Review

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Microbiome diurnal rhythmicity and its impact on host physiology and disease risk

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Abstract

Host–microbiome interactions constitute key determinants of host physiology, while their dysregulation is implicated in a wide range of human diseases. The microbiome undergoes diurnal variation in composition and function, and this in turn drives oscillations in host gene expression and functions. In this review, we discuss the newest developments in understanding circadian host–microbiome interplays, and how they may be relevant in health and disease contexts. We summarize the molecular mechanisms by which the microbiome influences host function in a diurnal manner, and inversely describe how the host orchestrates circadian rhythmicity of the microbiome. Furthermore, we highlight the future perspectives and challenges in studying this new and exciting facet of host–microbiome interactions. Finally, we illustrate how the elucidation of the microbiome chronobiology may pave the way for novel therapeutic approaches.

Keywords circadian; diurnal; microbiome; rhythm

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See the Glossary for abbreviations used in this article.

Introduction

The circadian clock

Diurnal changes of the environment, termed circadian to highlight their *circa diem* or approximate 24-h cycling period, have shaped life's evolution on Earth [1–5]. The ability to anticipate circadian environmental changes conferred an evolutionary advantage [3–5]. Under this evolutionary pressure, living organisms on Earth have developed molecular mechanisms that allowed them to synchronize biological processes with the changing time of day, and to time them with associated light and darkness conditions. These molecular mechanisms termed circadian clocks have unique properties that separate them from other oscillatory processes. Circadian clocks are defined as self-sustained, temperature-compensated, and entrainable oscillators. Structurally, circadian clocks consist of an input pathway that collects environmental cues and subsequently transmits them to the central oscillator that generates a 24-h rhythm based on the signals from the input pathway. This is linked to output pathways that synchronize circadian time between the central clocks and the periphery, controlling various metabolic, physiological, and behavioral processes [6–18]. In mammals, circadian clocks feature a two-tier hierarchical structure: a (i) master or "central" clocks that are located in the suprachiasmatic nucleus (SCN) of the hypothalamus that in turn orchestrates and synchronizes (ii) "peripheral" clocks within each cell of the body (Fig 1). At its core lies a negative feedback loop with a period of ~24 h controlled by CLOCK (circadian locomotor output cycles kaput) and ARNTL (aryl hydrocarbon receptor nuclear translocator-like protein 1), which regulate the rhythmic expression of up to 15% of the entire transcriptome of each mammalian cell [19]. Prokaryotic circadian clocks on the other hand are much less understood and have primarily been studied in light-responsive cyanobacteria. However, recent studies have identified circadian clocks also in light-independent prokaryotes [20]. In a remarkably simple and elegant system in cyanobacteria, a circadian clock of just three proteins (KaiA, KaiB, and KaiC) can be sustained even in the absence of transcription, although transcriptional feedback is required for its stability [21–24]. Circadian clocks of light-nonresponsive bacteria residing in diurnally changing environments are much less studied. In a pioneering study, Paulose *et al* [20] have described a circadian oscillator of *Enterobacter aerogenes*, a member of the human gut microbial community, also termed the human gut microbiome. The circadian oscillator of *E. aerogenes* is entrained by the human pineal and gastrointestinal hormone melatonin, having the same period as the human central circadian clocks of approximately 24 h. Furthermore, it is temperature compensated within the range of human body temperatures and it also regulates swarming activity of *E. aerogenes*. Bioinformatic analyses have revealed that circadian clocks of *E. aerogenes* are similar to those of cyanobacteria [20]. This suggests that other members of the human gut microbiome may also possess inherent circadian clocks. Indeed, the human gastrointestinal tract constitutes a diurnally changing environment, in which a multitude of circadian factors, including temperature, pH, nutrient availability, and gut motility, would make an adaptive circadian clock beneficial for some gut bacteria.

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Glossary

BMAL1	Brain and muscle ARNT-like protein
CLOCK	circadian locomotor output cycles kaput
E. aerogenes	*Enterobacter aerogenes*
GF	germ-free
HFD	high-fat diet
ILC	innate lymphoid cell
linc-RNA	large intergenic non-coding RNAs
NAD	nicotinamide adenine dinucleotide
NLR	nod-like receptor
PER	period
SCN	suprachiasmatic nucleus
SFB	segmented filamentous bacterium
SPF	specific pathogen-free mice
TLR	Toll-like receptor

Diurnally shifting host–microbiome interactions may also bear disease-relevant importance. Disruption of the circadian clock became a hallmark of modern lifestyle brought about by the invention of electric light that made shift-work widespread and the increase in global traveling activity across time zones resulting in jet-lag (Fig 2). Chronic disruption of the circadian clock has been associated with a multitude of diseases, including obesity, diabetes, cancer, cardiovascular, psychiatric, and neurodegenerative diseases, and susceptibility to infection [6,25–27]. Notably, these very conditions have been also associated with an aberrant composition of the intestinal microbiome community, termed microbiome dysbiosis, which is characterized by a sharp drop in diversity and reduced colonization resistance [28–31] (Fig 2). The association between circadian clock disruption and dysbiosis described in human studies can be recreated in animal models. Indeed, circadian clock disruption, either through genetic impairment of core clock components or their environmental disarrangement in rodent models, leads to gut microbiome dysbiosis [32,33]. Dysbiosis contributes to the adverse metabolic consequences of clock disruption, as antibiotic treatment ameliorates obesity and glucose intolerance in mice with environmental circadian clock disruption, while microbiome transfer from jet-lagged mice and humans into germ-free mice fully recapitulates metabolic disease manifestations in a new host [33–45]. These observations demonstrate that diseases induced by circadian clock disruption can be mediated by aberrations in microbiome composition and function. They also suggest that the microbiome might constitute a previously unrecognized link between the rise of microbiome-modulated diseases and misalignment between body clock and geophysical time.

