HHS Public Access

Author manuscript

Nat Rev Gastroenterol Hepatol. Author manuscript; available in PMC 2021 October 18.

Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2021 October ; 18(10): 679–689. doi:10.1038/s41575-021-00452-2.

Gut microbiota as a transducer of dietary cues to regulate host circadian rhythms and metabolism

Hyoann Choi[1,2], **Mrinalini C. Rao**[3], **Eugene B. Chang**[1,†]

[1]Department of Medicine, Knapp Center for Biomedical Discovery, Chicago, IL, USA.

[2]Department of Biological Engineering and The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA.

[3]Department of Physiology & Biophysics, University of Illinois at Chicago, Chicago, IL, USA.

Abstract

Certain members of the gut microbiota exhibit diurnal variations in relative abundance and function to serve as non-canonical drivers of host circadian rhythms and metabolism. Also known as microbial oscillators, these microorganisms entrain upon non-photic cues, primarily dietary, to modulate host metabolism by providing input to both circadian clock-dependent and clock-independent host networks. Microbial oscillators are generally promoted by plant-based, low-fat (lean) diets, and most are abolished by low-fiber, high sugar, high-fat (Western) diets. The changes in microbial oscillators under different diets then affect host metabolism by altering central and peripheral host circadian clock functions and/or by directly affecting other metabolic targets. Here, we review the unique role of the gut microbiota as a non-photic regulator of host circadian rhythms and metabolism. We describe genetic, environmental, dietary, and other host factors such as sex and gut immunity that determine the composition and behavior of microbial oscillators. The mechanisms by which these oscillators regulate host circadian gene expressions and metabolic states are further discussed. Because of the gut microbiota's unique role as a non-photic driver of host metabolism and circadian rhythms, the development and clinical application of novel gut microbiota-related diagnostics and therapeutics hold great promise for achieving and maintaining metabolic health.

Blurb

This Review explores how the gut microbiota acts as a driver and regulator of host circadian rhythms and metabolism, highlighting its unique role in transducing dietary cues. Key determinants of microbial oscillations and insights into microbial control of chronometabolism are discussed.

† echang@medicine.bsd.uchicago.edu .

Author contributions

All authors wrote and reviewed/edited the manuscript before submission. H.C. researched data for the article.

Competing interests

The authors declare no competing interests.

Introduction

The Earth's rotation on its axis dynamically exposes most organisms on the planet to sunlight. It creates oscillating light and dark cues that organisms need to adapt to in order to sustain their lives. The diurnal rhythms further affect environmental conditions such as temperature, water and nutrient availability to which most life forms adapt for survival and optimization of energy balance. Traditionally, photic cues have been thought to be the major, if not sole, drivers of organismal circadian rhythms, which are determined by a 24 h internal clock that controls both metabolic functions and behavior, such as sleep and wake cycles. For example, plants have circadian rhythms that are necessary for determining when and to what extent photosynthesis takes place, which is essential both for the plant's own survival, and for the survival of obligate herbivores[1,2]. These effects might then propagate through the food chain, via intricately intertwined predator–prey relationships as well as ecological cross talks based on mutualistic, commensal, or competitive interactions across various life-forms to affect their diurnal activities reciprocally[3].

Interestingly, the reciprocal regulation of diurnal variations between organisms of different kingdoms, has been also observed in the interactions of the gut microbiome with its mammalian host. Within the past decade, it has become apparent that a new player, the gut microbiota, contributes to the regulation of mammalian physiology. The gut microbiota is a complex admixture of bacteria, viruses, protists, Archaea and fungi that collectively comprise a symbiont-like organ for the host[4]. While the largest number of microorganisms reside in the large intestine, they also exist in other parts of the gut with their composition varying down the cephalocaudal axis and in their luminal versus mucosal location [5,6]. This composition creates a complex of ecological niches for the gut microbiota to differentially benefit from and affect the host's physiology. Unlike other organ systems, the gut microbial organ is acquired, selected and maintained by the host throughout life. In turn and like other organ systems, the gut microbiota supports physiological, behavioral and genetic processes that are important for development and general health of its host[7-9]. The influence of the gut microbiota on host physiology, however, is not static. In addition to the composition and function of the microorganisms, their diurnal variations have a major influence on host circadian network and metabolism.

In this Review, we specifically focus on the influence of the gut microbiota–host–clock interactions on metabolism proposing a unique role of the gut microbiota in transducing dietary cues to regulate diurnal oscillations of host metabolic functions even in the absence of photic signals. We describe factors that drive microbial oscillations that feed into and affect host circadian networks and metabolism.

Host circadian clock and the gut microbiota

In mammals, the internal circadian rhythms are important for coordinating the functions of different bodily organs to align with the external environmental signals and achieve maximum efficiency[10]. The circadian clocks in mammals, while cell-autonomous, through genetic networks that involve transcriptional autoregulatory feedback loops, depend on external cues to regulate physiological, behavioral and metabolic networks to ensure fitness

and survival[11]. Asynchrony between the external and internal clocks can be associated with diseases such as obesity, diabetes, ulcerative colitis, cancer and Alzheimer disease[12-17]. The circadian rhythms in mammals are mediated by a set of molecular components that are ubiquitously distributed inside all cells and are governed by clock genes. This intracellular machinery integrates the dynamics of external cues to create diurnal rhythms in the physiological processes of an organism. Of the many clock-controlled physiological and behavioral activities, metabolism is a primary target. The circadian genes modulate the metabolic signaling within a body, while nutrient factors can alter the expression of circadian genes[18]. Traditionally, the major light-mediated signals are believed to arise from the suprachiasmatic nuclei (SCN) located in the hypothalamic region of the brain[14,19]. However, the nutritional state and eating behavior are suggested to be additional primary 'zeitgebers', which are the environmental cues that can reprogram or modulate peripheral clocks. Thus, although some metabolic processes are controlled through SCN-dependent pathways that only receive light cues, a non-photic cue such as a food-entrainable oscillator (FEO) might be another mechanism of regulating peripheral oscillations, independent of SCN[20]. For instance, time-restricted feeding (TRF), which involves restriction of food intake to certain time intervals, for example 8-10 h of eating followed by fasting, controls nutritional cues that entrain the peripheral oscillations[21]. TRF helps maintain robust circadian rhythms and has been repeatedly shown to prevent and even reverse metabolic imbalances both in mice and humans[21]. Such dietary regimes improved health conditions of people with obesity or metabolic syndrome regardless of sexes and medication consumption[22,23]. Equally important, circadian eating behavior and metabolic processes have been preserved in SCN-ablated animals maintained under a TRF cycle, which implies that TRF might alter the peripheral gene oscillations without affecting the SCN[24].

In the gut microbiota, the majority of studies on diurnal variations have focused on the bacterial members as far less is known about fungi and Archaea in general. In contrast to eukaryotic organisms, bacteria are generally believed to lack autonomous circadian clock gene networks or canonical clock genes. However, genetic and proteomic mechanisms of an autonomous circadian clock have been reported for the cyanobacterium, *Synechococcus elongatus*, which utilizes light as a primary energy source, but which does not reside in the metazoan gut[25,26]. Even so, the cyanobacterial clock entrainment of light and dark signals is offset by metabolic signals[27]. Non-photosynthetic bacteria including those in the human gut are also reported to exhibit circadian rhythms that are dependent on non-photic environmental signals[4]. For example, the circadian rhythms in the swarming motility of an intestinal commensal, *Klebsiella aerogenes*, was shown *in vitro* to be mediated by temperature cycles[28] and melatonin levels[29]. Despite this evidence, knowledge on whether gut microorganisms have inherent clock mechanisms and to what extent their functions vary diurnally is still rudimentary. Instead, the studies published so far suggest that the biological rhythms of gut microorganisms might be dependent on specific environmental cues within the gut, which fluctuate according to dietary intake and host circadian rhythms and behaviors, rather than on cell-autonomous clock machinery. Reciprocally, their oscillations are integrated into the host circadian clock as another peripheral clock and can affect the circadian coordination of different host organs[30-32]. Clearly, a number of intriguing questions remain to be elucidated: do various gut microorganisms have inherent clock

mechanisms and can these affect the host's circadian clock? Alternatively, is the central host clock the mastermind behind the bacterial oscillators? For example, does feeding behavior, under the control of the photosensitive central host circadian clock, alter intake of nutrients that cause bacteria to release metabolites that then serve as cues to entrain peripheral clocks and therefore metabolism? How does the local gut milieu influence the effect of feeding behavior?

What causes the gut microorganisms' diurnal variations in microbial composition and abundance and, therefore, their influence on host metabolism has been of increasing interest. What is becoming clear is that not all members of the gut microbiota exhibit diurnal oscillations in their abundance and we and others term the small percentage that do as 'microbial oscillators'[30,32-34]. These so-called oscillators are present in both the small and large intestines of humans and mice[30,32-34]. Thus, in the dark lumen of the intestines, they might not directly entrain to photic cues, but primarily to dietary signals. They then transduce the signals to modulate host circadian networks and metabolism directly or indirectly, via presumed small molecule mediators including metabolites. By doing so, the gut microbiota provides information to the host about what, when, and how much of nutrients have been consumed, enabling the host to adjust overall energy balance accordingly—they could act as other zeitgebers to the host's circadian rhythms.

