathogens (Figure 1).

The intestine is colonized by ~100 trillion microbes (12), with a taxonomic profile dominated primarily by bacteria (13,14). The relationship between circadian rhythms and the diversity of microbes in the gut is an intriguing topic with implications for multiple GI diseases (7,8,15). Given the broad influence of circadian rhythms on GI functions, it follows that the composition and function of the gut microbiome would also display diurnal variations and that circadian disruptions, such as jet lag, would induce dysbiosis in mice and humans (7). Intriguingly, the gut microbiome also influences intestinal circadian rhythms. Antibiotic-induced depletion of gut microbiota results in lower amplitude circadian rhythms in mouse enterocytes (9). Moreover, the abundance, composition, and function of gut microbiota are dramatically altered in circadian arrhythmic *Per1*$^{-/-}$*;Per2*$^{-/-}$ double knockout mice (7), further indicating a bi-directional relationship between circadian rhythms and the gut microbiome.

Given the symbiotic relationship between humans and their resident gut bacteria, it is perhaps not surprising that the composition and function of the microbiome are intimately intertwined with circadian rhythms of mucosal immunity. Toll-like receptors (TLRs) in the small intestinal crypts play critical roles in innate immune recognition of foreign antigens, including commensal and pathogenic bacterial products, e.g TLR4 stimulation of α-defensin release in response to bacterial products such as lipopolysaccharide (LPS) (16,17). The circadian clock regulates diurnal release of defensins in the mouse small intestine, which is thought to augment mucosal defenses against bacteria ingested during feeding (18). RORα and REV-ERBα activate and repress BMAL1, respectively, and establish an interlocked transcriptional/translational feedback loop TTFL (Figure 1). Interestingly, RORα and REV-ERBα function as an activator and a suppressor of TLRs, respectively, leading to circadian expression of TLRs (9). Antibody-induced depletion of the microbiota increases the expression of REV-ERBα, resulting in a subsequent decrease in TLR expression (9). In addition, *Rev-erbα*$^{-/-}$ mice demonstrate elevated levels of the pro-inflammatory cytokine

IL-6, which normally displays a diurnal expression pattern (19). The circadian function of REV-ERBα extends beyond innate immunity to regulate the differentiation of interleukin-17-producing $CD4^+$ T helper (T_H17) cells, which function in adaptive gut immunity. REV-ERBα represses NFIL3, which suppresses the development of T_H17 cells (20). A higher frequency of T_H17 cell development is observed in naïve $CD4^+$ T cells isolated during the day vs. night. This diurnal pattern in T_H17 cell development is abolished in *Nfil3*$^{-/-}$ mice, which indicates that time of day specific function of REV-ERBα crosstalk with *Nfil3* dictates, in part, the development potential for T_H17 in a circadian manner (20). Macrophages maintain robust circadian rhythms, which results in rhythmic expression of proinflammatory cytokines (21). These intriguing data show the critical role of circadian clock in regulating the timing of the host immune response. Dysregulation of circadian function due to shift work, or perturbed microbiota signaling in the gut, might therefore have drastic effects on the level of inflammation within the GI tract. Moreover, the timing of injury or perturbation is likely an important contributor to the severity of the damage in the GI tract.