The gut microbiome

The human body is colonized by a diverse community of microorganisms, termed the human microbiome. The complexity and composition of microbiomes colonizing various barrier tissues of the human body varies, even more so between individuals [46,47]. Recent years have seen a remarkable progress in our understanding of the role the human microbiome plays in health and disease (for comprehensive reviews see [48–53]). Briefly, the "forgotten organ" of gut microbiome was shown to be involved in almost all facets of human health: in the maturation and continued education of the host immune response [54], as an effector of host metabolism, including energy harvest from food [55] and changing host propensity toward weight gain [56], as a contributor to host metabolic homeostasis [57], but also in providing protection against pathogen overgrowth and influencing host-cell proliferation [58] and gut vascularization [59], regulating neurologic signaling [60] and bone density [61], providing a source of energy biogenesis [62], biosynthesizing vitamins [63] and neurotransmitters [60], metabolizing bile salts, thereby reacting to or modifying drugs, and eliminating exogenous toxins [64,65]. The importance of the gut microbiome for human health is exemplified by observations that a disrupted microbiome exacerbates immune disorders such as autoinflammatory colitis [66], while a healthy microbiome can be used therapeutically to cure life-risking infections [67]. While the gut microbiome strongly influences gastrointestinal homeostasis, in addition to local effects, the microbiome may exert its influence systemically. One such example is featured in a mouse model of autism, in which transfer of a healthy microbiome ameliorated some neurological features of this disease [68]. All of the above-mentioned microbiome-mediated effects on human health are subject to microbiome composition. One of the processes that shapes microbiome configuration is aging [69]. The initial colonization of an infant by the microbiome occurs at birth, upon passing through the birth canal. Subsequently, the composition of the neonatal microbiome is primarily shaped by microbial transfer from maternal microbiome communities, including the ones colonizing skin and oral cavity. The neonatal gut microbiome is also strongly influenced by lactic compounds, which promote the growth of advantageous bacteria. As a result of the microbial transfer from the mother, the microbiome of the infant increases in heterogeneity and diversity [70], which in turn [70] leads to enhanced immune maturity and specificity [71]. Upon reaching adulthood, microbiome composition stabilizes, with a retention rate of approximately 60% over 5 years [72]. However, the composition of the microbiome maintains a rate of change well into old age, with distinct microbiomes noted in elderly subjects [73]. The factors that affect long-term microbiome stability include perturbations as exposure to major dietary changes [74,75], drugs, antibiotics [76], and food supplements [76,77]. For example, a drastic change in the amount of dietary fiber consumption results in significant microbiome composition rearrangements within 24 h that last for several weeks [74]. In addition to diet content, a major change in overall nutritional caloric value also results in a systemic and persisting change of the microbiotic *Firmicutes*-to-*Bacteroidetes* ratio [78]. The effects of the microbiome mentioned above are governed by its composition and function, which is dynamic and potentially amenable to change in contrast to host genetics, making it a promising hub for therapeutic targeting.

Mechanisms of circadian host–microbiome crosstalk

The human–microbiome meta-organism constitutes a multi-domain ecosystem, in which the interaction of eukaryotic and prokaryotic symbionts must not only be precisely synchronized with one another, but also with the environmental changes over the course of a day.

Modulation of the gut microbiome by circadian clocks of the host

In recent years, a new facet of host–microbiome interactions has been identified in the gut, consisting of circadian fluctuations in the composition and function of the intestinal microbiome [33]. The