The connections between the gut microbiota, diet and metabolism have been the focus of intensive research. There is compelling evidence that the gut microbiota plays an important part in host metabolic homeostasis[9,35]. An obvious corollary is that circadian modulation by the gut microbiota also influences the host's nutritional state and feeding behavior.

Circadian clocks in mammals

In mammals, geophysical time is integrated into the body through the internal molecular clock composed of the central pacemakers, the SCN, and the auxiliary oscillators, both of which exist in the brain and the latter also exist in almost all peripheral tissues[10]. The canonical regulation of diurnal oscillations of physiological processes is mediated by the SCN, which integrate dynamic photic cues recognized by the retina and regulates other central and peripheral circadian networks[10].

The typical mammalian circadian clock consists of two sets of genes: the primary clock genes, *CLOCK* (Circadian locomotor output cycles kaput), *ARNTL* (encoding aryl hydrocarbon receptor nuclear translocator-like protein 1, also known as BMAL1), *PER* (Period), *CRY* (Cryptochrome), which comprise the first feedback loop; and the secondary circadian effectors that include *NR1D1* (encoding nuclear receptor subfamily 1 group D member 1, also known as REV-ERBα) and retinoic acid receptor–related orphan receptors such as *RORA*, and the downstream clock-controlled genes such as *DBP* (D site-binding protein) and *PPARGC1A* (encoding peroxisome proliferator-activated receptor gamma coactivator 1-alpha) (Fig. 1). These molecular components feedback on one another to maintain circadian rhythms in mammals (Box 1). These core circadian mediators then control many downstream genes relevant to physiological functions, including energy balance[11,14,18]. The centrality of circadian rhythms in regulating myriads of biological

processes is underscored by the following: almost all cells possess the primary clock genes, and the products of these genes serve as master-regulators via the E-Box of thousands of genes, many of which are themselves regulators thereby affecting a cascade of biological processes.

Determinants of microbial oscillations

Microbial oscillators

Although many gut microbiome studies report the analysis of samples obtained at single, defined time points, sufficient evidence from studies involving sampling at multiple time points reveals that the composition of gut microbiota is not fixed, but dynamic throughout the day[30,33,34,36,37]. However, only a small percentage of these microorganisms are oscillators; that is, exhibit oscillations in abundance. Up to 20% of taxa in the cecal and fecal microbiota from mice, especially Clostridiales, Lactobacillales and Bacteroidales, exhibit diurnal variation in relative abundance[30,34]. The composition of the human gut microbiota was also found to vary depending on the time of defecation[34,37]. Although studies interconnecting the circadian clock, motility and the microbiota are as yet sparse, it has been suggested that changes in diurnal bowel function in chronic constipation is associated with altered colonic microflora in humans[38]. Nutrient availability and consumption fluctuate with time and are clearly contributing factors. Furthermore, metagenomic analysis indicates that certain microbial genes diurnally fluctuate, which could potentially affect host targets and functional activities in both mice and humans[34,37]. The time of day when expression of oscillating microbial genes peaked also varied, possibly as a result of phasic shifts of the host environment, such as body temperature, substrate availability or the activities of the immune system. The biogeography and metabolome of the gut microbiota also changes during the course of a day. Thaiss et al. showed that different microorganisms in mice rhythmically adhere to the host gut epithelium in varying abundances [31]. In the same study, the rhythmicity of intestinal metabolites such as ornithine and proline detected in serum was lost in antibiotic-treated and germ-free (GF) mice[31]. The time-specific exposure of the host intestinal cells to microorganisms and their products could potentially be another mechanism by which gut microorganisms influence host health.

Genetics, sex and environmental manipulation

Factors contributing to the circadian fluctuations of the gut microbiome have been identified. In addition to sex, the genetic or environmental disruption of the host's circadian clock alters microbial composition and functional capacity in the gut[34,36,39]. The deletion of one of the core circadian genes, *Bmal1*, altered diurnal variations of the gut microbiome at the genus level in mice. For example, both the amplitude and phase of the diurnal oscillations in relative and inferred absolute abundance of members of the Lactobacillaceae family at certain times were altered by *Bmal1* deletion[36].The deficiency of other core circadian genes, *Per1* and *Per2*, was further shown to abrogate compositional, functional, biogeographical and metabolomic fluctuations of the gut microbiome in mice[31,34]. The influence of *Bmal1* deletion on the gut microbiome was also sex-dependent; thus, although the diurnal oscillations of the relative abundance of gut (fecal) microbiota was more prominent in female than in male mice, this rhythmicity was absent in *Bmal1*-deleted

mice. Furthermore the relative abundance of various bacteria was altered by *Bmal1* deletion and this bacterial composition showed sex-related differences[36]. Diurnal expression of host hepatic genes, including those of metabolic pathways exhibit sexual dimorphism and the sex-related differences disappear in germ-free animals[40]. In terms of environmental manipulations, a mouse model of jet lag, mimicked by an 8h shift of light and dark cues, exhibited reduced number of diurnally oscillating bacteria and greater bacterial composition shift as compared to the control mice, that is, an increase in beta diversity between the two groups of mice. [34]. Conversely, the induction of circadian disruption by a 12h shift of light signals, reversing the day and night, altered the diurnal expression of *Per2* in mice, but was not sufficient to alter the gut microbiota composition in another study[39]. Instead, this study showed that feeding mice a high-fat, high-sugar diet in addition to the light–dark cycle reversal led to substantial microbial composition alterations decreasing the similarity between the two diet groups [39]. Further studies focusing on other circadian clock genes and the molecular basis for the influence of host sex and environmental disruption of the circadian clock network are needed for more comprehensive conclusions on how host clock affects microbial oscillations in the gut.

Dietary patterns and diet composition

In addition to the canonical clock genes and photic cues, other alterations in gut ecology influence the oscillations of the gut microbiome. For example, the diurnal oscillations exhibited by the mucosa-associated microbial community are mediated by the host circadian rhythms of feeding times, and production of mucus and antimicrobial peptides (AMPs)[31]. Notably, dietary patterns are shown to be the main driver of the circadian oscillations of the gut microorganisms as well as the animals' physiological processes. The influence of feeding regimens, the circadian clock and animal physiology have been well-studied[41,42]. In terms of animal physiology, rhythmic food intake, guided by SCN-driven cues, accounts for 70% of rhythmic hepatic gene expression and is largely independent of the hepatic clock[43]. When mice fed only in the light phase were compared to mice fed in the dark phase only, the oscillatory patterns, including amplitude and peak and/or trough fluctuations, of some microorganisms such as *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) were similar. However, one critical difference was that the dark-phase-feeding and light-phase-feeding exhibited a 12h-difference in the relative abundance of those microorganisms. The dark-phase-only feeding recapitulated the types and rhythmic patterns of oscillating microorganisms observed in the *ad libitum* fed mice, reflecting that mice are nocturnal eaters. Furthermore, in *Per1/2*-deficient mice, TRF, either only during the light or the dark phase, restored the microorganisms' diurnal dynamics that were vastly reduced in mice fed *ad libitum*[34]. TRF-mediated alteration in gut microbiota composition was replicated in healthy male humans whose gut microbiome composition and richness differed between the TRF group (n = 56) and non-TRF group (n = 24) [44]. However, TRF did not change the gut microbiota composition and richness in 14 people with obesity[45].

As mentioned previously, the host diet strongly influences the cyclic fluctuations of the gut microbiome. It can determine what microbial oscillators are present and whether they exhibit diurnal variation. For example, members of Clostridiales, are abundant and exhibit diurnal variation in mice fed a regular chow diet, but both features are lost with high-fat diet

(HFD) feeding. On the other hand, a relative increase in abundance of *Ruminococcus* is seen in mice with HFD without exhibiting any oscillatory activity[30]. An increase in oscillation only under HFD, but not regular chow-feeding, is infrequently seen in some operational taxonomic units (OTUs) such as *Lactococcoccus* representing the oscillatory changes in relative abundance of bacteria resolved to a genus-level[30] although how, if at all, this change influences metabolism is unknown. In another study, mice fed normal chow *ad libitum* exhibited circadian oscillations of microorganisms in major phyla including Firmicutes, Bacteroidetes and Verrucomicrobia. The diurnal dynamics, however, were diminished in mice fed *ad libitum* with a HFD[32]. Moreover, the oscillation of the alpha diversity , which represents the bacterial membership within the local community, peaked during the daytime in mice fed normal chow *ad libitum* was not observed in mice on a HFD regardless of whether the HFD feeding was time-restricted or *ad libitum*. By contrast, the number of cycling microorganisms that undergo oscillations in abundance also decreased in both *ad libitum* and the dark-phase-only HFD-fed-mice[32]. This finding shows that the composition of the diet can complicate the effects of TRF on microbial oscillations. Nevertheless, the subphylum types of cycling microorganisms and their oscillatory patterns differed between mice fed with a HFD *ad libitum* versus those fed in the dark phase only. Further beta diversity analysis also revealed that the TRF of HFD led to much more temporal fluctuations in the microorganisms than the *ad libitum* feeding of HFD. Interestingly, the TRF even rescued some of the changes in cyclical behavior of obesity-related microorganisms observed in the *ad libitum* HFD-fed mice[32]. The rhythmicity of the gut microbiota is further influenced by the route of feeding. For example, parenteral nutrition abrogated the effect of oral HFD feeding on the diurnal oscillations of the gut microbiota in mice[30]. This finding underscores that the composition, route, time of feeding and sex need to be further explored as important contributors in the interplay between the host's circadian network and the diurnal oscillations of the gut microorganisms. Although complex, such studies are easier to perform in defined animal models than in humans. What is clear in human studies is that some gut microorganisms exhibit diurnal rhythmicity and that this rhythmicity is disrupted in individuals who are obese (BMI >30)[40]. In addition to species and sex differences, the difficulties of extrapolation to human studies are compounded by considerations of underlying physiological status.

