Review

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

Samuel Philip Nobs[1,†], Timur Tuganbaev[1,†] & Eran Elinav[1,2,*] ID

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.

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.

1 Immunology Department, Weizmann Institute of Science, Rehovot, Israel
2 Cancer-Microbiome Division, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany
*Corresponding author. Tel: +972 (08) 934-4014; E-mails: eran.elinav@weizmann.ac.il; e.elinav@dkfz-heidelberg.de
†These authors contributed equally to this work

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 auto-inflammatory 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
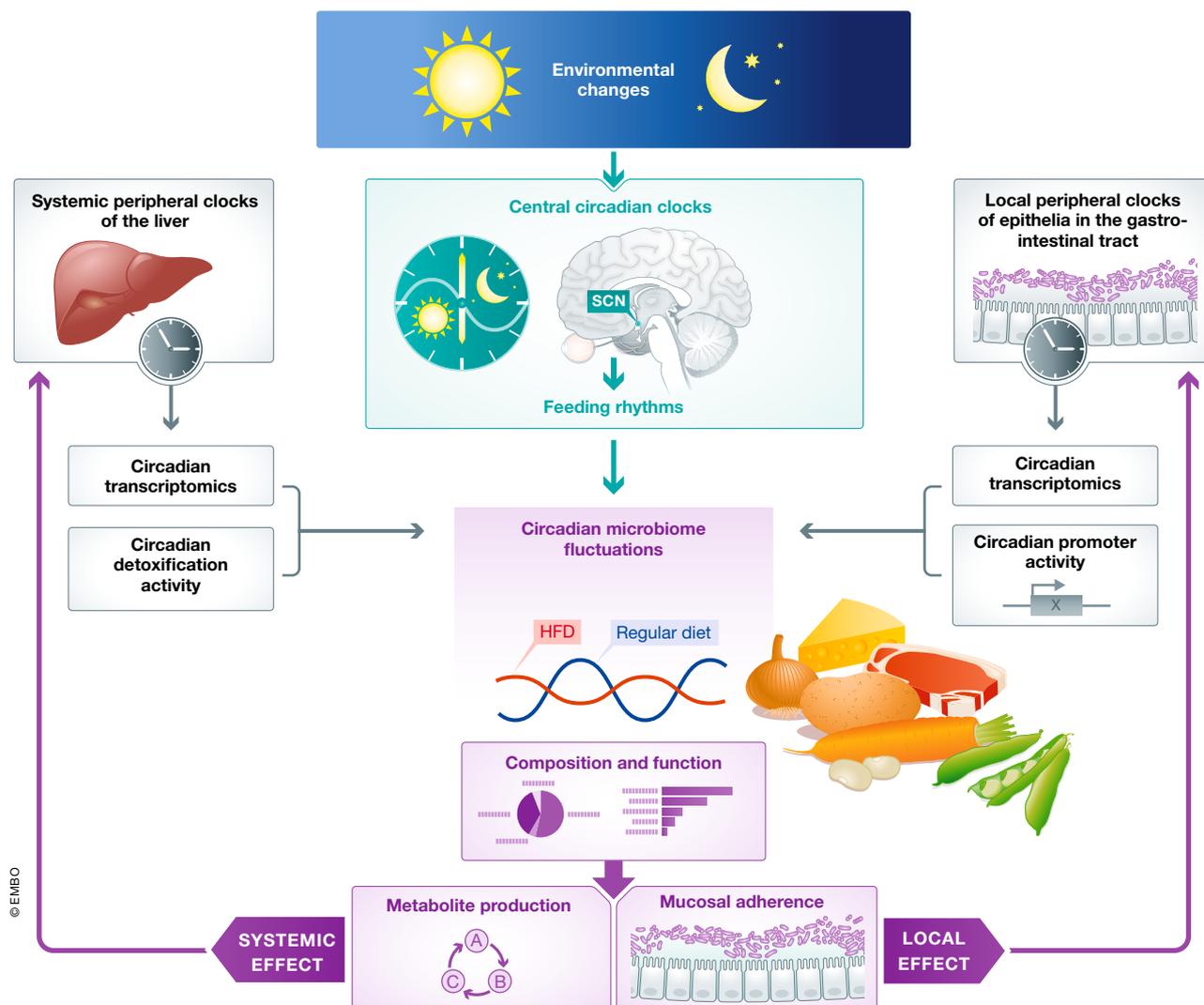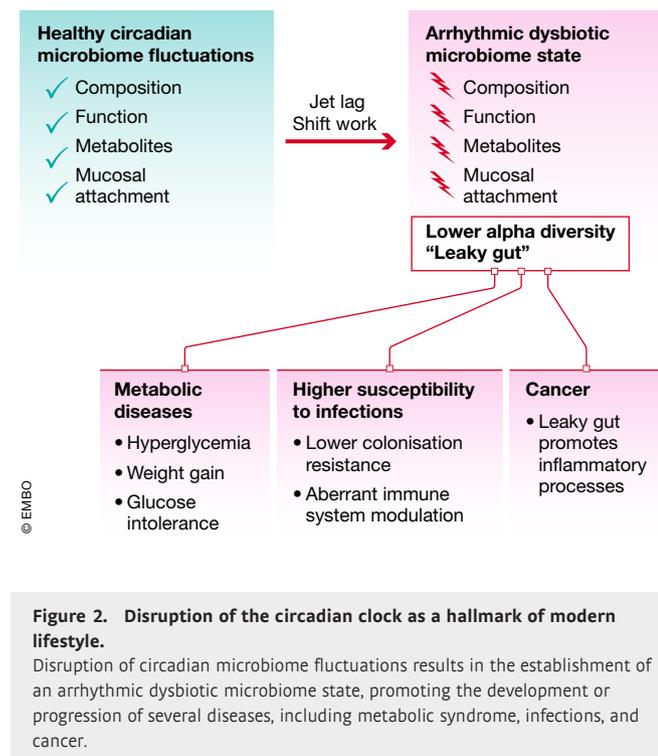
Figure 1. The two-tier hierarchical structure of mammalian circadian clocks.
The gut microbiome undergoes diurnal changes in composition and function that are entrained by feeding rhythms. In turn, microbiome circadian fluctuations of the microbiome modulate circadian activity of peripheral mammalian 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
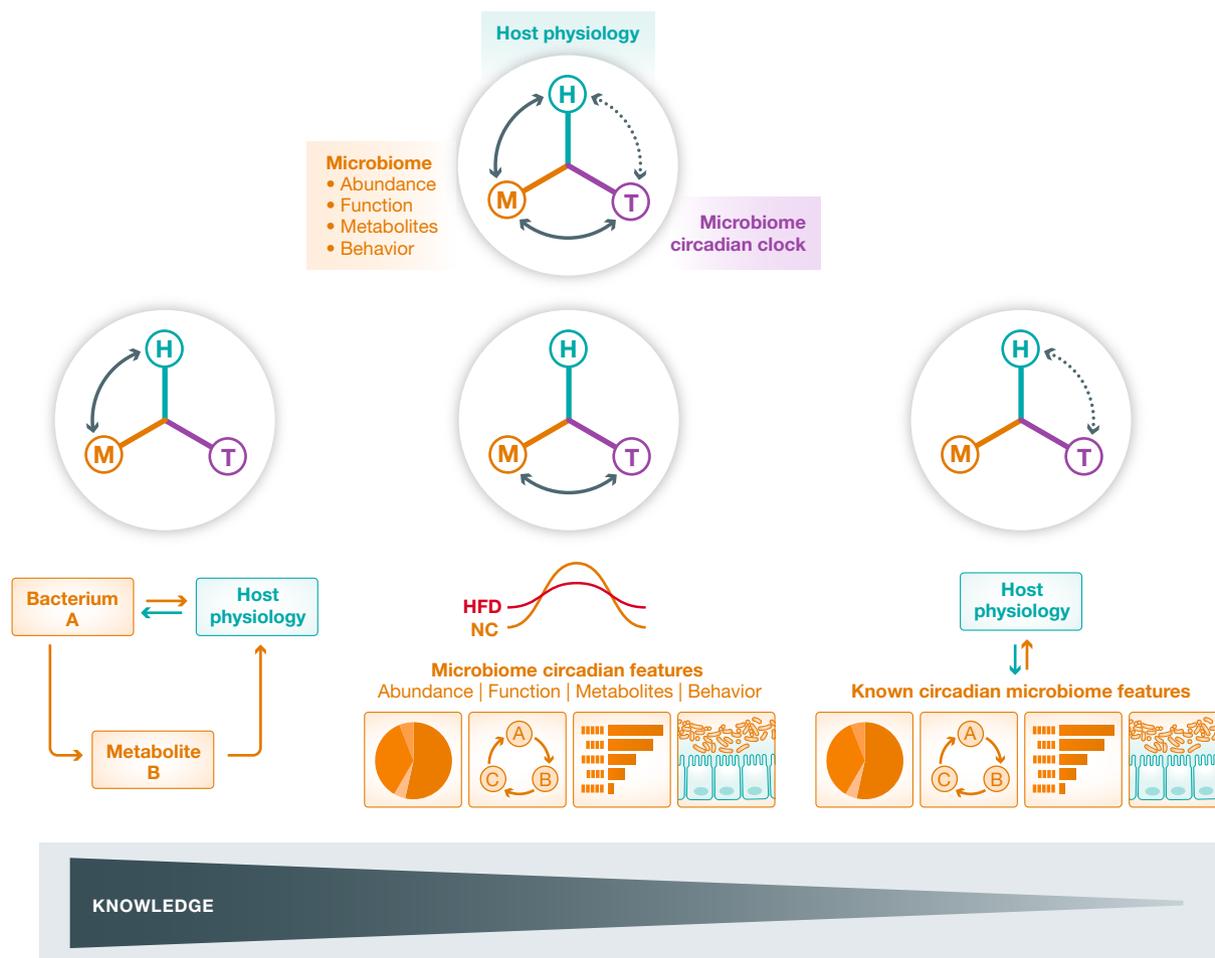
Figure 3. Time constitutes an underappreciated but important dimension of host–microbiome interactions.
A plethora of factors involving bacterial species, bacterial metabolites, and specific bacterial behaviors have been shown to affect host physiology, and at the same time, many of them have been demonstrated 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