CIRCADIAN RHYTHMS AND GASTROINTESTINAL INFECTIONS

Despite recent developments in understanding the influence of circadian rhythms on the gut microbiota and immunity, few studies have directly examined the circadian regulation of host response to GI pathogens. Intriguingly, Bellet and colleagues have recently shown that infection with *Salmonella enterica* serovar *Typhimurium* (*S. Typhimurium*) at different times of day provokes differential colonization and immune responses in mice (4). Infection of mice with *S. Typhimurium* during the onset of the rest phase (subjective morning) leads to a greater immune response and augmented colonization by *S. Typhimurium* compared with infection during the active phase (subjective night) (4). In contrast, *Clock/Clock* mutant mice, which demonstrate altered circadian rhythmicity (22), display reduced cytokine production from macrophages, arrhythmic inflammatory responses, and a loss of time-dependent differential colonization of *S. Typhimurium* (4). Macrophages from *Clock/Clock* mice lacked a robust immune response at all time points upon LPS challenge *in vitro*. Moreover, dramatically reduced secretion of IL-6 and IL-1β was observed in *Clock/Clock* mice using both a normal strain of *Salmonella* and a strain that avoids TLR4 detection, indicating the need for a functional clock to coordinate TLR4 signaling responses to *Salmonella* infection (4). These findings in mice provide important *in vivo* data in support of earlier findings in mouse intestinal cells showing that compromised circadian rhythms lead to altered TLR regulation (9). The degree to which these findings will translate to patient is unknown, yet it is intriguing to speculate that: 1) time of day influences human susceptibility to enteric pathogens, 2) circadian disruption (e.g., shift work, jet lag) lowers human resistance to enteric pathogens, and 3) jet lag, not just differences in sanitation and hygiene, is a risk factor for traveler's diarrhea.

Increased epithelial turnover is a key intestinal mucosal defense mechanism against parasite infection. The rates of both epithelial proliferation and turnover are increased in response to infection of gastrointestinal dwelling nematode, *Trichuris muris*, which facilitates the expulsion of the parasite (23). Intriguingly, circadian rhythms synchronize cell cycle phase and regulate intestinal stem cell regeneration upon damage (5). Hence, it is reasonable to

hypothesize that one mechanism by which the circadian clock influences host susceptibility to GI pathogens is by influencing differential rates of epithelial turnover based on the time of day at which the initial infection occurs.

CONCLUSION

Circadian rhythms, the immune system, and gut microbiota communicate and influence each other (Figure 2). A lack of microbiota decreases the activity of TLRs due to the absence of bacterial metabolites including LPS, which upregulates REV-ERBα and triggers subsequent arrhythmic gene expression of TLRs (9). The transcription of cytokines such as *Il-6* and *Il-1β* demonstrate circadian expression, and defective TLR signaling in *Clock/Clock* mutant mice results in reduced secretion of IL-6 and IL-1β upon *Salmonella* infection, which further compromises immune status. Intriguingly, gut *Per2* transcription progressively decreases in response to a *Salmonella* challenge (4), which suggests that pathogens may disrupt gut timekeeping to promote colonization and immune evasion. An analogous scenario from the plant and fungi kingdoms has been shown in the pathogenic fungus, *Botrytis cinerea*, which suppresses the circadian rhythms of its plant host, *Arabidopsis thaliana*, to augment its own virulence (24). Much more work remains to be done on elucidating the impact of pathogens and their circadian rhythms to the host circadian rhythms and immune response.

The clinical implications of understanding how circadian rhythms influence the gut microbiome and host responses to gut infections are far ranging. Might chronotherapy and improved sleep habits improve the prevention of intestinal infections? Is there an optimal time of day to administer live oral vaccines against enteric pathogens? For treatment of diarrhea, would probiotics be more effective if given in the morning vs. evening? What effect do antibiotics have on circadian rhythms of the GI tract? All of these questions will require research approaches that take into account circadian rhythms as a central component in the important relationship between humans, their microbiome, and microbial threats. Systematic understanding of the microbiota, circadian rhythms and immune response and how they are connected will ultimately lead to precision therapies directed at previously overlooked targets or organs in a time specific manner.

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Key Points

- The gut microbiota and gastrointestinal physiology display circadian oscillations of function, leading to synchronization between the two.
- Immune and inflammatory response show distinct time of day dependence and appear to be regulated by circadian mechanisms.
- Time of infection plays an important role in the damage response and may be an underappreciated facet in multiple GI pathologies.
- Microbiota signaling to the immune system plays an important role in preparing the body for potential pathogen infection.
- Proliferation of stem cells is synchronized by circadian clock genes and important in the damage response.