Mucus and antimicrobial peptide Reg3γ

The previous studies were based on the luminal gut microbiota from fecal or cecal samples. Other investigations on the intestinal mucus layer in mice demonstrated that microbial occupation of the mucus also exhibits diurnal dynamics[31]. In addition, the mice exhibited cycling alterations in the thickness of the mucus layer[31]. The changes in the bacteria residing in the mucosa of micewere expected because the mucus and the AMP, Reg3γ, regulate the spatial segregation that reduces the exposure of host epithelium to microorganisms[46]. Predictably, the diurnal rhythmicity in the mucosa-associated bacteria was not found in Reg3γ-deficient mice[31]. Although the latter study hinted at the effects of mucus and AMP on the circadian dynamics of the mucosa-associated gut microbiota, it did not explore whether these host factors also affect the diurnal oscillations of the luminal microbiota and how that compares to the changes in the mucosa-associated microbiota.

Although diurnal oscillations of gut microbiota are influenced by various factors, it has also been observed that not all bacteria fluctuate diurnally[30,33,37]. Importantly, what determines oscillating and non-oscillating microbial composition in the gut and how they differentially affect host health are just beginning to be elucidated. A study published as a Preprint in 2020 investigated the role of Reg3γ in regulating the composition of microbial oscillators in the small intestine of mice[33]. The study demonstrated that the membership of oscillating OTUs was greatly changed between *Reg3g*$^{+/-}$ and *Reg3g*$^{-/-}$ mice. In addition to an increase in the number of oscillating OTUs, the dominant oscillating microbial family was changed from Bacteriodales in *Reg3g*$^{+/-}$ mice to Clostridiales in *Reg3g*$^{-/-}$mice[33]. These changes were dampened by HFD and the total number of oscillating OTUs was decreased on a HFD compared to a regular chow diet. Interestingly, despite a reduction in the total number of OTUs, on a HFD, Clostridiales remains the dominant family and their OTUs were greater in *Reg3g*$^{-/-}$ mice compared with *Reg3g*$^{+/-}$ mice. This finding implies that in the small intestine diet could be the major driver of oscillations with Reg3γ having a supporting role[33]. The influence of other host factors, including other types of AMPs, on the composition of microbial oscillators remains to be investigated.

Intestinal immune system

The close interactions between the host immune system and the gut microbiota[47] are well-documented. It is also well-established that the immune system is controlled by circadian rhythms[48] and a disruption in this circadian control has been related to many inflammatory diseases such as arthritis[49-51]. It is therefore important to understand how the circadian behavior of host immune responses determines, and is mediated by, the rhythmicity of the gut microbiota. However, this field is largely understudied and lack investigations in humans. Instead, studies have highlighted the importance of the clocks in monocytes and macrophages during the responses to pathogens[52]. For example, lipopolysaccharide (LPS) treatment of human macrophages *in vitro* or mice *in vivo* led to circadian secretion of some cytokines, especiallyIL6, which was lost with deletion of the core circadian gene, *Bmal1* in the mice[53]. Jet-lagged mice exhibited hyper-response of serum cytokines such as IL6, IL-1β, and IL-12 to LPS *in vivo*[12]. This finding shows that the response to endotoxin administration can be influenced by the time of day. The circadian regulation in immune cells has been extensively studied and reviewed elsewhere[48] (Box 2). Besides LPS, it would be interesting to study how other gut microbiota-derived-compounds interact with the circadian dynamics of the host immune system to vary the immunological health states of the host. It is possible that many inflammatory diseases could be triggered by the breakdown of diurnally homeostatic interactions between the gut immune system and the gut microbiota. Other host factors that are known to alter the composition of gut microbiota, such as secretion of bile acids, age, or disease-induced changes in gut transit time, could also contribute to gut circadian and microbial rhythmicity and need further study.

The processes by which diurnal oscillations of the gut microbiota influence and are influenced by a variety of host parameters ranging from nutrient composition, food intake timing and route, to gut mucus and AMP production, and to systemic and gut-associated immune systems are just beginning to be understood. These explorations will help elucidate the interindividual and intra-individual variations in the relationship between the gut

microbiota and host health. It will lead to improved design of nutritional strategies to treat metabolic diseases such as obesity and diabetes, and to help better understand the complex underpinnings of inflammatory diseases such as arthritis and inflammatory bowel disease.

Microbial control of chronometabolism

The gut microbiota regulates the circadian rhythms of host activities; lack of the gut microbiota altered both the transcriptional and the epigenetic status of the host genes[31]. RNA-seq analysis of colonic cells of mice indicated that the oscillating genes were different between control and antibiotic-treated mice[31]. However, although major transcriptional oscillations were found in both groups of mice, the average expression level for each gene did not differ greatly[31]. The rhythmicity of the host genes was also found to be altered in GF mice compared with conventionally raised control mice and, interestingly, some of these genes are involved in metabolic pathways and inflammatory processes such as fatty acid metabolism, cholesterol synthesis, and leukocyte cytolysis[40]. The change in the rhythmicity of metabolic pathways was also shown in antibiotic-treated mice; for example, genes in colonic cells for pyruvate metabolism gained new rhythmicity in antibiotic-treated mice compared to wild-type nontreated control mice[31]. This finding was suggested to be a way for the host to maintain the circadian regulation of metabolism that could no longer be controlled by the rhythmic behavior of microorganisms in the control mice. Similarly, perturbations of the gut microbiota by antibiotics also altered epigenetic configurations of host genes[31]. Chromatin immunoprecipitation (CHIP)-seq analysis for histone modification showed that rhythmic promoter and enhancer activities differed between the control and antibiotic-treated mice, whereas the rhythmicity itself was evident in both groups of mice[31].

The effects of microbiota absence on the host peripheral clock genes seem to be tissue-specific[30,54-56,40]. For example, Leone et al. showed that the germ-free status and the diet of the host mice differentially affected the expression of the core clock genes, *Arntl*, *Clock*, *Per2*, and *Cry1*, in mediobasal hypothalamus as compared to the liver[30]. Similarly, another group reported alteration in the rhythmicity of hepatic core clock genes and nuclear receptors in germ-free mice, with an accompanying alteration in the hepatic metabolome profile[57]. In mice treated with a variety of specific antibiotics, Oh et al. also found altered expression of specific clock genes such as *Bmal1* and *Per2*[58]. Furthermore, rhythmicity in gene transcripts was differentially affected in duodenum and ileum compared with liver and white adipose tissue (WAT): more transcripts gained rhythmicity in the former tissues, whereas more transcripts lost rhythmicity in the latter tissues when GF mice were compared with conventionally raised control mice[40]. In this study, the authors reported only subtle changes in phase and abundance of mRNA of clock genes across these tissues. In another study, when the microbiota was ablated by antibiotic treatment in mice, although the rhythmic transcription of a number of host genes were affected, there was no change in the rhythmicity of expression of the hepatic clock gene *Per2*[31]. Although it is clear that there are tissue-specific differences in whether the microbiota influences clock genes, the cause for the variability in findings within a tissue needs to be further examined. For example, in these studies the mechanisms of ablating the microbiota (antibiotic treatment versus GF) could influence the results as antibiotic treatment might not achieve a complete ablation while GF mice exhibit altered physiology such as enlarged cecum compared to wild-type mice[59].

Another compounding factor could be the basis of analysis, quantitative RT-PCR[30] in which the data were normalized to a house keeping gene versus RNA-seq analysis[31,40].