<div style="background:#e8f0f7;padding:1em;">

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?

</div>

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, ubiquitinition, 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.

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.

References

1. Farre EM, Liu T (2013) The PRR family of transcriptional regulators reflects the complexity and evolution of plant circadian clocks. *Curr Opin Plant Biol* 16: 621–629

2. Merrow M, Maas MF (2009) Circadian clocks: evolution in the shadows. *Curr Biol* 19: R1042–R1045

3. Millar AJ (2016) The intracellular dynamics of circadian clocks reach for the light of ecology and evolution. *Annu Rev Plant Biol* 67: 595–618

4. Roenneberg T, Merrow M (2002) "What watch?...such much!" Complexity and evolution of circadian clocks. *Cell Tissue Res* 309: 3–9

5. Shindey R, Varma V, Nikhil KL, Sharma VK (2016) Evolution of robust circadian clocks in *Drosophila melanogaster* populations reared in constant dark for over 330 generations. *Naturwissenschaften* 103: 74

6. Brown SA, Kowalska E, Dallmann R (2012) (Re)inventing the circadian feedback loop. *Dev Cell* 22: 477–487

7. Buhr ED, Takahashi JS (2013) Molecular components of the mammalian circadian clock. *Handb Exp Pharmacol* 217: 3–27

8. Fitzgerald GA, Yang G, Paschos GK, Liang X, Skarke C (2015) Molecular clocks and the human condition: approaching their characterization in human physiology and disease. *Diabetes Obes Metab* 17(Suppl 1): 139–142

9. Brandstaetter R (2004) Circadian lessons from peripheral clocks: is the time of the mammalian pacemaker up? *Proc Natl Acad Sci USA* 101: 5699–5700

10. Brown SA, Zumbrunn G, Fleury-Olela F, Preitner N, Schibler U (2002) Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr Biol* 12: 1574–1583

