Bacteria Need "Sleep" Too?: Microbiome Circadian Rhythmicity, Metabolic Disease, and Beyond

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Abstract

Humans contain an "organ" composed of two to six pounds of bacteria, primarily residing in the gastrointestinal tract, which has gone largely unappreciated until recently. The gastrointestinal microbiome is a dynamic "organ" that performs important physiological functions for its host and can also be a factor in disease. Currently, animal models of germ-free mice, zebrafish, and fruit flies are utilized to address cause and effect relationships between resident microbes and their host. Like almost all living organisms, the gut microbiota regulate many of their changes in cellular activity and behavior over 24-hour cycles known as the circadian clock or rhythm. The microbiome has been shown to rhythmically fluctuate in both community composition and gene expression in a circadian-dependent manor with respect to host feeding schedules. Disruption of the microbiome circadian rhythm is associated with metabolic disease in mice. Furthermore, host immune cells have been shown to respond to the resident microbiota in a circadian-fashion. These findings provide insight to a microbiome feature that may be important for early diagnosis and therapeutic intervention in the management of metabolic diseases and potentially many others, as well as how frequent flyers cope with changes in sleep patterns. The microbiome circadian rhythm is a largely underexplored field that will likely have profound implications to both the understanding of how bacterial symbiosis affects human physiology and how manipulation of the microbiome may be translated into therapeutic treatment or diagnostic for human disease(s).

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Introduction

The human body is initially colonized during traditional birth by microorganisms from the mother's genital tract.[1-3] Subsequent colonization and dynamic changes result due to the newborn's exposure to breast milk and environmental microbes.[2, 3] The diverse bacterial communities of young children typically mature to resemble the distinct and stable profiles of an adult by one to two years of age.[2, 3] Microbes most abundantly colonize the human gastrointestinal tract. The adult gastrointestinal tract has been estimated to contain ten-fold more bacteria than total human cells (depending on host body mass) by absolute count[4] and encodes 100-fold more gene functions than its human host.[5] Thus, it is not surprising that these bacterial communities1, known as the microbiota, play important roles in shaping their host's physiology. However, until the recent decreased costs and increased throughput of nucleotide sequencing, comprehensive investigations of the gastrointestinal microbiota were not possible. Today, the interest and tools to explore the human microbiome (microbiota and gene functions) have become sufficient to investigate many of the fundamental questions regarding the importance of these host-microbe interactions to human health.

Animal Models for Understanding Complex Host-Microbe Interactions

Vertebrates, insects, fungi, and bacteria all possess circadian clocks to temporally regulate behavior and physiology in response to environmental signals.[6] Circadian rhythms in gene expression and behavior are evolutionarily set to fluctuate cyclically over a 24-hour period. The importance of circadian rhythms can be appreciated from the fact that multi-tissue analysis has shown that expression of more than one-third of the mammalian genome is dependent on the circadian clock.[7]

The complexity of circadian rhythms and host-microbe interactions make questions in these fields of studies particularly challenging to answer in humans. However, animal models may provide valuable insights into these processes for

the time being. For instance, the squid known as *Euprymna scolopes*, has a monotonous relationship with the bacterium, *Vibrio fischerri*. *Vibrio fischerri* enables the squid to cyclically regulate its bioluminescence with circadian rhythmicity.[8] *Vibrio fischerri* has also been shown to regulate the expression of the squid's host circadian clock machinery.[8] Thus, this well-characterized and relatively "simple" relationship could be a valuable tool for better understanding the role that certain bacterial symbiotic species play in the modulation of mammalian circadian rhythmicity. However, the importance of interspecies bacterial-host interactions in shaping mammalian physiology within the gastrointestinal tract still must be evaluated. The power of colonizing germ-free animals such as fruit flies, zebrafish, or rodents with whole or selective human microbiota samples still remain important study tools. The squid-*V. fischerri* mutualistic relationship and recent murine experimental findings[9-12] provide evidence that the crosstalk between host and microbial circadian rhythmicity are integral to host physiological homeostasis. The need for investigation and potential translation of these findings to humans still remains.