The interactions between gut microbiota and host circadian network are extended to affect host metabolic phenotypes. The changes in the gut microbiota composition in HFD-fed mice reprogrammed the hepatic clocks and resulted in metabolic aberrations such as weight gain compared with mice fed a normal chow or a low-fat diet. The circadian expressions of liver core clock genes such as *Arntl* and *Per2* remained similar between mice fed different diets, but the time-specific transcription of lipid-metabolism-related genes (such as those involved in the biosynthesis of unsaturated fatty acids and the peroxisome proliferator-activated receptor (PPAR) signaling pathway) were altered in mice fed HFD compared to those fed a control diet. Interestingly, these effects were replicated in GF mice that received a fecal transplant of the gut microbiota derived from HFD-fed mice[30,55]. Furthermore, the gut microbiota also influences sex-specific differences in rhythmicity of gene expression in metabolic organs. Some of the sex-dependent rhythmic transcriptional differences in gut, liver, and WAT were abrogated in GF mice and the affected genes were related to metabolic phenotypes. For example, the rhythmic and sexually dimorphic expression of glycolysis and bile acid metabolism[40] are abrogated and the hepatic genes related to lipid and xenobiotic metabolism are altered in GF mice. Notably, the absence of the gut microbiota led to alterations in growth hormone (GH) secretion and sexual maturation in mice, leading to attenuation of sexual dimorphism, a pattern also seen in obese mice or those lacking circadian clock genes (for example, Bmal1-knockout mice). Intriguingly, secretion of ghrelin, the GH regulator, was also decreased and injection of ghrelin restored hepatic sexual dimorphism in feminized GF male mice[40].

The gut microbiota is also involved in abnormal metabolic symptoms induced by disruption of circadian rhythms. The antibiotic-treated mice were resistant to obesity and glucose intolerance exhibiting no increase in weight or body fat content and normal glucose response, even when the sleep cycle was shifted by induction of a jet lag. Additionally, the transfer of the gut microbiota from either jet-lagged mice or humans transmitted metabolic imbalances to GF mice resulting in weight gain and glucose intolerance[34]. Despite these associations of the bidirectional interactions between the host circadian clock and the gut microbiota, the extent to which, and the molecular basis of how, the microbiota affects the host circadian clock network are unclear. Furthermore, the mechanisms underlying the tissue-specificity of the effect of the microbiota on the peripheral clocks are just being unraveled and need to be further elucidated.

Mediators of chronometabolism

Microbial metabolites

The studies on how the gut microbiota regulates host physiology through modulation of the host circadian clock network have focused on metabolism. Several of the studies showed that the diurnal oscillations of the gut microbiota-derived metabolites affect the host's circadian control of metabolism. One study showed that hepatic circadian gene expressions are regulated by a microorganism-derived short chain fatty acid (SCFA), butyrate, whose circadian production patterns are different in the HFD-fed and low-fat-diet fed mice[30]. The

HFD feeding altered the oscillatory behavior of a few microorganisms responsible for SCFA production. Specifically, the oscillation of Lachnospiraceae, a known butyrate producer, was greatly dampened by HFD feeding as compared with low-fat-diet feeding in mice. Accordingly, the diurnal oscillations of butyrate levels disappeared upon HFD feeding[30]. When the effect of this change on the rhythmicity of butyrate on the host was tested *in vitro* using hepatic organoids established from specific-pathogen free mice and *in vivo* using GF mice, the butyrate treatment changed both the rhythmicity and amplitude of the circadian clock genes, especially *Per2* and *Arntl*. Acetate, another SCFA, also altered the expression of circadian clock genes, most notably that of *Per2* in the hepatic organoids. Furthermore, the exposure to NaHS, which increases microbial H_2S production, blunted the circadian gene oscillations, especially those of *Per2*[30]. Thus, this study showed that a variety of microbiota-derived metabolites have the potential to modulate the host circadian clock.

Another study by different investigators suggested unconjugated bile acids to be another mediator by which the gut microbiota entrains host peripheral circadian clocks[60]. Bile acids are largely secreted as conjugated compounds by the liver and are then deconjugated by gut microorganisms[61]. In the Caco-2 intestinal epithelial cell line model in which cells were synchronized to have the same circadian rhythms, treatment with unconjugated bile acids, deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA), markedly altered the amplitude and cyclic behavior of both the primary and secondary clock genes including *CLOCK, ARNTL* (encoding BMAL1*), PER, CRY, RORA, NRID1 (*encoding REV-ERBα), *DBP, and E4BP4* as compared with treatment with conjugated bile acids or vehicle[60]. The changes in the expression levels of the circadian genes were also observed *in vivo* in C57BL/6 mice. Interestingly, the direction of changes in the expression levels of some genes were the opposite in different organs. For example, unconjugated bile acids increased *Cry1* expression in ileum and colon whilst decreasing it in the liver[60]. Thus, again, the effects of microorganisms on the host circadian clock might be tissue-specific, although the reason for the tissue-specificity is unknown.

Another interesting metabolite group are the polyamines, produced by both the mammalian hosts and gut microbiota. It has been suggested that the diurnal rhythmicity of polyamines in serum is affected by the absence of gut microbiota. Conversely, a diet lacking in polyamines altered hepatic circadian transcriptome with the changes paralleling those observed in antibiotic-treated mice[31]. Polyamines, in turn, are known to regulate circadian oscillations by affecting the interactions between the core clock proteins, PER2 and CRY1 in mice[62]. Whether the lack of gut microbial polyamine production directly caused the observed changes in the diurnal oscillations was not tested. Nevertheless, these observations underscore the necessity to investigate the range of microbial metabolites whose effects on host metabolism have been well-documented.

Microorganism-derived components

Bacterial components such as flagellin and LPS are also thought to mediate the host circadian metabolic homeostasis, as exemplified by the work of Wang et al[56] (Fig. 2). They demonstrated that gut microorganisms are involved in diurnal dynamics of body weight in miceby regulating the amplitude of circadian rhythms of the transcription factor,

NFIL3. The regulation of NFIL3 was specific for the signals of Gram-negative bacteria such as LPS and flagellin. NFIL3 regulates body fat and ablation of its gene resulted in lower body weight in mice fed either a normal diet or a HFD. The expression of *Nfil3* was also higher in control mice than in antibiotic-treated or germ-free mice[56]. Through the use of knockout of various genes in mouse models, the authors further demonstrated that the microbiota-induced increase in the expression of *Nfil3* involved decreased expression of REV-ERBα protein (Box 1 and 2); the effect was mediated through *Myd88* signaling in $CD11c^+$ dendritic cells. Briefly, flagellin and LPS are recognized by TLRs on dendritic cells, which signal to group 3 innate lymphoid cells (ILC3s) via IL-23. ILC3s in turn signal via IL-22 to phosphorylate the transcription factor signal transducer and activator of transcription 3 (STAT3) in intestinal epithelial cells (IECs). Phosphorylated STAT3 decreases the expression of REV-ERBα protein. Whereas REV-ERBα mediates the circadian rhythms of NFIL3, the microbiota via STAT3 fine-tune the amplitude of these rhythms. Consequently, compared with GF mice, the microbiota-dependent increase in the epithelial NFIL3 levels affected the expression of the genes associated with lipid uptake and metabolism in the control mice. This process increased lipid absorption by IECs and lipid export to the bloodstream for fat storage in the adipose tissue[56].

Another study demonstrated that gut microorganisms could be involved in diurnal dynamics of murine corticosterone production, whose excessive level can lead to diabetes[54]. Using a model of antibiotic-treated mice, the authors demonstrated that in the absence of the gut microbiota, as there is no Toll-like receptor (TLR) activation, the expression of PPARα is increased and not inhibited. PPARα increases the expression of *Cyp11a1*, the rate-limiting enzyme in corticosterone synthesis[54]. PPARα activation also increases the expression of REV-ERBα, which decreases the binding of its competitive clock regulator, RORα, to RORE. As RORα normally represses *Cyp11a1* via NFIL3, the net result is an overproduction of corticosterone and increased glucocorticoid receptor activity. Accordingly, blood glucose levels were increased in the antibiotic-treated mice. Altered levels ofREV-ERBα and RORα proteins and their recruitment to RORE also could affect the first clock loop genes, *Bmal1*, *Per* and *Cry* and reduce TLR expression. Although a promising demonstration of how circadian clock genes rhythmically transmit their signals to host metabolic genes, these observations remain to be fully substantiated. For example, Zarrinpar et al. showed a decrease in blood glucose level with antibiotic treatment in mice[63] and Murakami et al. showed an increase in blood glucose level in mice on a HFD, that was not seen in antibiotic-treated mice on HFD[55]. Using a variety of specific antibiotic cocktails, Oh et al. did not see any effect on the expression of hepatic glucose metabolism genes in mice[58]. The discrepancy in whether glucose levels are affected in microbiota-depleted mice might have arisen from use of different antibiotic combinations and doses, the composition of baseline gut microbiota, and different sampling times. The studies on microbiota effects on corticosterone are largely centered on the hypothalamic pituitary adrenal axis; while they demonstrate that the manipulation of the microbiota alters corticosterone in mice, the results are discrepant and clearly need to be further explored in mice and extended to human studies[64-66]. For example, Luo et al. found that GF animals exhibited anti-anxiety and anti-depressant-like behavior with no change in serum cortisol level, but an upregulation of six glucocorticoid pathway related genes, as compared to specific pathogen free mice[64].

By contrast, Neufeld et al. reported anti-anxiety behavior in GF mice, but this behavior was associated with an increase in serum corticosterone level[65]. Yan et al. further showed that rats on high salt diet exhibited a reduction in levels of *Bacteroides fragilis*, which led to an increase in serum and intestinal corticosterone levels[66]. The final changes in the metabolic states in the host caused by the influence of the altered gut microbiota on host circadian clock, therefore, should take into consideration the compounding effects of other factors that can influence the relaying pathways.