11. Hastings M, Maywood ES (2000) Circadian clocks in the mammalian brain. *BioEssays* 22: 23–31

12. Husse J, Eichele G, Oster H (2015) Synchronization of the mammalian circadian timing system: light can control peripheral clocks independently of the SCN clock: alternate routes of entrainment optimize the alignment of the body's circadian clock network with external time. *BioEssays* 37: 1119–1128

13. Ikonomov OC, Stoynev AG, Shisheva AC (1998) Integrative coordination of circadian mammalian diversity: neuronal networks and peripheral clocks. *Prog Neurobiol* 54: 87–97

14. Saini C, Suter DM, Liani A, Gos P, Schibler U (2011) The mammalian circadian timing system: synchronization of peripheral clocks. *Cold Spring Harb Symp Quant Biol* 76: 39–47

15. Susaki EA, Stelling J, Ueda HR (2010) Challenges in synthetically designing mammalian circadian clocks. *Curr Opin Biotechnol* 21: 556–565

16. Ueda HR (2007) Systems biology of mammalian circadian clocks. *Cold Spring Harb Symp Quant Biol* 72: 365–380

17. Ueda HR, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, Iino M, Hashimoto S (2005) System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet* 37: 187–192

18. Ukai-Tadenuma M, Kasukawa T, Ueda HR (2008) Proof-by-synthesis of the transcriptional logic of mammalian circadian clocks. *Nat Cell Biol* 10: 1154–1163

19. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109: 307–320

20. Paulose JK, Wright JM, Patel AG, Cassone VM (2016) Human gut bacteria are sensitive to melatonin and express endogenous circadian rhythmicity. *PLoS One* 11: e0146643

21. Teng SW, Mukherji S, Moffitt JR, de Buyl S, O'Shea EK (2013) Robust circadian oscillations in growing cyanobacteria require transcriptional feedback. *Science* 340: 737–740

22. O'Neill JS, van Ooijen G, Dixon LE, Troein C, Corellou F, Bouget FY, Reddy AB, Millar AJ (2011) Circadian rhythms persist without transcription in a eukaryote. *Nature* 469: 554–558

23. Johnson CH, Stewart PL, Egli M (2011) The cyanobacterial circadian system: from biophysics to bioevolution. *Annu Rev Biophys* 40: 143–167

24. Johnson CH, Zhao C, Xu Y, Mori T (2017) Timing the day: what makes bacterial clocks tick? *Nat Rev Microbiol* 15: 232–242

25. Golombek DA, Casiraghi LP, Agostino PV, Paladino N, Duhart JM, Plano SA, Chiesa JJ (2013) The times they're a-changing: effects of circadian desynchronization on physiology and disease. *J Physiol Paris* 107: 310–322

26. Bechtold DA, Gibbs JE, Loudon AS (2010) Circadian dysfunction in disease. *Trends Pharmacol Sci* 31: 191–198

27. Archer SN, Laing EE, Moller-Levet CS, van der Veen DR, Bucca G, Lazar AS, Santhi N, Slak A, Kabiljo R, von Schantz M *et al* (2014) Mistimed sleep disrupts circadian regulation of the human transcriptome. *Proc Natl Acad Sci USA* 111: E682–E691

28. Sears CL, Garrett WS (2014) Microbes, microbiota, and colon cancer. *Cell Host Microbe* 15: 317–328

29. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D *et al* (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490: 55−60

30. Karlsson FH, Tremaroli V, Nookaew I, Bergstrom G, Behre CJ, Fagerberg B, Nielsen J, Backhed F (2013) Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498: 99−103

31. Cox LM, Blaser MJ (2013) Pathways in microbe-induced obesity. *Cell Metab* 17: 883−894

32. Voigt RM, Forsyth CB, Green SJ, Mutlu E, Engen P, Vitaterna MH, Turek FW, Keshavarzian A (2014) Circadian disorganization alters intestinal microbiota. *PLoS One* 9: e97500

33. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, Abramson L, Katz MN, Korem T, Zmora N *et al* (2014) Transkingdom control of microbiota diurnal oscillations promotes metabolic home-ostasis. *Cell* 159: 514−529

34. Chaix A, Zarrinpar A, Miu P, Panda S (2014) Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab* 20: 991−1005

35. Zarrinpar A, Chaix A, Yooseph S, Panda S (2014) Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab* 20: 1006−1017

36. Chen X, Devaraj S (2018) Gut microbiome in obesity, metabolic syndrome, and diabetes. *Curr Diab Rep* 18: 129

37. Fava F, Gitau R, Griffin BA, Gibson GR, Tuohy KM, Lovegrove JA (2013) The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. *Int J Obes (Lond)* 37: 216−223

38. Feng X, Uchida Y, Koch L, Britton S, Hu J, Lutrin D, Maze M (2017) Exer-cise prevents enhanced postoperative neuroinflammation and cognitive decline and rectifies the gut microbiome in a rat model of metabolic syndrome. *Front Immunol* 8: 1768

39. Ferrarese R, Ceresola ER, Preti A, Canducci F (2018) Probiotics, prebi-otics and synbiotics for weight loss and metabolic syndrome in the microbiome era. *Eur Rev Med Pharmacol Sci* 22: 7588−7605

40. Kovatcheva-Datchary P, Arora T (2013) Nutrition, the gut microbiome and the metabolic syndrome. *Best Pract Res Clin Gastroenterol* 27: 59−72

41. Mazidi M, Rezaie P, Kengne AP, Mobarhan MG, Ferns GA (2016) Gut microbiome and metabolic syndrome. *Diabetes Metab Syndr* 10: S150−S157

42. Parekh PJ, Balart LA, Johnson DA (2015) The influence of the gut micro-biome on obesity, metabolic syndrome and gastrointestinal disease. *Clin Transl Gastroenterol* 6: e91

43. Shapiro H, Suez J, Elinav E (2017) Personalized microbiome-based approaches to metabolic syndrome management and prevention. *J Diabetes* 9: 226−236

44. Tutkova M, Ruda-Kucerova J (2018) Microbiome in connection with metabolic syndrome and the therapeutic potential of its influencing. *Ceska Slov Farm* 67: 71−80