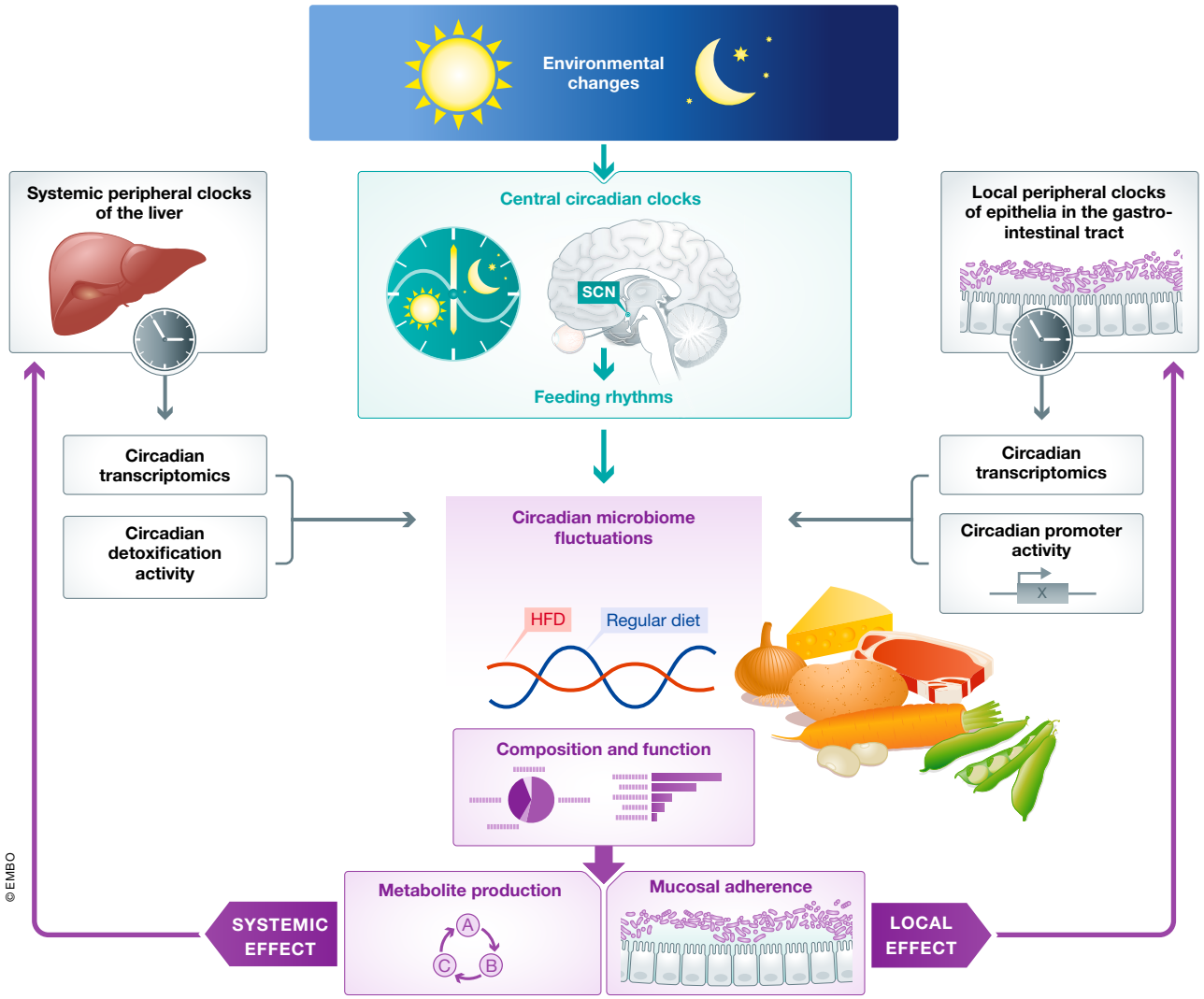

Figure 1. The two-tier hierarchical structure of mammalian circadian clocks.
The gut microbiome undergoes diurnal changes in composition and function that are entrained by feeding rhythms. In turn, microbiome circadian fluctuations of the microbiome modulate circadian activity of peripheral mammalian clocks, both locally (right), through mucosal attachment, and systemically (left), through the production of metabolites.

relative abundance of 20% of all microbiome species in both mice and humans undergoes diurnal fluctuations. As a result, time of day-specific configurations of the intestinal microbiota can be identified (Fig 1). For example, relative abundance of the commensal genus *Lactobacillus* declines during the active phase and increases during the resting phase in both mice and humans [33,35]. Interestingly, this phenomenon does not appear to be dependent on a particular microbiota configuration, as diurnal microbiome oscillations can be observed in mice and humans that feature inter-individual variability in the composition of their baseline commensal gut bacteria. In addition to compositional fluctuations, microbiome communities appear to be performing specific tasks predominantly during one or the other time-period: detoxification and chemotaxis during the resting phase, and energy harvest, DNA repair, and cell growth during the active phase of the host (Fig 1). Furthermore, the gut microbiome exhibits rhythmic biogeographic localization, adherence to the intestinal epithelium, and rhythmic metabolite secretion (Fig 1). These diurnal fluctuations have both local and systemic consequences for the host: Through rhythmic adherence to the intestinal epithelium, the microbiome is able to modulate chromatin and transcriptional oscillations in intestinal cells, while far-reaching metabolites allow the microbiome to shape hepatic circadian gene expression and detoxification reactions [33,79,80] (Fig 1). These microbiome oscillations depend on the cues from the host's circadian clocks, as mice lacking two major components of the mammalian molecular clock, PER1 and PER2, do not feature daily rhythmicity in bacterial composition and function [33]. This indicates that the activity of the meta-organism is synchronized through entrainment of microbial oscillations by circadian clocks of the host. The exact mechanism behind microbiome compositional and

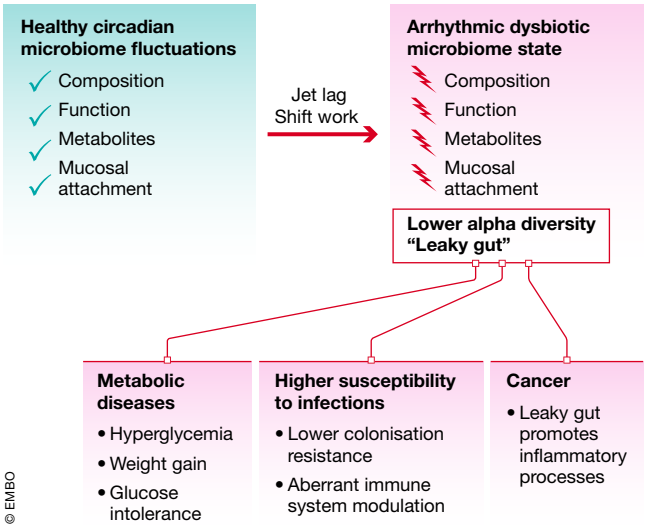

Figure 2. Disruption of the circadian clock as a hallmark of modern lifestyle.
Disruption of circadian microbiome fluctuations results in the establishment of an arrhythmic dysbiotic microbiome state, promoting the development or progression of several diseases, including metabolic syndrome, infections, and cancer.