Nuclear receptors

These studies, which suggest that nuclear receptors seem to be the molecular mediators relaying microbial signals from the lumen to influence the host's circadian control of metabolism, segue into the well-documented observations that nuclear receptors regulate the circadian clock in a variety of mammals, including humans, and thereby are important for human health[67]. For example, in human and mouse hepatic cell lines *in vitro*, the deficiency of the core clock gene, *Bmal1*, leads to N^6-methyladenosine (m6A) modification on PPARα mRNA, to increase the expression and/or alter the rhythmic expression of some lipid metabolism-associated transcripts[68]. Another important nuclear receptor, in the circadian control of host metabolism and that is regulated by gut microbiota is PPARγ. HFD-induced generation of new circadian oscillations in the hepatic transcriptional network of mice was mediated by the activity of PPARγ[69]. In a separate study, the absence of gut microbiota in mice was shown to change PPARγ signaling and the downstream gene transcripts it controls in IECs and liver[55]. Either HFD treatment or fecal transplant of HFD microbiota led to the upregulation of liver PPARγ and its downstream genes, *Cidec*, *Acot2*, *Pltp*, and *Pcx*, all of which are involved in lipid metabolism[55]. PPARγ protein in HFD microbiota-recipient mice had expression peaks at a zeitgeber time that corresponds to 6 pm and gained cyclic activities. Both treatments, however, conferred only mild changes in the circadian clock genes, including *Per2* expression and the phosphorylation state of *Bmal1*. These effects were reversed when HFD mice were treated with antibiotics. The antibiotic-treated mice on a HFD exhibited less weight gain and had decreased serum glucose level as compared with non-treated control mice on the same HFD diet[55]. More interestingly, another study revealed that REV-ERBα mediates gut-microbiota-dependent rhythmic recruitment of histone deacetylase 3 (HDAC3) to host-metabolism-associated target genes[70]. The genetic and epigenetic mechanisms of how the gut microbiota controls circadian rhythms in genes and proteins associated with host metabolism warrants further investigation.

Importance of microbial chronobiology

Although the role of gut microbiota in human health has been extensively studied, its effects on diurnal rhythms are under-investigated. Only in the past 6 years, the studies on the cross-talks between host circadian rhythms and the gut microbiota have noted the importance of microorganism-mediated chronometabolism. The gut microbiota contributes to the circadian rhythms of host metabolism either by affecting the major circadian clock components (such as *Arntl* and *Per*) or by directly affecting the activity of the effectors downstream of the core circadian clock genes. Both the gut microbiota and circadian rhythm

studies further hint that the diurnal oscillations of microorganisms are likely to be the result of the circadian dynamics of host factors, such as food intake, host-derived metabolites and immune system, which are known to regulate the composition, function, and biogeography of the gut microbiota. Additionally, it is important to note that ‘gut microbiota’ composition is far from uniform, and varies down the length of the gastrointestinal tract, with subtle niches within a region[5]. For example, it cannot be fully represented by stool samples, which exclude mucosa-associated microorganisms. The luminal and mucosa-associated microorganisms could affect host metabolism differently. It is further likely that metabolism mediated by the more aerotolerant microorganisms of the small intestine might exhibit very different dynamics than that elicited by the anaerobic residents of the colon. Future studies of gut microbial chronometabolism should consider these differences.

Although many questions regarding the bidirectional interactions between host circadian clock and gut microbiota remain unanswered, these interactions should be considered when designing microbiome-based interventions to improve human health. The diurnal fluctuations of the gut microbiota might be important in defining the healthy and in delivering prebiotics, probiotics, postbiotics and fecal transplantation in a time-controlled manner. Presence of microorganisms at an inopportune time can reduce the communities’ beneficial function or render them harmful to the host. Comparing microbial oscillations and circadian oscillations of host cells could also provide a clear view of the types of microorganisms required for different physiological processes in the host and whether the major drivers of a particular process are the microbial oscillations, the host clock or a combination. The intraindividual variations in the gut microbiota throughout a day will further help explain the interindividual variability in disease progression and therapeutic responses. Indeed, a study investigated human fecal samples from 1,976 individuals to predict type 2 diabetes mellitus (T2DM) and validated the results from this group by examining two additional cohorts of 1,070 individuals or 1,399 individuals. The number of rhythmic OTUs was shown to decrease in T2DM in the group of 1,976 individuals. In the validation cohorts, 13 selected arrhythmic OTUs were able to distinguish T2DM in the samples from the cohort of 1,070 indiividuals, but not in the samples from the cohort of 1,399 individuals[37]. Although the predictive performance was not validated in the second cohort, translationally, this finding implies a potential use of gut microbial oscillators as biomarkers to predict health and disease states of humans just by collecting fecal samples. Thus, further studies that integrate the rhythmicity of the gut microbiota to predict diseases states would also advance the use of gut microbiome markers for personalized medicine.

Conclusions

This Review primarily focuses on the links between the gut microbiota and the circadian control of metabolism. However, the links with the circadian control of other physiological and behavioral events are also worth investigating. The human body is a complex collection of organs whose functions need to be regulated in an intricately timed manner. This regulation helps the body interact with the dynamic surroundings most efficiently. Thus, the influences on host health by the diurnal dynamics of the gut microbiota cannot be overemphasized. The presence and function of the microorganisms at inappropriate times can adversely affect the host circadian networks and metabolism, contributing to the

breakdown of the coordination of different host body functions and the host's adaptation to the environment. Further studies are needed to better understand the potential influence of microbial oscillators and their mechanisms of action on host metabolism and circadian rhythms. The knowledge gained will promote the development of microbiome-based interventions for maintaining human health and for countering metabolic disorders.

Acknowledgements

The authors' research is supported by the following grants from NIDDK (R01DK115221, and the Center for Interdisciplinary Study of Inflammatory Intestinal Diseases (P30 DK42086).

References

1. Dodd AN et al. Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage. Science 309, 630, doi:10.1126/science.1115581 (2005). [PubMed: 16040710]
2. Fenske MP, Nguyen LP, Horn EK, Riffell JA & Imaizumi T Circadian clocks of both plants and pollinators influence flower seeking behavior of the pollinator hawkmoth Manduca sexta. Sci Rep 8, 2842–2842, doi:10.1038/s41598-018-21251-x (2018). [PubMed: 29434312]
3. Helm B et al. Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. Philos Trans R Soc Lond B Biol Sci 372, doi:10.1098/rstb.2016.0246 (2017).
4. Sartor F et al. Are There Circadian Clocks in Non-Photosynthetic Bacteria? Biology (Basel) 8, doi:10.3390/biology8020041 (2019).
5. Donaldson GP, Lee SM & Mazmanian SK Gut biogeography of the bacterial microbiota. Nature Reviews Microbiology 14, 20–32, doi:10.1038/nrmicro3552 (2016). [PubMed: 26499895]
6. Gorkiewicz G & Moschen A Gut microbiome: a new player in gastrointestinal disease. Virchows Archiv 472, 159–172, doi:10.1007/s00428-017-2277-x (2018). [PubMed: 29243124]
7. Cryan JF & Dinan TG Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 13, 701–712, doi:10.1038/nrn3346 (2012). [PubMed: 22968153]
8. Guinane CM & Cotter PD Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol 6, 295–308, doi:10.1177/1756283X13482996 (2013).
9. Martinez-Guryn K et al. Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. Cell Host Microbe 23, 458–469 e455, doi:10.1016/j.chom.2018.03.011 (2018). [PubMed: 29649441]
10. Takahashi JS, Hong HK, Ko CH & McDearmon EL The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet 9, 764–775, doi:10.1038/nrg2430 (2008). [PubMed: 18802415]
11. Rijo-Ferreira F & Takahashi JS Genomics of circadian rhythms in health and disease. Genome Med 11, 82, doi:10.1186/s13073-019-0704-0 (2019). [PubMed: 31847894]
12. Castanon-Cervantes O et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol 185, 5796–5805, doi:10.4049/jimmunol.1001026 (2010). [PubMed: 20944004]
13. Chen ST et al. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. Carcinogenesis 26, 1241–1246, doi:10.1093/carcin/bgi075 (2005). [PubMed: 15790588]
14. Maury E, Ramsey KM & Bass J Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. Circ Res 106, 447–462, doi:10.1161/CIRCRESAHA.109.208355 (2010). [PubMed: 20167942]
15. Turek FW et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science 308, 1043–1045, doi:10.1126/science.1108750 (2005). [PubMed: 15845877]
16. Wulff K, Gatti S, Wettstein JG & Foster RG Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. Nat Rev Neurosci 11, 589–599, doi:10.1038/nrn2868 (2010). [PubMed: 20631712]