45. Ussar S, Fujisaka S, Kahn CR (2016) Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Mol Metab* 5: 795−803

46. Human Microbiome Project C (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486: 207−214

47. Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, Brady A, Creasy HH, McCracken C, Giglio MG *et al* (2017) Strains,

functions and dynamics in the expanded Human Microbiome Project. *Nature* 550: 61−66

48. Buford TW (2017) (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. *Microbiome* 5: 80

49. Dinan TG, Cryan JF (2017) The microbiome-gut-brain axis in health and disease. *Gastroenterol Clin North Am* 46: 77−89

50. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK (2011) Under-standing the role of gut microbiome-host metabolic signal disruption in health and disease. *Trends Microbiol* 19: 349−359

51. Ihekweazu FD, Versalovic J (2018) Development of the pediatric gut microbiome: impact on health and disease. *Am J Med Sci* 356: 413−423

52. Liang D, Leung RK, Guan W, Au WW (2018) Involvement of gut micro-biome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathog* 10: 3

53. Shreiner AB, Kao JY, Young VB (2015) The gut microbiome in health and in disease. *Curr Opin Gastroenterol* 31: 69−75

54. Fulde M, Hornef MW (2014) Maturation of the enteric mucosal innate immune system during the postnatal period. *Immunol Rev* 260: 21−34

55. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G (2010) Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature* 464: 908−912

56. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR *et al* (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 341: 1241214

57. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444: 1027−1031

58. Ijssennagger N, Belzer C, Hooiveld GJ, Dekker J, van Mil SW, Muller M, Kleerebezem M, van der Meer R (2015) Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. *Proc Natl Acad Sci USA* 112: 10038−10043

59. Reinhardt C, Bergentall M, Greiner TU, Schaffner F, Ostergren-Lunden G, Petersen LC, Ruf W, Backhed F (2012) Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. *Nature* 483: 627−631

60. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* 161: 264−276

61. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I *et al* (2012) Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 488: 621−626

62. Canfora EE, Jocken JW, Blaak EE (2015) Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 11: 577−591

63. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP *et al* (2012) Human gut microbiome viewed across age and geography. *Nature* 486: 222−227

64. Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R *et al* (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143: 913−916 e917

65. Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK (2009) Pharma-cometabonomic identification of a significant host-microbiome

metabolic interaction affecting human drug metabolism. *Proc Natl Acad Sci USA* 106: 14728–14733

66. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI *et al* (2011) NLRP6 inflamma-some regulates colonic microbial ecology and risk for colitis. *Cell* 145: 745–757

67. Rohlke F, Stollman N (2012) Fecal microbiota transplantation in relapsing Clostridium difficile infection. *Therap Adv Gastroenterol* 5: 403–420

68. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF *et al* (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155: 1451–1463

69. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. *PLoS Biol* 5: e177

70. Schwartz S, Friedberg I, Ivanov IV, Davidson LA, Goldsby JS, Dahl DB, Herman D, Wang M, Donovan SM, Chapkin RS (2012) A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. *Genome Biol* 13: r32

71. Scholtens PA, Oozeer R, Martin R, Amor KB, Knol J (2012) The early settlers: intestinal microbiology in early life. *Annu Rev Food Sci Technol* 3: 425–447

72. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL *et al* (2013) The long-term stability of the human gut microbiota. *Science* 341: 1237439

73. Mariat D, Firmesse O, Levenez F, Guimaraes V, Sokol H, Dore J, Corthier G, Furet JP (2009) The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* 9: 123

74. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R *et al* (2011) Linking long-term dietary patterns with gut microbial enterotypes. *Science* 334: 105–108

75. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA *et al* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505: 559–563

76. Suez J, Zmora N, Zilberman-Schapira G, Mor U, Dori-Bachash M, Bashiardes S, Zur M, Regev-Lehavi D, Ben-Zeev Brik R, Federici S *et al* (2018) Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. *Cell* 174: 1406–1423 e1416

77. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB *et al* (2018) Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. *Cell* 174: 1388–1405 e1321

78. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* 444: 1022–1023

79. Thaiss CA, Levy M, Korem T, Dohnalova L, Shapiro H, Jaitin DA, David E, Winter DR, Gury-BenAri M, Tatirovsky E *et al* (2016) Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* 167: 1495–1510 e1412

80. Thaiss CA, Zeevi D, Levy M, Segal E, Elinav E (2015) A day in the life of the meta-organism: diurnal rhythms of the intestinal microbiome and its host. *Gut Microbes* 6: 137–142

81. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U (2008) SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134: 317–328

82. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P (2008) The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134: 329–340

83. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science* 324: 654–657

84. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C *et al* (2009) Circadian clock feed-back cycle through NAMPT-mediated NAD+ biosynthesis. *Science* 324: 651–654

85. Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S (2009) Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci USA* 106: 21453–21458

86. Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, Baggs JE, Panda S, Hogenesch JB (2009) Harmonics of circadian gene transcription in mammals. *PLoS Genet* 5: e1000442

87. Wu G, Tang W, He Y, Hu J, Gong S, He Z, Wei G, Lv L, Jiang Y, Zhou H *et al* (2018) Light exposure influences the diurnal oscillation of gut microbiota in mice. *Biochem Biophys Res Commun* 501: 16–23

88. Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J (2007) High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* 6: 414–421

89. Eckel-Mahan KL, Patel VR, de Mateo S, Orozco-Solis R, Ceglia NJ, Sahar S, Dilag-Penilla SA, Dyar KA, Baldi P, Sassone-Corsi P (2013) Reprogramming of the circadian clock by nutritional challenge. *Cell* 155: 1464–1478

90. Ramanan D, Cadwell K (2016) Intrinsic defense mechanisms of the intestinal epithelium. *Cell Host Microbe* 19: 434–441

91. Tahara Y, Yamazaki M, Sukigara H, Motohashi H, Sasaki H, Miyakawa H, Haraguchi A, Ikeda Y, Fukuda S, Shibata S (2018) Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue. *Sci Rep* 8: 1395

92. Yamada K, Gherasim C, Banerjee R, Koutmos M (2015) Structure of human B12 trafficking protein CblD reveals molecular mimicry and identifies a new subfamily of nitro-FMN reductases. *J Biol Chem* 290: 29155–29166