functional oscillations remains unknown. This synchronization may confer a range of evolutionary advantages for the host–microbiome meta-organism, including colonization resistance toward pathogenic species and the detoxification of noxious xenobiotics. The probability of an encounter of the mentioned noxious xenobiotics and pathogenic species is not constant through the day, suggesting that anticipatory circadian mechanisms may play an important role in maintaining homeostasis. Among the most important diurnal environmental changes is nutrient availability. Indeed, circadian clock machinery and metabolic pathways are closely intertwined, with the NAD pathway being directly coupled to core components of mammalian clocks [81–84]. The interconnectivity of metabolic pathways and the circadian clock is necessary to synchronize the anabolic and catabolic pathways of energy turnover with diurnal variations in nutrient intake. Indeed, time of nutrient consumption is a central driver of peripheral body clocks: Feeding in restricted periods of the light–dark cycle uncouples peripheral clocks from the central master clock in the SCN; mice with a genetically impaired circadian clock not only lose most transcriptome oscillations, but also their strict nocturnal feeding pattern. However, restricting access to food to the dark part of the cycle rescues, at least in part, loss of transcriptional and behavioral rhythms in these mice, suggesting that oscillatory processes exist which are entrained by feeding rhythms, and can persist in the absence of a functional circadian clock [85,86]. This paradigm of food-entrainable clocks is crucial when understanding the host's circadian interaction with the intestinal microbiome. Restricting food access in mice to the dark or light phase induced a phase shift in microbiome oscillations, while the overall number of oscillatory commensals remained constant. Furthermore, the loss of bacterial oscillations in arrhythmic Per1/2-deficient mice was restored by subjecting them to scheduled feeding [33]. Therefore, feeding times are a dominant driver of the temporal orchestration of microbiome activity (Fig 1). Supporting the notion that microbiome circadian changes are controlled by central circadian clocks, Wu *et al* [87] recently described the effects of exposure to light on circadian microbiome fluctuations.

In addition to the time of consumption, the type of food also has an effect on the clock machinery. Mice fed a high-fat diet exhibit attenuated clock gene oscillations, alterations in locomotor activity rhythms, and massive reprogramming of the circadian transcriptome [88,89]. In addition, high-fat diet alters the temporal organization of the microbiome: Cyclic bacteria species are not identical between mice on HFD and controls, and the overall number of cyclic bacteria species in mice on HFD is reduced [35]. The type of food therefore seems to determine the extent and amplitude of microbiome oscillations (Fig 1). Interestingly, time-restricted feeding rescues the adverse metabolic effects of high-fat feeding on the host [34], suggesting that not the content of the diet *per se*, but rather the mistiming of feeding is driving the onset of metabolic disease. Therefore, apart from the entrainment of the host circadian clocks, feeding rhythmicity also shapes the symbiotic community of microorganisms colonizing the mammalian intestine.

The impact of circadian microbiome fluctuations on the host

The complex network of host–microbiome interactions is in many cases bilateral. As such, the host impacts the diurnal activity of the gut microbiome through its clock machinery and feeding behavior (see above), while the diurnally shifting microbiome conversely impacts the host circadian activity. Indeed, different members of the microbiota impact the host in varying ways, depending on their location, metabolic activity, abundance, and other factors. The microbiome was shown to be essential for driving host transcriptomic oscillations in the intestine and in the liver, as complete ablation using antibiotics or germ-free mice leads to a change of expression patterns of circadian transcripts in the host [79] (Fig 1). Interestingly, in the absence of the microbiome, *de novo* transcriptomic oscillations are generated. Mechanistically, both contact-dependent and contact-independent interactions between the host and the microbiome regulate these phenomena. Contact-dependent interactions involve adherence of commensals of the mucosal microbiome such as segmented filamentous bacteria (SFB) to intestinal epithelial cells [79]. Interestingly, mucosal-associated bacteria undergo robust diurnal oscillations in both composition and function [79], including their capacity to degrade mucus, which allows other members of the microbiota to dwell in this region of the intestine [90]. Indeed, rhythmic attachment of SFB *per se* induces robust transcriptomic oscillations in colonic epithelial cells [79], while the rhythmically varying number of non-adhering rat SFB induced significantly weaker transcriptomic oscillations [79]. How bacterial proximity induces these oscillations remains to be elucidated, but intestinal epithelial cells and myeloid cells, such as dendritic cells, express a large array of pattern recognition receptors like Toll-like receptors (TLRs) or NOD-like receptors (NLRs), which bind conserved microbial molecules and thus may directly detect microbes in their vicinity. Apart from direct attachment and PRR ligands, microbial metabolites may exert local and systemic effects on the host by modulating the function of epithelial, immune, and other cells. Indeed, circadian fluctuations of many metabolites are dependent on the microbiome, leading to transcriptomic and

consequently functional oscillations in the intestine and peripheral sites [79]. Rhythmic oscillations of the intestinal microbiome are associated with similar oscillations in serum metabolite levels [79]. These in turn, drive circadian gene expression patterns in the liver, impacting many important functions, including oxidative phosphorylation and other catabolic pathways [79]. Furthermore, administration of bacterial metabolites at specific time points directly entrained peripheral host clocks [91], suggesting that bacteria are an important factor in maintaining host circadian rhythmicity in general (Fig 1).