17. Vinogradova IA, Anisimov VN, Bukalev AV, Semenchenko AV & Zabezhinski MA Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in rats. Aging (Albany NY) 1, 855–865, doi:10.18632/aging.100092 (2009). [PubMed: 20157558]
18. Huang W, Ramsey KM, Marcheva B & Bass J Circadian rhythms, sleep, and metabolism. J Clin Invest 121, 2133–2141, doi:10.1172/JCI46043 (2011). [PubMed: 21633182]
19. Peek CB et al. Circadian regulation of cellular physiology. Methods Enzymol 552, 165–184, doi:10.1016/bs.mie.2014.10.006 (2015). [PubMed: 25707277]
20. Stephan FK The "other" circadian system: food as a Zeitgeber. J Biol Rhythms 17, 284–292, doi:10.1177/074873040201700402 (2002). [PubMed: 12164245]
21. Chaix A, Manoogian ENC, Melkani GC & Panda S Time-Restricted Eating to Prevent and Manage Chronic Metabolic Diseases. Annu Rev Nutr 39, 291–315, doi:10.1146/annurev-nutr-082018-124320 (2019). [PubMed: 31180809]
22. Sutton EF et al. Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes. Cell Metabolism 27, 1212–1221.e1213, doi:10.1016/j.cmet.2018.04.010 (2018). [PubMed: 29754952]
23. Wilkinson MJ et al. Ten-Hour Time-Restricted Eating Reduces Weight, Blood Pressure, and Atherogenic Lipids in Patients with Metabolic Syndrome. Cell Metabolism 31, 92–104.e105, doi:10.1016/j.cmet.2019.11.004 (2020). [PubMed: 31813824]
24. Damiola F et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev 14, 2950–2961, doi:10.1101/gad.183500 (2000). [PubMed: 11114885]
25. Cohen SE & Golden SS Circadian Rhythms in Cyanobacteria. Microbiology and Molecular Biology Reviews 79, 373, doi:10.1128/MMBR.00036-15 (2015). [PubMed: 26335718]
26. Tseng R et al. Structural basis of the day-night transition in a bacterial circadian clock. Science 355, 1174, doi:10.1126/science.aag2516 (2017). [PubMed: 28302851]
27. Pattanayak GK, Lambert G, Bernat K & Rust MJ Controlling the Cyanobacterial Clock by Synthetically Rewiring Metabolism. Cell Rep 13, 2362–2367, doi:10.1016/j.celrep.2015.11.031 (2015). [PubMed: 26686627]
28. Paulose JK, Cassone CV, Graniczkowska KB & Cassone VM Entrainment of the Circadian Clock of the Enteric Bacterium Klebsiella aerogenes by Temperature Cycles. iScience 19, 1202–1213, doi:10.1016/j.isci.2019.09.007 (2019). [PubMed: 31551197]
29. Paulose JK, Wright JM, Patel AG & Cassone VM Human Gut Bacteria Are Sensitive to Melatonin and Express Endogenous Circadian Rhythmicity. PLoS One 11, e0146643, doi:10.1371/journal.pone.0146643 (2016). [PubMed: 26751389]
30. Leone V et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host Microbe 17, 681–689, doi:10.1016/j.chom.2015.03.006 (2015). [PubMed: 25891358] This study highlighted the gut microbiota as transducers of dietary cues to affect host metabolism and suggested microbial metabolites as one of the mediators.
31. Thaiss CA et al. Microbiota Diurnal Rhythmicity Programs Host Transcriptome Oscillations. Cell 167, 1495–1510 el412, doi:10.1016/j.cell.2016.11.003 (2016). [PubMed: 27912059] This study extensively investigated the relationship between microbial oscillations and host molecular network and metabolome.
32. Zarrinpar A, Chaix A, Yooseph S & Panda S Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. Cell Metab 20, 1006–1017, doi:10.1016/j.cmet.2014.11.008 (2014). [PubMed: 25470548]
33. Frazier K et al. High fat diet disrupts diurnal interactions between REG3γ and small intestinal gut microbes resulting in metabolic dysfunction. bioRxiv, 2020.2006.2017.130393, doi:10.1101/2020.06.17.130393 (2020).
34. Thaiss CA et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 159, 514–529, doi:10.1016/j.cell.2014.09.048 (2014). [PubMed: 25417104]
35. Martinez KB, Leone V & Chang EB Western diets, gut dysbiosis, and metabolic diseases: Are they linked? Gut Microbes 8, 130–142, doi:10.1080/19490976.2016.1270811 (2017). [PubMed: 28059614]

36. Liang X, Bushman FD & FitzGerald GA Rhythmicity of the intestinal microbiota is regulated by gender and the host circadian clock. Proceedings of the National Academy of Sciences 112, 10479, doi:10.1073/pnas.1501305112 (2015).

37. Reitmeier S et al. Arrhythmic Gut Microbiome Signatures Predict Risk of Type 2 Diabetes. Cell Host Microbe 28, 258–272.e256, doi:10.1016/j.chom.2020.06.004 (2020). [PubMed: 32619440] This study demonstrated translational potential of microbial oscillations as predictive makers for a metabolic disease by investigating human samples.

38. Khalif IL, Quigley EMM, Konovitch EA & Maximova ID Alterations in the colonic flora and intestinal permeability and evidence of immune activation in chronic constipation. Digestive and Liver Disease 37, 838–849, doi:10.1016/j.dld.2005.06.008 (2005). [PubMed: 16169298]

39. Voigt RM et al. Circadian disorganization alters intestinal microbiota. PLoS One 9, e97500, doi:10.1371/journal.pone.0097500 (2014). [PubMed: 24848969]

40. Weger BD et al. The Mouse Microbiome Is Required for Sex-Specific Diurnal Rhythms of Gene Expression and Metabolism. Cell Metab 29, 362–382.e368, doi:10.1016/j.cmet.2018.09.023 (2019). [PubMed: 30344015] This study demonstrated that sex-specific characteristics of host circadian gene expression in different organs can be affected by gut microbiome.

41. Froy O & Miskin R Effect of feeding regimens on circadian rhythms: Implications for agingand longevity. Aging (Albany NY) 2, 7–27, doi:10.18632/aging.100116 (2010). [PubMed: 20228939]

42. Longo VD & Panda S Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. Cell Metabolism 23, 1048–1059, doi:10.1016/j.cmet.2016.06.001 (2016). [PubMed: 27304506]

43. Greenwell BJ et al. Rhythmic Food Intake Drives Rhythmic Gene Expression More Potently Than the Hepatic Circadian Clock in Mice. Cell Rep 27, 649–657.e645, doi:10.1016/j.celrep.2019.03.064 (2019). [PubMed: 30995463]

44. Zeb F et al. Effect of time-restricted feeding on metabolic risk and circadian rhythm associated with gut microbiome in healthy males. British Journal of Nutrition 123, 1216–1226, doi:10.1017/S0007114519003428 (2020).

45. Gabel K et al. Effect of time restricted feeding on the gut microbiome in adults with obesity: A pilot study. Nutrition and Health 26, 79–85, doi:10.1177/0260106020910907 (2020). [PubMed: 32228124]

46. Loonen LM et al. REG3gamma-deficient mice have altered mucus distribution and increased mucosal inflammatory responses to the microbiota and enteric pathogens in the ileum. Mucosal Immunol 7, 939–947, doi:10.1038/mi.2013.109 (2014). [PubMed: 24345802]

47. Belkaid Y & Hand TW Role of the microbiota in immunity and inflammation. Cell 157, 121–141, doi:10.1016/j.cell.2014.03.011 (2014). [PubMed: 24679531]

48. Scheiermann C, Gibbs J, Ince L & Loudon A Clocking in to immunity. Nature Reviews Immunology 18, 423–437, doi:10.1038/s41577-018-0008-4 (2018).

49. Hand LE et al. The circadian clock regulates inflammatory arthritis. FASEB J 30, 3759–3770, doi:10.1096/fj.201600353R (2016). [PubMed: 27488122]

50. Pagel R et al. Circadian rhythm disruption impairs tissue homeostasis and exacerbates chronic inflammation in the intestine. FASEB J 31, 4707–4719, doi:10.1096/fj.201700141RR (2017). [PubMed: 28710114]

51. Yu X et al. TH17 cell differentiation is regulated by the circadian clock. Science 342, 727–730, doi:10.1126/science.1243884 (2013). [PubMed: 24202171]

52. Tognini P, Thaiss CA, Elinav E & Sassone-Corsi P Circadian Coordination of Antimicrobial Responses. Cell Host Microbe 22, 185–192, doi:10.1016/j.chom.2017.07.007 (2017). [PubMed: 28799904]

53. Gibbs JE et al. The nuclear receptor REV-ERBalpha mediates circadian regulation of innate immunity through selective regulation of inflammatory cytokines. Proc Natl Acad Sci U S A 109, 582–587, doi:10.1073/pnas.1106750109 (2012). [PubMed: 22184247]

54. Mukherji A, Kobiita A, Ye T & Chambon P Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs. Cell 153, 812–827, doi:10.1016/j.cell.2013.04.020 (2013). [PubMed: 23663780] This study suggested Toll-like receptors to be

important interface where the interplay between host circadian clock and gut microbiota is regulated, resulting in metabolic changes in the host.