93. Kellermayer R, Dowd SE, Harris RA, Balasa A, Schaible TD, Wolcott RD, Tatevian N, Szigeti R, Li Z, Versalovic J *et al* (2011) Colonic mucosal DNA methylation, immune response, and microbiome patterns in Toll-like receptor 2-knockout mice. *FASEB J* 25: 1449–1460

94. Paul B, Barnes S, Demark-Wahnefried W, Morrow C, Salvador C, Skibola C, Tollefsbol TO (2015) Influences of diet and the gut microbiome on epigenetic modulation in cancer and other diseases. *Clin Epigenetics* 7: 112

95. Ciarlo E, Heinonen T, Herderschee J, Fenwick C, Mombelli M, Le Roy D, Roger T (2016) Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections *in vivo*. *Sci Rep* 6: 37944

96. Remely M, Aumueller E, Merold C, Dworzak S, Hippe B, Zanner J, Pointner A, Brath H, Haslberger AG (2014) Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. *Gene* 537: 85–92

97. Mullineaux-Sanders C, Suez J, Elinav E, Frankel G (2018) Sieving through gut models of colonization resistance. *Nat Microbiol* 3: 132–140

98. Curtis AM, Bellet MM, Sassone-Corsi P, O'Neill LA (2014) Circadian clock proteins and immunity. *Immunity* 40: 178–186

99. Ehlers A, Xie W, Agapov E, Brown S, Steinberg D, Tidwell R, Sajol G, Schutz R, Weaver R, Yu H *et al* (2018) BMAL1 links the circadian clock

to viral airway pathology and asthma phenotypes. *Mucosal Immunol* 11: 97–111

100. Gibbs J, Ince L, Matthews L, Mei J, Bell T, Yang N, Saer B, Begley N, Poolman T, Pariollaud M *et al* (2014) An epithelial circadian clock controls pulmonary inflammation and glucocorticoid action. *Nat Med* 20: 919–926

101. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A (2013) Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. *Science* 341: 1483–1488

102. Jacobson A, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K *et al* (2018) A gut commensal-produced metabolite mediates colonization resistance to salmonella infection. *Cell Host Microbe* 24: 296–307.e7

103. Steed AL, Christophi GP, Kaiko GE, Sun L, Goodwin VM, Jain U, Esaulova E, Artyomov MN, Morales DJ, Holtzman MJ *et al* (2017) The microbial metabolite desaminotyrosine protects from influenza through type I interferon. *Science* 357: 498–502

104. Thaiss CA, Zmora N, Levy M, Elinav E (2016) The microbiome and innate immunity. *Nature* 535: 65–74

105. Levy M, Thaiss CA, Zeevi D, Dohnalova L, Zilberman-Schapira G, Mahdi JA, David E, Savidor A, Korem T, Herzig Y *et al* (2015) Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* 163: 1428–1443

106. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, Merad M (2014) Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 343: 1249288

107. Hepworth MR, Monticelli LA, Fung TC, Ziegler CG, Grunberg S, Sinha R, Mantegazza AR, Ma HL, Crawford A, Angelosanto JM *et al* (2013) Innate lymphoid cells regulate CD4$^+$ T-cell responses to intestinal commensal bacteria. *Nature* 498: 113–117

108. Oh JZ, Ravindran R, Chassaing B, Carvalho FA, Maddur MS, Bower M, Hakimpour P, Gill KP, Nakaya HI, Yarovinsky F *et al* (2014) TLR5-mediated sensing of gut microbiota is necessary for antibody responses to seasonal influenza vaccination. *Immunity* 41: 478–492

109. Khosravi A, Yanez A, Price JG, Chow A, Merad M, Goodridge HS, Mazmanian SK (2014) Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host Microbe* 15: 374–381

110. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A *et al* (2015) Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature* 517: 205–208

111. Tognini P, Murakami M, Sassone-Corsi P (2017) Interplay between microbes and the circadian clock. *Cold Spring Harb Perspect Biol* 10: a028365

112. Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J, Ubags N, Fajas L, Nicod LP, Marsland BJ (2018) Dietary fiber confers protection against flu by shaping Ly6c(−) patrolling monocyte hematopoiesis and CD8(+) T cell metabolism. *Immunity* 48: 992–1005 e1008

113. Ando H, Kumazaki M, Motosugi Y, Ushijima K, Maekawa T, Ishikawa E, Fujimura A (2011) Impairment of peripheral circadian clocks precedes metabolic abnormalities in ob/ob mice. *Endocrinology* 152: 1347–1354

114. Barandas R, Landgraf D, McCarthy MJ, Welsh DK (2015) Circadian clocks as modulators of metabolic comorbidity in psychiatric disorders. *Curr Psychiatry Rep* 17: 98

115. Challet E (2013) Circadian clocks, food intake, and metabolism. *Prog Mol Biol Transl Sci* 119: 105–135

116. Coomans CP, Lucassen EA, Kooijman S, Fifel K, Deboer T, Rensen PC, Michel S, Meijer JH (2015) Plasticity of circadian clocks and consequences for metabolism. *Diabetes Obes Metab* 17(Suppl 1): 65–75

117. Delezie J, Challet E (2011) Interactions between metabolism and circadian clocks: reciprocal disturbances. *Ann N Y Acad Sci* 1243: 30–46

118. Gimble JM, Sutton GM, Bunnell BA, Ptitsyn AA, Floyd ZE (2011) Prospective influences of circadian clocks in adipose tissue and metabolism. *Nat Rev Endocrinol* 7: 98–107

119. Paschos GK (2015) Circadian clocks, feeding time, and metabolic homeostasis. *Front Pharmacol* 6: 112

120. Scott EM (2015) Circadian clocks, obesity and cardiometabolic function. *Diabetes Obes Metab* 17(Suppl 1): 84–89

121. He B, Chen Z (2016) Molecular targets for small-molecule modulators of circadian clocks. *Curr Drug Metab* 17: 503–512

122. Mukherji A, Kobiita A, Damara M, Misra N, Meziane H, Champy MF, Chambon P (2015) Shifting eating to the circadian rest phase misaligns the peripheral clocks with the master SCN clock and leads to a metabolic syndrome. *Proc Natl Acad Sci USA* 112: E6691–E6698