Equally interesting is the mechanism by which the gut microbiome globally influences the transcriptome. It is becoming increasingly clear that the microbiota has a major role in regulating the epigenetic landscape in host cells, which in turn leads to changes in gene expression patterns. The gut microbiota produces key micronutrients for DNA modifications including B vitamins, which are needed for DNA methylation [92], while changes in the gut microbiome in mice deficient for specific PRRs such as TLR2 lead to differences in DNA methylation patterns [93]. Furthermore, the gut microbiota was shown to provide several cofactors needed for enzymes involved in epigenetic modifications such as acetyl-CoA and NAD^+ [94]. For histone-deacetylases (HDAC) in particular, gut microbiome-derived short-chain fatty acids such as butyrate or propionate were shown to be key regulators of activity [95], which in turn can have many downstream effects in mediating human disease, such as type 2 diabetes or obesity [96]. One key mechanism by which the microbiome regulates host circadian transcriptomic oscillations is through the modulation of the chromatin landscapes. Indeed, the epigenetic landscape of genes with a circadian expression pattern in intestinal epithelial cells is profoundly affected by antibiotic treatment, including gain and loss of histone modifications such as H3K4me3, H3K27 acetylation at active promoters, and H3K27 acetylation at enhancers [79]. Overall rhythmicity of histone marks is not affected by depletion of the microbiome, but the loci undergoing oscillations in histone marks are significantly changed, consistent with the changes in transcriptomic oscillations observed under similar conditions [79]. Apart from directly interacting with the host, commensals may also indirectly interact with it by suppressing the colonization of pathogens through direct secretion of anti-bacterial molecules such as bacteriocins or indirectly by competing for the same nutrients. In addition, following their capacity to influence the inflammatory response described above, some commensals may modulate inflammation to prevent favorable conditions for pathogen colonization [97]. Despite these advances, many of the molecular mechanisms of host–microbiome interactions remain unknown and more research is necessary to unravel more about how they are regulated in health and disease.

Impact of host–microbiome circadian crosstalk on health and disease

The rhythmic adaptation to environmental fluctuations over the course of a day extends to the entire meta-organism of both host and symbiotic communities in health and disease. In this section, we will highlight examples of how diurnal host–microbiome interactions impact host physiology and risk of disease.

Infection

Circadian rhythmicity in general has long been recognized as a major factor controlling the host response to infection [98], and disruption of the circadian cycle was found to lead to increased susceptibility to viral [99], as well as bacterial infections [100], including foodborne pathogens [101]. However, while significant evidence is emerging that circadian rhythmicity of the microbiota regulates host metabolism, understanding how commensal microbial-intrinsic clocks influence host resistance to infection remains unclear. The microbiota contributes to the host's resistance to infection by a number of distinct mechanisms. This includes colonization resistance, where commensals occupy distinct biological niches and thereby prevent pathogens from establishing themselves in the microbial ecosystem [97] (Fig 2). In addition, in some instances members of microbiota, e.g., of the *Bacteroides* family, will directly inhibit pathogen growth by secretion of metabolites such as the short-chain fatty acid propionate [102]. Indeed, microbiota-derived metabolites were shown to modulate susceptibility to bacterial infections, e.g., through disruption of intracellular pH homeostasis of the pathogen [102], and of viral infection, e.g., through boosting of type I interferon signaling [103], both in the intestinal tract and in peripheral organs. Another major mechanism by which the microbiota contributes to host resistance to infection is the crosstalk with the immune system (Fig 2) [104]. Microbial components induce production of anti-microbial peptides by intestinal epithelial cells [105], promote activation of innate immune cells such as ILCs [106], and induce adaptive T- [107] and B-cell responses [108]. Furthermore, the microbiome is essential for a proper development in particular of myeloid cells, which in turn promotes resistance to bacterial infection [109].

While it has not been shown to date that circadian rhythmicity of the microbiome affects susceptibility to infection, there is circumstantial evidence that this may be the case. First, mice featuring a disrupted circadian cycle have a reduced microbiome diversity [33], which in turn was shown to decrease resistance to infection [110]. Furthermore, given the well-known effect of the microbiome on the immune system and increased susceptibility to infection of jet-lagged mice [111], it is very likely that the absence of circadian rhythmicity affects infection susceptibility in this case (Fig 2). Finally, levels of specific bacterial metabolites, which are known to mediate infection resistance [112], are dysregulated in mice with a disrupted circadian cycle [91] (Fig 2). It is therefore possible, but remains to be investigated, that the well-established circadian variations of microbiota-derived metabolite levels will influence infection resistance in these cases. In addition, fluctuations in commensal composition will potentially affect colonization resistance to bacterial pathogens due to the changing availability of a suitable niche (Fig 2). However, no studies to date have addressed the impact of circadian rhythmicity of the microbiota on susceptibility to infection in the intestinal tract or peripheral sites, so it remains to be seen to what extent this phenomenon regulates infection resistance (Fig 2).

Metabolic disease

Disruption of circadian clocks has long been associated with a range of metabolic disorders including obesity [113–120], metabolic syndrome [114,121–124], and type II diabetes [29,114,115, 117,118,120,122,124–128]. Recently, microbiome dysbiosis has emerged as the mechanistic link connecting metabolic diseases and

circadian clock disruption (Fig 2). Indeed, mice with either genetic or environmental impairment of circadian clocks develop a number of metabolic derangements similar to human metabolic syndrome: higher weight, hyperglycemia, insulin resistance, and higher body fat composition, compared to circadian clock-sufficient controls [33,35,129,130]. Importantly, antibiotic treatment protected mice from developing circadian clock disruption-mediated metabolic syndromes. Mechanistically, circadian clock disruption triggers a compositional and functional change in the gut microbiome community, leading to the dysbiotic microbiome state. Microbial transfer of this dysbiotic community from either mice or humans into germ-free mice fully recapitulates metabolic derangements of the donor [33,129]. These observations demonstrate that metabolic diseases induced by circadian clock disruption can be contributed by aberrations in microbiome composition and function. They also suggest that the microbiome might constitute a previously unrecognized link between the rise of metabolic diseases and misalignment between the circadian clock and the geophysical time.