55. Murakami M et al. Gut microbiota directs PPARgamma-driven reprogramming of the liver circadian clock by nutritional challenge. EMBO Rep 17, 1292–1303, doi:10.15252/embr.201642463 (2016). [PubMed: 27418314]
56. Wang Y et al. The intestinal microbiota regulates body composition through NFIL3 and the circadian clock. Science 357, 912–916, doi:10.1126/science.aan0677 (2017). [PubMed: 28860383] This study identified a specific molecular pathway through which the gut microbiota regulates host metabolism, which also involves innate lymphoid cells, suggesting that the intestinal immune system can be involved in the interactions between gut microbiota and host circadian control of metabolism.
57. Montagner A et al. Hepatic circadian clock oscillators and nuclear receptors integrate microbiome-derived signals. Sci Rep 6, 20127, doi:10.1038/srep20127 (2016). [PubMed: 26879573]
58. Oh HYP et al. Depletion of Gram-Positive Bacteria Impacts Hepatic Biological Functions During the Light Phase. International Journal of Molecular Sciences 20, 812 (2019).
59. Kennedy EA, King KY & Baldridge MT Mouse Microbiota Models: Comparing Germ-Free Mice and Antibiotics Treatment as Tools for Modifying Gut Bacteria. Frontiers In Physiology 9, doi:10.3389/fphys.2018.01534 (2018).
60. Govindarajan K et al. Unconjugated Bile Acids Influence Expression of Circadian Genes: A Potential Mechanism for Microbe-Host Crosstalk. PLoS One 11, e0167319, doi:10.1371/journal.pone.0167319 (2016). [PubMed: 27907092]
61. Martinez KB, Pierre JF & Chang EB The Gut Microbiota: The Gateway to Improved Metabolism. Gastroenterol Clin North Am 45, 601–614, doi:10.1016/j.gtc.2016.07.001 (2016). [PubMed: 27837775]
62. Zwighaft Z et al. Circadian Clock Control by Polyamine Levels through a Mechanism that Declines with Age. Cell Metabolism 22, 874–885, doi:10.1016/j.cmet.2015.09.011 (2015). [PubMed: 26456331]
63. Zarrinpar A et al. Antibiotic-induced microbiome depletion alters metabolic homeostasis by affecting gut signaling and colonic metabolism. Nature Communications 9, 2872, doi:10.1038/s41467-018-05336-9 (2018).
64. Luo Y et al. Gut microbiota regulates mouse behaviors through glucocorticoid receptor pathway genes in the hippocampus. Translational Psychiatry 8, 187, doi:10.1038/s41398-018-0240-5 (2018). [PubMed: 30194287]
65. Neufeld KM, Kang N, Bienenstock J & Foster JA Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterology & Motility 23, 255–e119, doi:10.1111/j.1365-2982.2010.01620.x (2011). [PubMed: 21054680]
66. Yan X et al. Intestinal Flora Modulates Blood Pressure by Regulating the Synthesis of Intestinal-Derived Corticosterone in High Salt-Induced Hypertension. Circulation Research 126, 839–853, doi:doi:10.1161/CIRCRESAHA.119.316394 (2020). [PubMed: 32078445]
67. Yang X et al. Nuclear receptor expression links the circadian clock to metabolism. Cell 126, 801–810, doi:10.1016/j.cell.2006.06.050 (2006). [PubMed: 16923398]
68. Zhong X et al. Circadian Clock Regulation of Hepatic Lipid Metabolism by Modulation of m(6)A mRNA Methylation. Cell Rep 25, 1816–1828 e1814, doi:10.1016/j.celrep.2018.10.068 (2018). [PubMed: 30428350]
69. Eckel-Mahan KL et al. Reprogramming of the circadian clock by nutritional challenge. Cell 155, 1464–1478, doi:10.1016/j.cell.2013.11.034 (2013). [PubMed: 24360271]
70. Kuang Z et al. The intestinal microbiota programs diurnal rhythms in host metabolism through histone deacetylase 3. Science 365, 1428–1434, doi:10.1126/science.aaw3134 (2019). [PubMed: 31604271]
71. Solt LA, Kojetin DJ & Burris TP The REV-ERBs and RORs: molecular links between circadian rhythms and lipid homeostasis. Future Med Chem 3, 623–638, doi:10.4155/fmc.11.9 (2011). [PubMed: 21526899]
72. Solt LA et al. Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature 485, 62–68, doi:10.1038/nature11030 (2012). [PubMed: 22460951]

73. Chen L & Yang G PPARs Integrate the Mammalian Clock and Energy Metabolism. PPAR Res 2014, 653017, doi:10.1155/2014/653017 (2014). [PubMed: 24693278]
74. Guerre-Millo M et al. PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes 50, 2809–2814, doi:10.2337/diabetes.50.12.2809 (2001). [PubMed: 11723064]
75. Rosen ED et al. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell 4, 611–617, doi:10.1016/s1097-2765(00)80211-7 (1999). [PubMed: 10549292]
76. Barak Y et al. Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. Proc Natl Acad Sci U S A 99, 303–308, doi:10.1073/pnas.012610299 (2002). [PubMed: 11756685]
77. Luquet S et al. Roles of PPAR delta in lipid absorption and metabolism: a new target for the Treatment of type 2 diabetes. Biochim Biophys Acta 1740, 313–317, doi:10.1016/j.bbadis.2004.11.011 (2005). [PubMed: 15949697]
78. Monsalve FA, Pyarasani RD, Delgado-Lopez F & Moore-Carrasco R Peroxisome proliferator-activated receptor targets for the treatment of metabolic diseases. Mediators Inflamm 2013, 549627, doi:10.1155/2013/549627 (2013). [PubMed: 23781121]
79. Bellet MM et al. Circadian clock regulates the host response to *Salmonella*. Proceedings of the National Academy of Sciences 110, 9897, doi:10.1073/pnas.1120636110 (2013).
80. Carroll RG, Timmons GA, Cervantes-Silva MP, Kennedy OD & Curtis AM Immunometabolism around the Clock. Trends Mol Med 25, 612–625, doi:10.1016/j.molmed.2019.04.013 (2019). [PubMed: 31153819]

Box 1 |

Interlocked circadian clock loops in mammals.

Mammalian circadian clock genes are ubiquitously distributed in almost all cell types. The clocks largely consist of two interlocked loops that feedback on each other. In the first loop, the CLOCK–BMAL dimer induces the expression of PER and CRY and, in turn, the PER–CRY dimer represses CLOCK and ARNTL (also known as BMAL) expression. CLOCK–ARNTL also activate the transcription of a number of other clock-controlled genes (CCGs). Importantly, the cyclic activity of the CLOCK–ARNTL and PER–CRY dimers regulate the expression of the nuclear receptors, REV-ERBα and RORα, which form the second clock loop. RORα and REV-ERBα competitively bind to RORE (ROR/REV-ERB-response element), present in the *ARNTL* (encoding BMAL1) promoter to respectively increase or inhibit *ARNTL* transcription (Fig. 1). Equally important, these nuclear receptors are integral drivers of regulation and rhythmicity of thousands of processes, including metabolism. For example, administration of a REV-ERBα agonist increased energy expenditure and decreased fat mass in mice[71,72]. Other nuclear receptors, specifically PPARs (Peroxisome proliferator-activated receptors), are also linked to the circadian control of metabolism. The expression of PPARα and PPARγ are diurnally controlled and they in turn affect the expression of circadian clock genes[73]. High-fat diet (HFD) feeding did not induce insulin resistance in *Ppara*-knockout mice[74]. PPARγ is required for adipocyte generation[75], whereas *Ppard*–knockout reduced adipose mass in mice[76]. Thus, PPARs are generally related to lipid metabolism, insulin sensitivity, and glucose homeostasis and their agonists are being widely used as therapeutics in various metabolic diseases such as type 2 diabetes mellitus [77,78].

Box 2.

Circadian control in the immune system.

The immune system, like many other physiological systems, is controlled by circadian clock networks in mammals. Both innate and adaptive immunities are documented to exhibit circadian oscillations in some key processes and are influenced by their cell-intrinsic clocks and by cell-extrinsic oscillations in signals from the microenvironment. Several innate immune cells including monocytes, macrophages and neutrophils have intrinsic clocks that mediate their function. The abundance and trafficking of these cells in blood and immune organs exhibit daily fluctuations depending on both their cell-intrinsic and cell-extrinsic signals. The response of innate immune cells to pathogens including bacteria, virus and parasites show a diurnal pattern. For example, the intestinal immunity response to infection by *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) showed a diurnal variation, with a higher level of infection in the light phase compared with the level in the mid-dark phase[79]. The circadian expression of the antimicrobial peptide, lipocalin-2 (LCN-2), was demonstrated to regulate this rhythmic colonization of *S*. Typhimurium in the colon. This finding is because *S*. Typhimurium is relatively resistant to LCN-2, and a higher expression of LCN-2 during the day gives *S*. Typhimurium a competitive survival advantage[79].