123. Sancar G, Brunner M (2014) Circadian clocks and energy metabolism. *Cell Mol Life Sci* 71: 2667–2680

124. Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, Wu X, Goh BC, Mynatt RL, Gimble JM (2006) Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 55: 962–970

125. Ando H (2013) [Circadian clocks and lifestyle-related diseases]. *Rinsho Byori* 61: 1044–1050

126. Berenbaum F, Meng QJ (2016) The brain-joint axis in osteoarthritis: nerves, circadian clocks and beyond. *Nat Rev Rheumatol* 12: 508–516

127. Challet E (2014) Circadian clocks and energy metabolism in rodents. *Biol Aujourdhui* 208: 269–274

128. Heden TD, Kanaley JA (2018) Syncing exercise with meals and circadian clocks. *Exerc Sport Sci Rev* 47: 22–28

129. Leone V, Gibbons SM, Martinez K, Hutchison AL, Huang EY, Cham CM, Pierre JF, Heneghan AF, Nadimpalli A, Hubert N *et al* (2015) Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* 17: 681–689

130. Jiang P, Turek FW (2018) The endogenous circadian clock programs animals to eat at certain times of the 24-hour day: what if we ignore the clock? *Physiol Behav* 193: 211–217

131. de Goede P, Sen S, Su Y, Foppen E, Poirel VJ, Challet E, Kalsbeek A (2018) An ultradian feeding schedule in rats affects metabolic gene expression in liver, brown adipose tissue and skeletal muscle with only mild effects on circadian clocks. *Int J Mol Sci* 19: E3171

132. Gatfield D, Schibler U (2008) Circadian glucose homeostasis requires compensatory interference between brain and liver clocks. *Proc Natl Acad Sci USA* 105: 14753–14754

133. Radziuk JM (2013) The suprachiasmatic nucleus, circadian clocks, and the liver. *Diabetes* 62: 1017–1019

134. Saini C, Liani A, Curie T, Gos P, Kreppel F, Emmenegger Y, Bonacina L, Wolf JP, Poget YA, Franken P *et al* (2013) Real-time recording of circadian liver gene expression in freely moving mice reveals the phase-setting behavior of hepatocyte clocks. *Genes Dev* 27: 1526–1536

135. Schibler U (2003) Circadian rhythms. Liver regeneration clocks on. *Science* 302: 234–235

136. Sosniyenko S, Parkanova D, Illnerova H, Sladek M, Sumova A (2010) Different mechanisms of adjustment to a change of the photoperiod in

the suprachiasmatic and liver circadian clocks. *Am J Physiol Regul Integr Comp Physiol* 298: R959 – R971

137. Tognini P, Murakami M, Liu Y, Eckel-Mahan KL, Newman JC, Verdin E, Baldi P, Sassone-Corsi P (2017) Distinct circadian signatures in liver and gut clocks revealed by ketogenic diet. *Cell Metab* 26: 523 – 538 e525

138. Lin EA, Barlow GM, Mathur R (2014) The microbiome in non-alcoholic fatty liver disease: associations and implications. *Ann Gastroenterol* 27: 181 – 183

139. Nobili V, Putignani L, Mosca A, Chierico FD, Vernocchi P, Alisi A, Stronati L, Cucchiara S, Toscano M, Drago L (2018) Bifidobacteria and lactobacilli in the gut microbiome of children with non-alcoholic fatty liver disease: which strains act as health players? *Arch Med Sci* 14: 81 – 87

140. Saltzman ET, Palacios T, Thomsen M, Vitetta L (2018) Intestinal microbiome shifts, dysbiosis, inflammation, and non-alcoholic fatty liver disease. *Front Microbiol* 9: 61

141. Aqel B, DiBaise JK (2015) Role of the gut microbiome in nonalcoholic fatty liver disease. *Nutr Clin Pract* 30: 780 – 786

142. Bischoff SC (2017) The intestinal microbiome and metabolic diseases: from obesity to diabetes and nonalcoholic steatohepatitis. *Internist (Berl)* 58: 441 – 448

143. Boursier J, Diehl AM (2016) Nonalcoholic fatty liver disease and the gut microbiome. *Clin Liver Dis* 20: 263 – 275

144. Hara E (2015) Relationship between obesity, gut microbiome and hepatocellular carcinoma development. *Dig Dis* 33: 346 – 350

145. Leung DH, Yimlamai D (2017) The intestinal microbiome and paediatric liver disease. *Lancet Gastroenterol Hepatol* 2: 446 – 455

146. Neuman MG, French SW, Zakhari S, Malnick S, Seitz HK, Cohen LB, Salaspuro M, Voinea-Griffin A, Barasch A, Kirpich IA *et al* (2017) Alcohol, microbiome, life style influence alcohol and non-alcoholic organ damage. *Exp Mol Pathol* 102: 162 – 180

147. Panasevich MR, Peppler WT, Oerther DB, Wright DC, Rector RS (2017) Microbiome and NAFLD: potential influence of aerobic fitness and lifestyle modification. *Physiol Genomics* 49: 385 – 399

148. Puri P, Sanyal AJ (2018) The intestinal microbiome in nonalcoholic fatty liver disease. *Clin Liver Dis* 22: 121 – 132

149. Schnabl B, Brenner DA (2014) Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 146: 1513 – 1524

150. Wankhade UD, Zhong Y, Kang P, Alfaro M, Chintapalli SV, Thakali KM, Shankar K (2017) Enhanced offspring predisposition to steatohepatitis with maternal high-fat diet is associated with epigenetic and microbiome alterations. *PLoS One* 12: e0175675

151. Woodhouse CA, Patel VC, Singanayagam A, Shawcross DL (2018) Review article: the gut microbiome as a therapeutic target in the pathogenesis and treatment of chronic liver disease. *Aliment Pharmacol Ther* 47: 192 – 202

152. Zhu L, Baker RD, Baker SS (2015) Gut microbiome and nonalcoholic fatty liver diseases. *Pediatr Res* 77: 245 – 251

153. Adolph TE, Effenberger M, Tilg H (2018) How our understanding of the microbiome has changed the way we look at liver diseases. *Biomark Med* 12: 1203 – 1206