Liver function

The liver is a key organ orchestrating metabolic homeostasis with multiple functions that oscillate in response to food intake [131–137]. Through the portal vein that connects the liver to the gastrointestinal tract, the liver is exposed to the influence of the gut microbiome. Perturbations of the gut microbiome, coupled with disturbances in gut barrier function, have been associated with common liver disorders, such as non-alcoholic fatty liver disease [138–140], non-alcoholic steatohepatitis [139,141–152], alcoholic liver disease [139,140,142,145,147,148,150,151,153–164], and liver cirrhosis [165–170]. Thus, the microbiome serves as a modulator of liver rhythmic functions. Microbiome transfer into germ-free (GF) mice resulted in PPARgamma-mediated activation of new oscillatory transcriptional programs in the liver. Inversely, treatment of specific pathogen-free mice (SPF) with broad-spectrum antibiotics suppressed PPARgamma-driven transcription in the liver, underscoring the essential role of gut microbes in clock reprogramming and hepatic circadian homeostasis [137,171]. One of the ways by which the microbiome orchestrates hepatic transcriptome oscillations is through modulation of the generation and secretion of microbiome-derived metabolites. Microbial metabolites, specifically short-chain fatty acids, directly modulate diurnal expression of circadian clock genes in hepatocytes [129], while germ-free and antibiotic-treated mice display altered daily oscillation of clock gene expression in the liver [79,172]. In addition to aberrant clock gene expression in the absence of microbiome, global rhythmicity of the liver transcriptome undergoes a profound rearrangement with up to 70% *de novo* oscillating transcripts [79,173]. Interestingly, while SPF male and female mice feature sex-specific rhythmic liver transcriptome programs, these changes are largely attenuated in GF mice [173]. These results highlight the microbiome as being critical for maintaining the homeostatic rhythmic functions of the liver. Indeed, the capacity of the liver for the detoxification of acetaminophen (acetyl-para-aminophenol APAP) varied with time of day and depended on the presence of the microbiome. Remarkably, administration of APAP to SPF mice during the active period resulted in much greater damage to the liver compared to treatments during the resting period, while there was no time-specific difference in antibiotic-treated or germ-free groups [79]. Collectively, this suggests that homeostatic microbiota rhythms and microbiota-mediated maintenance of the circadian transcriptome are necessary to maintain normal diurnal activity in hepatic drug metabolism.

Cancer

There is evidence that both the microbiota and disruptions to the host circadian rhythm play a role in cancer pathogenesis. For both colorectal [174] and lung cancers [175], jet-lag was found to promote carcinogenesis in mice and humans and it is likely that disruption of microbial rhythmicity in addition to the host clock will influence disease development (Fig 2). Furthermore, there is evidence that apart from modulating malignancy directly or indirectly through modulation of chronic inflammation, the microbiota may modulate the response to chemotherapy [176] and irradiation therapy [177]. Whether treatment with chemotherapeutic agents, immune-modulating drugs, or irradiation is dependent on or influenced by microbial diurnal rhythmicity remains to be investigated in the future. However, due to the well-established roles of circadian rhythms and the microbiome in controlling inflammation it is likely that fluctuating microbiota-derived metabolite levels may impact anti-cancer immunity, and thus the response to "checkpoint blockade". Indeed, the phenomenon of the "leaky gut", which is associated with microbiota-mediated modulation of anti-tumor therapies due to their cell proliferation suppressive effects, is also induced by environmental circadian disturbances [176,178–184]. More research is necessary to unravel the relationship between microbiome rhythmicity, composition and function, and the molecular mechanisms promoting cancer development.

Conclusions and future perspectives

Many questions regarding the understanding of how circadian microbial clocks regulate host physiology in the steady state and in inflammation remain unanswered (Box 1), and represent a fascinating area of research in years to come (Fig 3).

Expanding the role of circadian diurnal rhythmicity to physiological and disease conditions

The diurnally changing microbiome and its impact on the host may influence other microbiome-associated physiological states and diseases. For example, aging is associated with distinct changes in the gut microbiome [69,185–204]. Moreover, the microbiome has been implicated to modulate various aspects of aging including longevity [205], and very old individuals were found to feature a microbiota composition enriched for "health-promoting" commensals [206]. While the microbiota is relatively stable in adulthood [72], the aging process is associated with a change in an individual's microbiome composition, which is thought to be mainly driven by environmental effects such as the use of antibiotics, changes in nutrition, and the development of chronic illnesses, including metabolic syndrome and inflammatory diseases [73,185–204]. The microbial diversity decreases significantly with age [207], while the inter-individual variability in microbiota increases significantly in elderly compared to younger adults [208]. Some of these effects are in part due to a shift in the circadian rhythm as people age. Indeed, it is well established that sleep patterns change with age, with older individuals generally