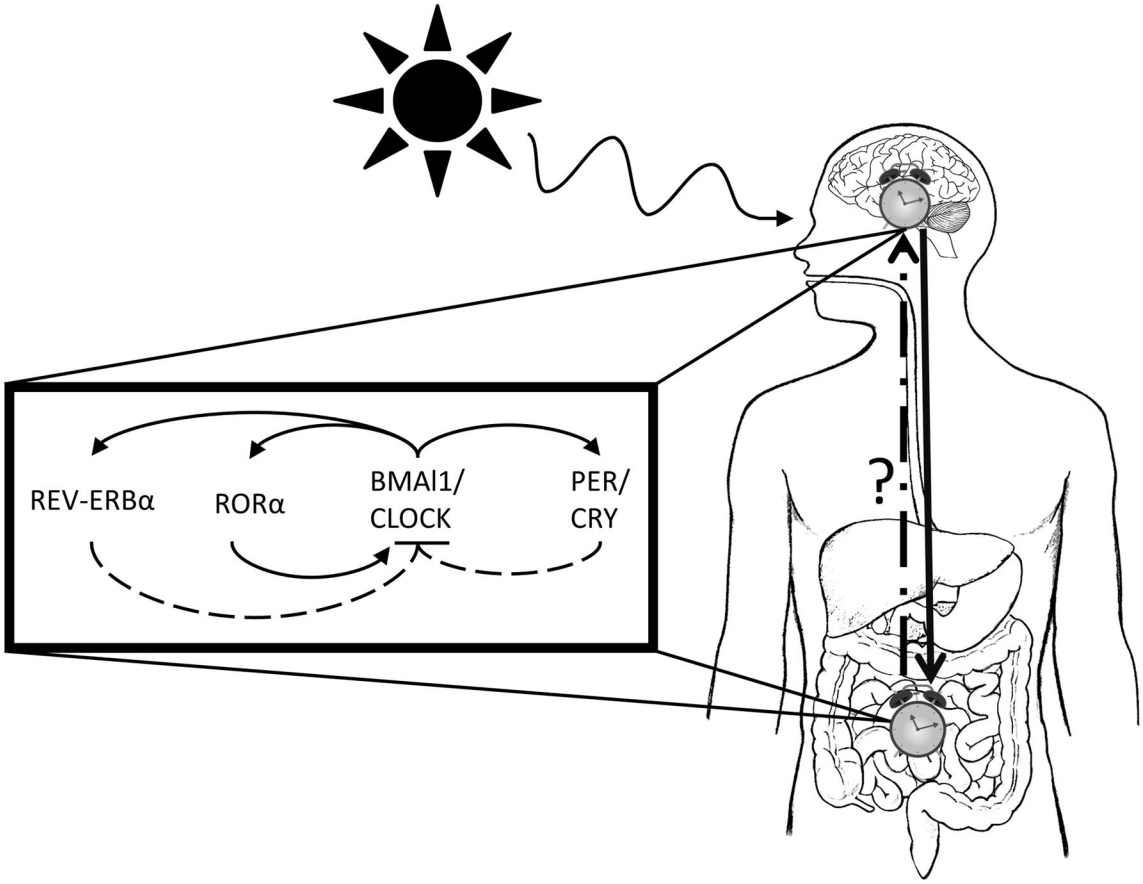

Figure 1.