154. Adolph TE, Grander C, Moschen AR, Tilg H (2018) Liver-microbiome axis in health and disease. *Trends Immunol* 39: 712 – 723

155. Aitbaev KA, Murkamilov IT, Fomin VV (2017) Liver diseases: the pathogenetic role of the gut microbiome and the potential of treatment for its modulation. *Ter Arkh* 89: 120 – 128

156. Capurso G, Lahner E (2017) The interaction between smoking, alcohol and the gut microbiome. *Best Pract Res Clin Gastroenterol* 31: 579 – 588

157. Castano-Rodriguez N, Mitchell HM, Kaakoush NO (2017) NAFLD, *Helicobacter* species and the intestinal microbiome. *Best Pract Res Clin Gastroenterol* 31: 657 – 668

158. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK *et al* (2017) Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab* 25: 1054 – 1062 e1055

159. Maruvada P, Leone V, Kaplan LM, Chang EB (2017) The human microbiome and obesity: moving beyond associations. *Cell Host Microbe* 22: 589 – 599

160. Omer E, Atassi H (2017) The microbiome that shapes us: can it cause obesity? *Curr Gastroenterol Rep* 19: 59

161. Puri P, Liangpunsakul S, Christensen JE, Shah VH, Kamath PS, Gores GJ, Walker S, Comerford M, Katz B, Borst A *et al* (2018) The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. *Hepatology* 67: 1284 – 1302

162. Szabo G (2018) Gut-liver axis beyond the microbiome: how the fungal mycobiome contributes to alcoholic liver disease. *Hepatology* 68: 2426 – 2428

163. Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R (2018) Publisher correction: the gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 15: 785

164. Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R (2018) The gut-liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 15: 397 – 411

165. Fukui H (2017) Gut microbiome-based therapeutics in liver cirrhosis: basic consideration for the next step. *J Clin Transl Hepatol* 5: 249 – 260

166. Jacobs JP, Dong TS, Agopian V, Lagishetty V, Sundaram V, Noureddin M, Ayoub WS, Durazo F, Benhammou J, Enayati P *et al* (2018) Microbiome and bile acid profiles in duodenal aspirates from patients with liver cirrhosis: the microbiome, microbial markers and liver disease study. *Hepatol Res* 48: 1108 – 1117

167. Lu H, Qian G, Ren Z, Zhang C, Zhang H, Xu W, Ye P, Yang Y, Li L (2015) Alterations of *Bacteroides* sp., *Neisseria* sp., *Actinomyces* sp., and *Streptococcus* sp. populations in the oropharyngeal microbiome are associated with liver cirrhosis and pneumonia. *BMC Infect Dis* 15: 239

168. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L *et al* (2014) Alterations of the human gut microbiome in liver cirrhosis. *Nature* 513: 59 – 64

169. Roderburg C, Luedde T (2014) The role of the gut microbiome in the development and progression of liver cirrhosis and hepatocellular carcinoma. *Gut Microbes* 5: 441 – 445

170. Schulz C, Schutte K, Kropf S, Schmitt FC, Vasapolli R, Kliegis LM, Riegger A, Malfertheiner P (2016) RiMINI – the influence of rifaximin on minimal hepatic encephalopathy (MHE) and on the intestinal microbiome in patients with liver cirrhosis: study protocol for a randomized controlled trial. *Trials* 17: 111

171. Murakami M, Tognini P, Liu Y, Eckel-Mahan KL, Baldi P, Sassone-Corsi P (2016) Gut microbiota directs PPARgamma-driven reprogramming of the liver circadian clock by nutritional challenge. *EMBO Rep* 17: 1292 – 1303

172. Montagner A, Korecka A, Polizzi A, Lippi Y, Blum Y, Canlet C, Tremblay-Franco M, Gautier-Stein A, Burcelin R, Yen YC *et al* (2016) Hepatic circadian clock oscillators and nuclear receptors integrate microbiome-derived signals. *Sci Rep* 6: 20127

173. Weger BD, Gobet C, Yeung J, Martin E, Jimenez S, Betrisey B, Foata F, Berger B, Balvay A, Foussier A *et al* (2018) The mouse microbiome is

required for sex-specific diurnal rhythms of gene expression and metabolism. *Cell Metab* 29: 362–382.e8

174. Wang X, Ji A, Zhu Y, Liang Z, Wu J, Li SQ, Meng S, Zheng XY, Xie LP (2015) A meta-analysis including dose-response relationship between night shift work and the risk of colorectal cancer. *Oncotarget* 6: 25046–25060

175. Papagiannakopoulos T, Bauer MR, Davidson SM, Heimann M, Subbaraj L, Bhutkar A, Bartlebaugh J, Vander Heiden MG, Jacks T (2016) Circadian rhythm disruption promotes lung tumorigenesis. *Cell Metab* 24: 324–331

176. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP *et al* (2018) Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 359: 91–97

177. Cui M, Xiao H, Luo D, Zhang X, Zhao S, Zheng Q, Li Y, Zhao Y, Dong J, Li H *et al* (2016) Circadian rhythm shapes the gut microbiota affecting host radiosensitivity. *Int J Mol Sci* 17: E1786

178. Summa KC, Voigt RM, Forsyth CB, Shaikh M, Cavanaugh K, Tang Y, Vitaterna MH, Song S, Turek FW, Keshavarzian A (2013) Disruption of the circadian clock in mice increases intestinal permeability and promotes alcohol-induced hepatic pathology and inflammation. *PLoS One* 8: e67102

179. Bashiardes S, Tuganbaev T, Federici S, Elinav E (2017) The microbiome in anti-cancer therapy. *Semin Immunol* 32: 74–81

180. Biancheri P, Divekar D, Watson AJM (2018) Could fecal transplantation become part of PD-1-based immunotherapy, due to effects of the intestinal microbiome? *Gastroenterology* 154: 1845–1847

181. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, Prieto PA, Vicente D, Hoffman K, Wei SC *et al* (2018) Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359: 97–103

182. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, Luke JJ, Gajewski TF (2018) The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* 359: 104–108

183. Santoni M, Piva F, Conti A, Santoni A, Cimadamore A, Scarpelli M, Battelli N, Montironi R (2018) Re: Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Eur Urol* 74: 521–522