The T and B cells of adaptive immunity also exhibit circadian oscillations in their clock genes, which along with cell-extrinsic signals control their development, differentiation, and trafficking. For example, the frequencies of RORγ^+ T helper 17 (Th17) cells increased during circadian disruption in jet-lagged mice induced from light cycle perturbation[51]. This change during jet-lag induction was not replicated in mice lacking Rev-erbα (*Nr1d1*$^{-/-}$ mice) because Rev-erbα normally represses the expression of *Nfil3*, which is an inhibitor of *Rorγt* expression[51]. The diurnal oscillations of migratory signals and expression of their receptors on T and B cells mediate their rhythmic homing and egress from lymph nodes[48]. The circadian expression of pattern-recognition receptors, such as Toll-like receptors, by antigen presenting-cells (including dendritic cells and macrophages) leads to diurnal proliferation of and cytokine production by lymphocytes[48]. Although not fully defined, some studies revealed that the circadian control of metabolic pathways is also present in immune cells. For example, glycolysis is regulated by the molecular clock, which further affects diurnal immune responses of dendritic cells and macrophages. Timely targeting of metabolic pathways in immune cells has been suggested to be a new treatment approach for chronic inflammatory diseases including cardiovascular disease and arthritis [80].

Key points

- The gut microbiome has an essential role in transducing dietary cues used by central and peripheral host circadian clocks to regulate and adapt to shifts in energy balance.
- Low-fat (lean) diets promote diurnal ‘oscillations’ of certain microbial populations that are metabolically-relevant circadian drivers.
- Western diets high in fat and refined sugars, and low in fiber influence key microbial oscillators to disrupt host circadian rhythms and metabolism to promote obesity.
- The effects of microbial oscillators on host circadian networks and metabolism might involve the production of bioactive small molecules and metabolites.
- Activation of nuclear receptors by microbiome-derived mediators is one of many mechanisms to regulate host transcriptional and epigenetic pathways that influence host circadian control of energy balance.

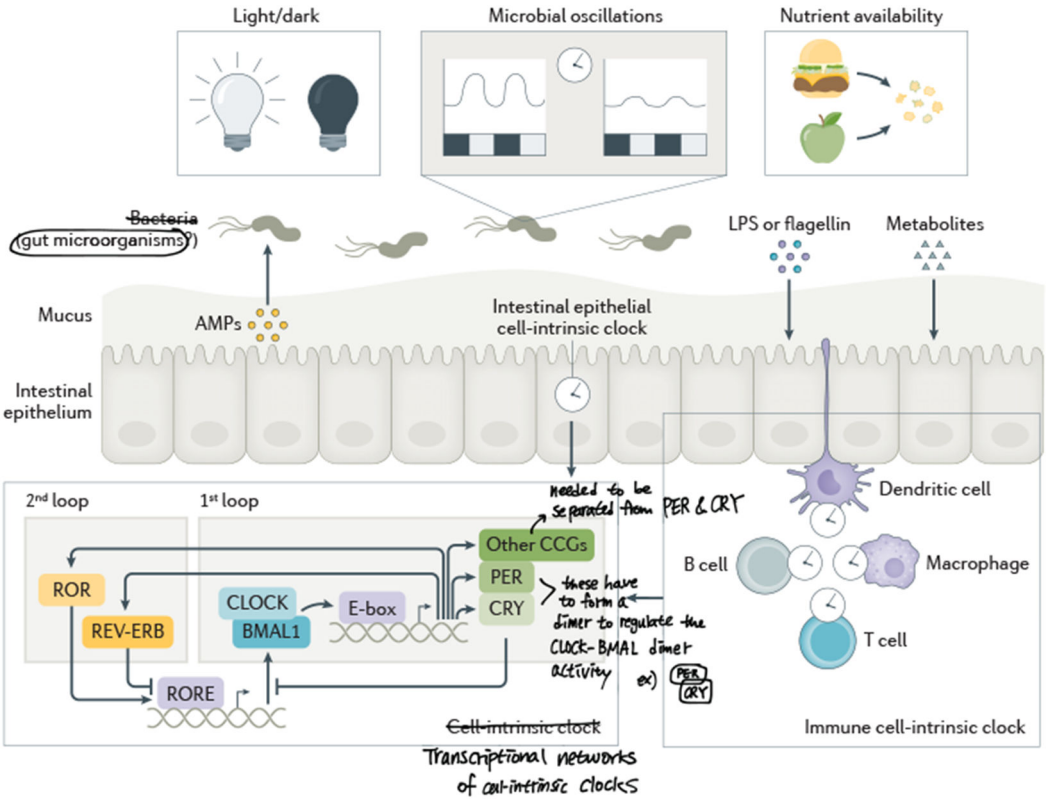

Fig. 1 |. Crosstalk between host circadian clock and the gut microbiota.

Cell-intrinsic clocks are ubiquitously distributed in host cells, including intestinal epithelial cells and immune cells, and are composed of two loops of core clock regulators. In the first loop, the heterodimeric complex CLOCK–ARNTL (also known as BMAL1) acts on E-box elements to regulate the expression of other circadian regulators, PER, CRY, REV-ERB, and ROR and other clock-controlled genes (CCGs). In turn, PER forms a heterodimer with CRY to repress the expression of ARNTL. RORs and REV-ERBs comprise the second circadian loop by competitively binding to the RORE element and regulating BMAL1 expression. The E-box elements regulate the expression of many other regulatory genes (CCGs and others not shown), to influence multiple functions. Microbial components (such as lipopolysaccharide (LPS) and flagellin) and microbial metabolites (such as short-chain fatty acids and unconjugated bile acids) also regulate the molecular clocks and the CCGs, thereby affecting a variety of physiological processes in host cells. In turn, the circadian profile of host factors such as light–dark cycle, feeding time and route, diet composition and production of mucus and antimicrobial peptides (AMPs) regulates the diurnal oscillations of the gut microbiota (upper inset). While not all gut bacteria exhibit diurnal oscillations in relative abundance, some exhibit strong or weak oscillations, which are primarily altered by host diets. (DC: dendritic cells; B, T: B and T lymphocytes; M: macrophages). The molecular clock (lower inset).

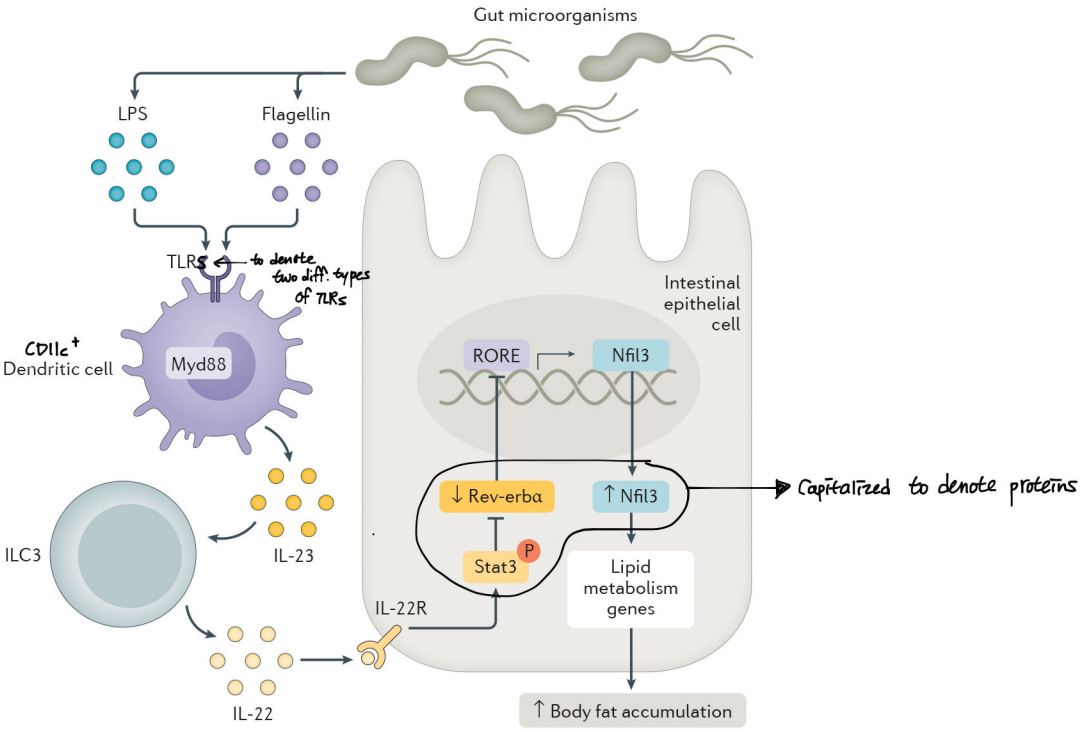

Fig. 2 |. Lipid metabolism regulated through the control of host circadian network by the gut microbial components.

Lipopolysaccharide (LPS) and flagellin from the gut microbiota can be recognized by either intestinal dendritic cells (DCs) or intestinal epithelial cells (IECs), each relaying the microbial signals to affect host lipid metabolism and corticosterone synthesis. Upon TLR activation, DCs releases IL-23, which signals type 3 innate lymphoid cells (ILC3s) to release IL-22. The IL-22 receptor on IECs phosphorylates STAT3, which then reduces REV-ERBα expression. Lack of RORE inhibitor, REV-ERBα, leads to increased level of NFIL3, which regulates various lipid metabolism genes such as *Cd36*, *Scd1*, *Cyp2e1*, and *Fabp4*. Correspondingly, *Nfil3*-knockout in mice fed with a high-fat diet (HFD) limited body fat accumulation compared with control wild-typemice on a HFD.