The master pacemaker is housed within the suprachiasmatic nucleus (SCN) of the hypothalamus, which receives light input via the optic chiasm from the retina. The master pacemaker coordinates peripheral clocks throughout the body for optimal physiological function. Autonomous circadian oscillations are generated by transcription-translation feedback loops (TTFL) at a single cell level in both master and peripheral clocks; the main feedback loops shown. A heterodimeric circadian transcription factor, CLOCK/BMAL1, activates negative elements *Per* and *Cry* where their protein products inhibit CLOCK/BMAL1, creating a time-delayed negative feedback loop. CLOCK/BMAL1, also activates a nuclear receptors, *Rorα* and *Rev-erbα*, which activates and represses the expression of BMAL1, respectively, forming additional feedback loops. Solid black arrows denote activations and dotted lines with blunt end represent negative feedback loops. The blocked dashed arrow from GI systems to the brain indicates hypothetical action of peripheral clocks on the master pacemaker in the SCN.

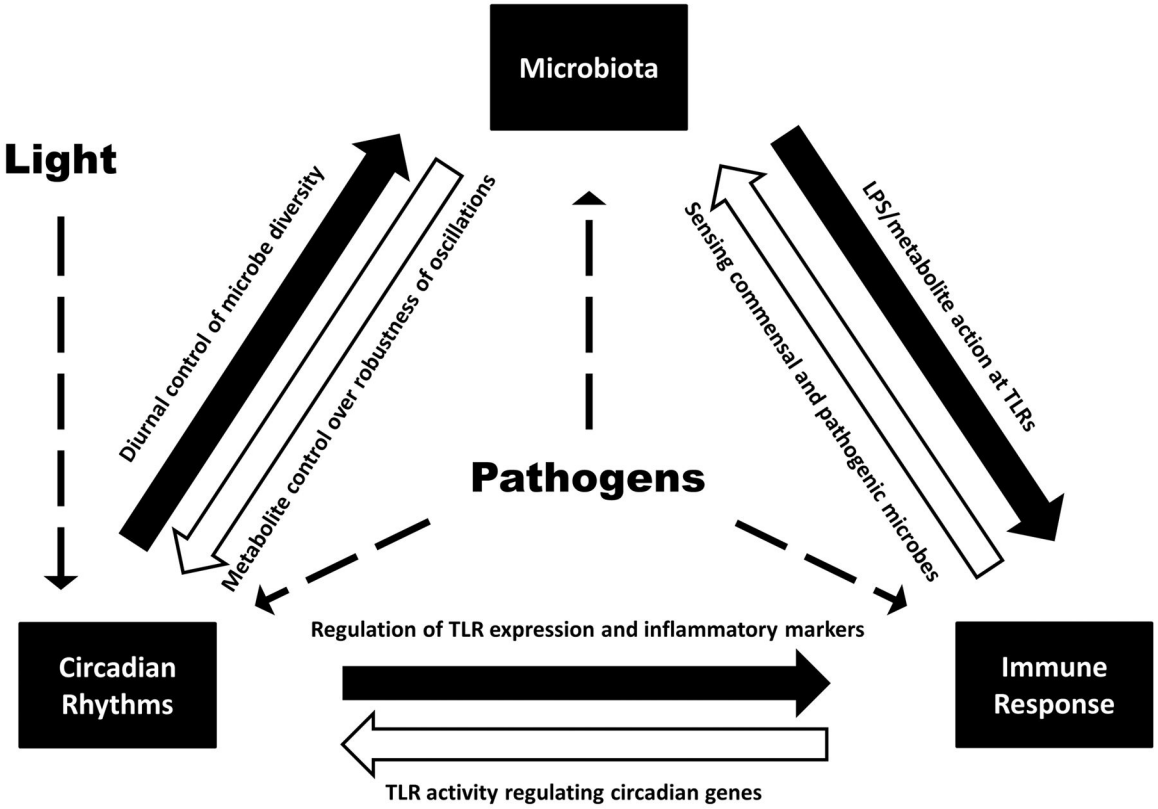

Figure 2.

Summary of the dynamic relationship between circadian rhythms, intestinal microbiota, and immune response. Dotted arrows denote the points of impact of light and pathogens on these three components. In this model, environmental factors trigger different outcomes depending on the timing of perturbations and signaling cascades through the interconnected network of circadian rhythms, the microbiota, and immune response.