184. Vetizou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP *et al* (2015) Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* 350: 1079–1084

185. Candela M, Biagi E, Brigidi P, O'Toole PW, De Vos WM (2014) Maintenance of a healthy trajectory of the intestinal microbiome during aging: a dietary approach. *Mech Ageing Dev* 136–137: 70–75

186. Chotirmall SH, Burke CM (2015) Aging and the microbiome: implications for asthma in the elderly? *Expert Rev Respir Med* 9: 125–128

187. Conley MN, Wong CP, Duyck KM, Hord N, Ho E, Sharpton TJ (2016) Aging and serum MCP-1 are associated with gut microbiome composition in a murine model. *PeerJ* 4: e1854

188. Desai SN, Landay AL (2018) HIV and aging: role of the microbiome. *Curr Opin HIV AIDS* 13: 22–27

189. Ebersole JL, Kirakodu SS, Novak MJ, Orraca L, Martinez JG, Cunningham LL, Thomas MV, Stromberg A, Pandruvada SN, Gonzalez OA (2016) Transcriptome analysis of B cell immune functions in periodontitis: mucosal tissue responses to the oral microbiome in aging. *Front Immunol* 7: 272

190. Fischer N, Relman DA (2018) Clostridium difficile, aging, and the gut: can microbiome rejuvenation keep us young and healthy? *J Infect Dis* 217: 174–176

191. Heintz C, Mair W (2014) You are what you host: microbiome modulation of the aging process. *Cell* 156: 408–411

192. Hughes GM, Leech J, Puechmaille SJ, Lopez JV, Teeling EC (2018) Is there a link between aging and microbiome diversity in exceptional mammalian longevity? *PeerJ* 6: e4174

193. Kiecolt-Glaser JK, Wilson SJ, Madison A (2018) Marriage and gut (microbiome) feelings: tracing novel dyadic pathways to accelerated aging. *Psychosom Med* https://doi.org/10.1097/PSY.0000000000000647

194. Kong F, Deng F, Li Y, Zhao J (2018) Identification of gut microbiome signatures associated with longevity provides a promising modulation target for healthy aging. *Gut Microbes* https://doi.org/10.1080/19490976.2018.1494102

195. Lamming DW (2014) Hot topics at the intersection of aging and energetics: diabetes/insulin resistance, sirtuins, and the microbiome. *F1000Res* 3: 257

196. Lewy T, Hong BY, Weiser B, Burger H, Tremain A, Weinstock G, Anastos K, George MD (2018) Oral microbiome in HIV-infected women: shifts in the abundance of pathogenic and beneficial bacteria are associated with aging, HIV load, CD4 count, and antiretroviral therapy. *AIDS Res Hum Retroviruses* https://doi.org/10.1089/AID.2017.0200

197. Maldonado-Contreras A, Gerstein RM (2017) Editorial: no Trp, no B: surprising connectivity of diet, microbiome, aging, and adaptive immunity. *J Leukoc Biol* 101: 807–809

198. Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, Kitzman DW, Kushugulova A, Marotta F, Yadav H (2018) Gut microbiome and aging: physiological and mechanistic insights. *Nutr Healthy Aging* 4: 267–285

199. Ni Lochlainn M, Bowyer RCE, Steves CJ (2018) Dietary protein and muscle in aging people: the potential role of the gut microbiome. *Nutrients* 10: E929

200. Paschos GK, FitzGerald GA (2017) Circadian clocks and metabolism: implications for microbiome and aging. *Trends Genet* 33: 760–769

201. Shoemark DK, Allen SJ (2015) The microbiome and disease: reviewing the links between the oral microbiome, aging, and Alzheimer's disease. *J Alzheimers Dis* 43: 725–738

202. Steves CJ, Bird S, Williams FM, Spector TD (2016) The microbiome and musculoskeletal conditions of aging: a review of evidence for impact and potential therapeutics. *J Bone Miner Res* 31: 261–269

203. Xiao Y, Dong J, Yin Z, Wu Q, Zhou Y, Zhou X (2018) Procyanidin B2 protects against d-galactose-induced mimetic aging in mice: metabolites and microbiome analysis. *Food Chem Toxicol* 119: 141–149

204. Zapata HJ, Quagliarello VJ (2015) The microbiota and microbiome in aging: potential implications in health and age-related diseases. *J Am Geriatr Soc* 63: 776–781

205. Matsumoto M, Kurihara S, Kibe R, Ashida H, Benno Y (2011) Longevity in mice is promoted by probiotic-induced suppression of colonic senescence dependent on upregulation of gut bacterial polyamine production. *PLoS One* 6: e23652

206. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, Consolandi C, Quercia S, Scurti M, Monti D *et al* (2016) Gut microbiota and extreme longevity. *Curr Biol* 26: 1480–1485

207. O'Toole PW, Jeffery IB (2015) Gut microbiota and aging. *Science* 350: 1214–1215

208. Claesson MJ, Cusack S, O'Sullivan O, Greene-Diniz R, de Weerd H, Flannery E, Marchesi JR, Falush D, Dinan T, Fitzgerald G *et al* (2011)

Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci USA* 108(Suppl 1): 4586−4591

209. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE *et al* (2017) Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci USA* 114: 10719−10724

210. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL *et al* (2017) Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA* 114: 10713−10718

211. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V *et al* (2016) Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 167: 1469−1480 e1412

212. Wang HB, Loh DH, Whittaker DS, Cutler T, Howland D, Colwell CS (2018) Time-restricted feeding improves circadian dysfunction as well as motor symptoms in the Q175 mouse model of Huntington's disease. *eNeuro* 5: 1−16

213. Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 28: 203−209

214. Buxton OM, Cain SW, O'Connor SP, Porter JH, Duffy JF, Wang W, Czeisler CA, Shea SA (2012) Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci Transl Med* 4: 129ra143

215. Suwazono Y, Dochi M, Sakata K, Okubo Y, Oishi M, Tanaka K, Kobayashi E, Kido T, Nogawa K (2008) A longitudinal study on the effect of shift work on weight gain in male Japanese workers. *Obesity (Silver Spring)* 16: 1887−1893