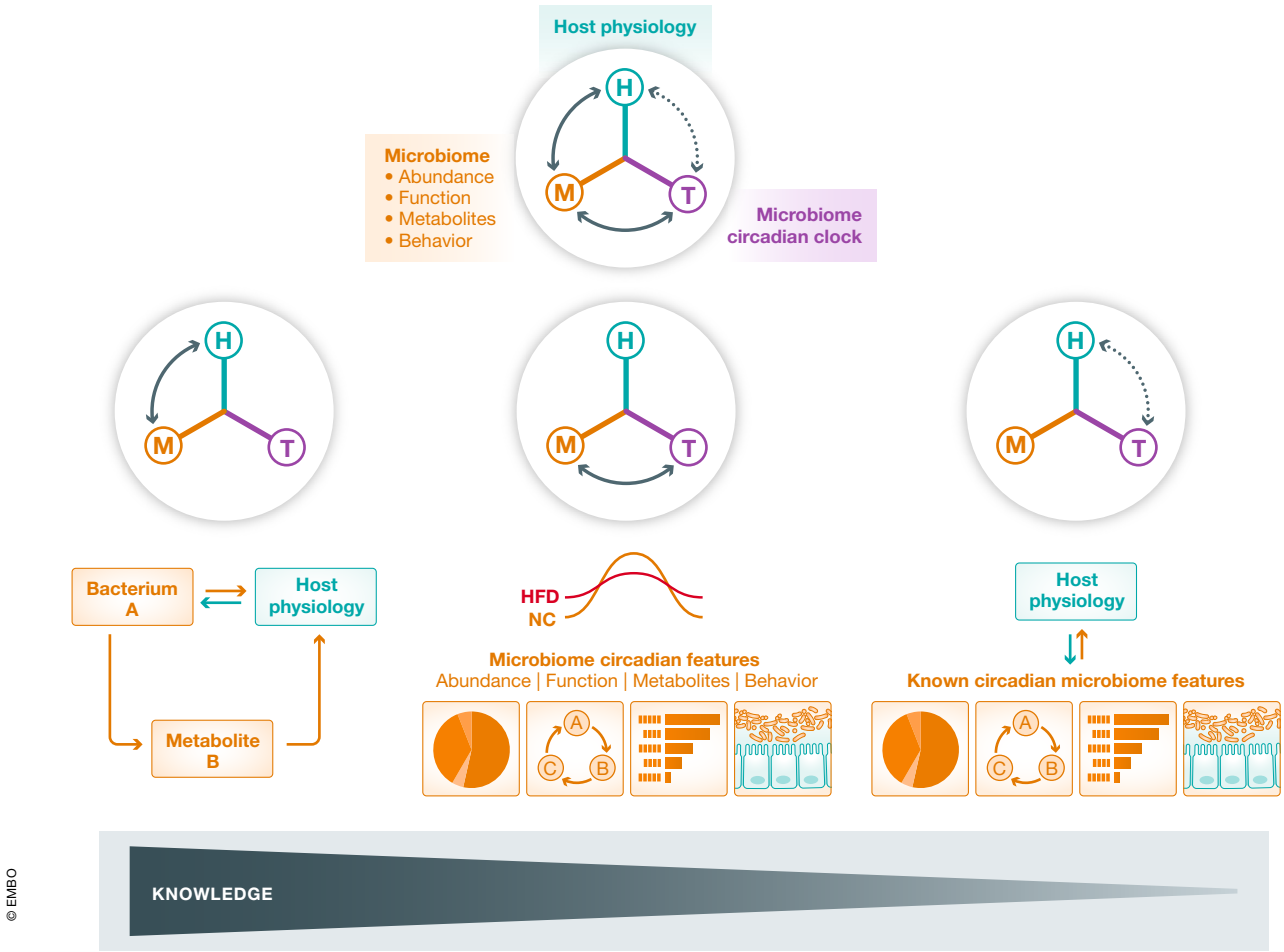

Figure 3. Time constitutes an underappreciated but important dimension of host–microbiome interactions.
A plethora of factors involving bacterial species, bacterial metabolites, and specific bacterial behaviors have been shown to affect host physiology, and at the same time, many of them have been demonstrated to exhibit circadian oscillations. Thus, the intersection of classical microbiome studies and temporal biology holds great potential for future investigation.

exhibiting shorter and lighter periods of sleep [200]. As sleep has been directly linked to regulating the microbiota, it is very likely that the shift in circadian rhythmicity with age has a significant impact on the microbiome. For the human commensal *E. aerogenes*, it was shown that the sleep hormone melatonin directly regulates swarming motility in the intestinal lumen [20], providing a first insight into how host rhythmicity may in turn control the functions of specific members of the microbiota. Microbiome-derived metabolites fluctuating in a circadian manner, such as short-chain fatty acids [91], in turn control the host clock in the intestinal epithelial cells, liver, and possibly other peripheral tissues (Fig 3). As described above, food intake is a major driver of circadian rhythmicity in microbiome composition and function, and it is likely that shifting meal habits commonly observed with increasing age will contribute to differences in metabolism between young adults and elderly. Due to the combined effects of metabolic changes and changes in the circadian rhythm in older individuals, these fluctuations in metabolite levels will likely have a significant impact on the aging process (Fig 3).

An example of a set of diseases potentially modifiable by the shifting gut microbiome–host interface is neurodegenerative diseases. While there is increasing evidence for an involvement of the microbiome in the development of neurological diseases including multiple sclerosis [209,210] and Parkinson's disease [211], it remains largely unclear to what extent circadian variations in microbiome composition or function contributes to this phenomenon (Fig 3). There is some indication that time-restricted feeding can modulate disease worsening in Huntingdon's disease [212], and as food uptake is known to orchestrate gut microbiome fluctuations [33], it is very likely that some of the effects seen in this model can be attributed to fluctuating microbial products (Fig 3). More work is necessary to determine whether human commensals such as *Bacteroides fragilis*, which have been shown to modulate neurodevelopmental disorders [68], fluctuate in a circadian manner. It has been suggested that microbial metabolites play an important role in regulating host physiology at peripheral sites to the intestine, including the brain. Indeed, the "gut–brain axis" is now recognized as an important interaction network

Box 1: In need of answers

(i) Does the host directly regulate microbial clocks other than by food intake? And if yes, what are the underlying molecular mechanisms?
(ii) What is the relationship between aging, shifts in the circadian rhythm and the microbiome?
(iii) How are diurnal post-translational modifications regulated by the microbiome?
(iv) How are non-coding elements of the genome such as microRNAs regulated by the diurnal rhythmicity of the microbiota?
(v) How is diurnal rhythmicity of the microbiome regulated at mucosal surfaces other than the intestine?
(vi) How does microbial circadian rhythmicity affect the response to microbiota-based therapies such as probiotics?

where the microbiome directly influences the peripheral and central nervous system [213].

Exploring further mechanisms of circadian microbiome control of host physiology

It is clear that more mechanistic evidence is required for global understanding of the diurnally shifting host–microbiome interface. Open questions include the cell types at mucosal surfaces or peripheral sites that are directly regulated by microbial factors, and how this modulation relates to their circadian variation in gene expression patterns and function over the course of a day. A second interesting yet not fully addressed question is the regulation of the molecular mechanisms driving diurnally shifting microbiome-mediated impacts on the host. While it has been shown that multiple host transcriptional fluctuations are driven by the microbiome, more needs to be done to understand the mechanisms driving these changes in gene expression, including epigenetic regulation. A third interesting unmet question involves the potential regulation of host post-translational modifications by the shifting microbiome, including hourly changing protein phosphorylation, ubiquitination, and sumoylation. Such mechanisms may contribute to a mechanistic understanding of observations linking the diurnally oscillating microbiome to host changes in cell cycle, secretion of exosomes, and more. A fourth unanswered key question, and an exciting area of future research, is the elucidation of mechanisms by which the circadian microbiota interacts with the immune system. As briefly described above, the bidirectional relationship of the microbiome and immunity is a very important component of host–microbiome interactions, and understanding how circadian fluctuations in microbiome composition and function control inflammation and key immune capacities, will be of great interest in future years. Examples of these topics include elucidation of possible diurnal microbial regulation of the activation of innate immunity, induction of T-cell responses, and establishment of memory (Fig 3). It will be necessary to disentangle the role of host circadian control of the immune responses and microbiome diurnal rhythmicity utilizing immune cell specific core circadian clock-knockout mice. Equally important is to unravel to which extent the diurnally rearranging microbiome contributes to the regulation or promotion of inflammation, possibly influencing resistance to infection and driving immune activation versus tolerance. In addition, further studies are necessary to investigate a role of the immune system in synchronizing bacterial intrinsic clocks with host circadian rhythms, and its relationship with food uptake.

Dissecting the role of specific members of the circadian microbiota

More research is also needed to uncover relationships between specific members of the microbiota, their rhythmicity in abundance or function, and particular host phenotypes. In particular, it will be important to understand which are the essential components in the circadian microbiota driving oscillations in gene expression and function in the host. Furthermore, the analysis of the microbiome oscillations in composition and function needs to be extended to microbiomes at other sites, in particular other barrier tissues such as the skin. The elucidation of the site-specific circadian regulation of host–microbiome interactions will be crucial to understand their role in microbiome-mediated diseases in peripheral tissues, including local fluctuations in microbial metabolites, and time-dependent localization of specific members of the microbiota (i.e., mucosal versus luminal). In this respect, one of the key challenges includes the development of methods to assess fluctuations in microbial composition and function at sites featuring a low biomass. In addition, another major question to be answered in this context is to what extent the regulation of bacterial intrinsic clocks is dependent on the environment (i.e., tissue). Furthermore, a crucial question to be answered is whether the host directly regulates the bacterial clock in certain disease contexts apart from the fluctuations induced by nutrient availability during food uptake.

Studying circadian microbiome rhythmicity in humans—translational aspects

Finally, many important questions remain unanswered regarding the applicability of the findings largely obtained in animal models to humans, potentially enabling future therapeutic exploitation. While it is challenging to directly show causal relationships between diurnal changes in the microbiome and effects on the human host, recent studies have provided some evidence into this direction [27,33,214,215]. The microbiome in humans was also suggested to vary in composition and function across the course of a day, although variations in transit and excretion times make such interpretations more complex in the human as compared to the rodent setting [33]. In addition, circadian disruption, such as the jet-lag induced by repeated re-adaptation to different time zones, leads to metabolic derangements once the human microbiota is transferred to germ-free mice [33]. Although it provides only a glimpse of how the circadian human microbiota controls host physiology, it may nonetheless be a starting point to mechanistically decode the microbiome-mediated effects of circadian disturbances in shift-work and jet-lag on human physiology. Indeed, shift-work has long been associated with increased risk to develop multiple diseases, including obesity, diabetes, and cancer [27,214,215]. Therefore, establishing the links between this phenomenon and the impact on the human microbiota will be an essential and exciting step toward identifying new therapeutic targets and preventing or ameliorating the adverse health effects of chronic circadian microbiome-mediated alterations. Furthermore, from a translational viewpoint, the key question to be addressed will be whether the timing of application of microbiota-based therapeutics, such as pre-, pro-, and post-biotics, will affect their efficacy. Based on the emerging evidence of microbial

circadian rhythmicity, it is possible that there will be diurnal variation of the response to these therapeutic approaches. Chronopharmacological studies of microbiota-based therapeutics would pave the way toward the possibility of manipulation of host peripheral clocks. This in turn can provide potential means to correct dysregulation of the host circadian rhythm associated with aging or chronic illnesses, such as metabolic syndrome, through the modification of the diurnally shifting microbiome.

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Conflict of interest

EE is a paid consultant at DayTwo and BiomX. None of the work reviewed here is related to these or any other commercial entity.

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