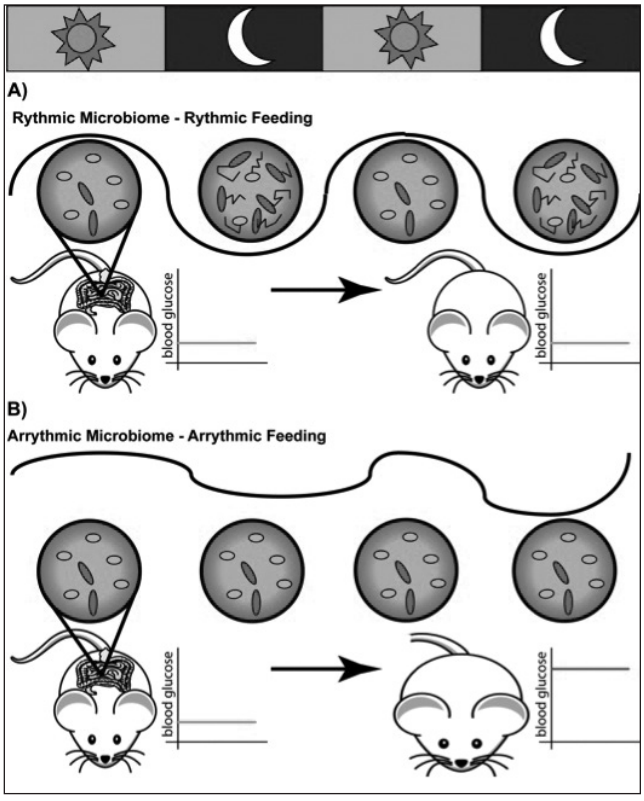

Figure 1. (A) Typically the "healthy" microbiome has been shown to undergo circadian fluctuations in bacterial community abundance and gene expression that are dependent on diet schedule. (B) Dysbiosis in the microbiome circadian rhythm by insults such as arrhythmic feeding leads to metabolic diseases such as diabetes and obesity in mouse models.

In the case of squid-*V. fischerri* mutualism, the notable microbe-host circadian interaction is host light-responsive circadian machinery changes due to bacterial bioluminescence.[8] For mammalian species, the mechanisms for host-microbe circadian clock interactions could be more complex and numerous. Firstly, expression of Toll-like receptors, which are important for transducing host cellular signals in response to microbe-associated molecular patterns, have been shown to vary with circadian rhythmicity in intestinal epithelial cells[13] and surrounding tissues.[14] Rhythmic Toll-like receptor signaling was associated with circadian machinery regulation via the activator protein-1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) inflammatory pathways.[13] However, it is still unknown whether Toll-like receptor cycling is regulated at the level of the host or microbiota. In other words, it is unclear whether the dynamic microbiota fluctuations induce host changes or whether the host Toll-like receptor rhythmicity is an innate host trait. Secondly, the microbiota could be producing compounds that influence host physiology.[15]

The importance of the gut-brain axis has been an exciting field for microbiome research.[16] There is potential that cycling bacterial communities within the gastrointestinal tract produce the ideal amount of active hormones, precursors, or metabolites for the regulation of host metabolism, energy expenditure, or host circadian rhythmicity. Perturbances in microbiome rhythmicity could exacerbate weight gain via a decreased bacterial utilization of consumed nutrients, upregulation of host nutrient uptake machinery, decreased host energy utilization, and/or increased appetite. Alternatively, it is interesting to speculate about how short timescale microbiome cycling may prevent excessive immune recruitment and inflammation to the gastrointestinal tract. It is plausible that impairments to this process may be an initiating factor to the development of obesity and diabetes. Many of these speculations can be examined by holistic multi-disciplinary longitudinal studies in animal models and humans experiencing circadian arrhythmicity (e.g. frequent long-distance travellers, shift workers, etc.).

Microbiome Circadian Rhythms and Metabolic Disease

A relatively new question has arisen: how important is the circadian rhythm of the gastrointestinal microbiota to host physiology? Despite diet and antibiotic usage, the microbiota has been suggested to display long-term resilience to change over months to years.[17] However, Thaiss and colleagues demonstrated that these organisms typically fluctuate in community composition and gene expression in a circadian-dependent manner.[10] This finding has since been replicated.[12] Despite the changes in cycling bacterial communities and gene expression being relatively small, impairment of these microbiome circadian fluctuations by arrhythmic feeding has been found to exacerbate the progression of metabolic diseases (Figure 1), such as diabetes and obesity.[10] Strong experimental evidence suggests that not only diet, but also feeding schedules are important determinants for microbiota composition and function.[10,12] Mice with disturbed circadian rhythms ("jet lag-like" condi-

tions) have been shown to display exacerbated progression of weight gain, blood glucose levels, and body fat compared to both standard rodent chow and high-fat/ "Western" diet controls.[10] The importance of the microbiota in metabolic disease progression was empirically observed by the fact that "jet-lagged" mice treated with broad-spectrum antibiotics did not display this metabolic pathology, but instead more closely resembled the control group. In addition, germ-free mice that were colonized with the microbiota of humans suffering from jet lag showed greater weight gain, impaired glucose tolerance, and increased fat content compared to controls. These findings corroborate with a similar study that showed antibiotic-induced microbiota disturbances caused elevations in blood levels of corticosterone, glucose, triglycerides, and free fatty acids in mice.[13] Taken together, these findings provide strong evidence that the circadian rhythm of the human microbiota may play an important role in the pathogenesis of metabolic diseases such as diabetes and obesity.

Research has begun to unveil the complex roles that microbes play in shaping host metabolism and nutrient availability. The observation that host circadian rhythm likely has notable effects on microbiota composition[13] has profound impacts to the study of host metabolism and nutrient absorption.[9] This also corroborates well with a substantial number of studies already noting the important influence that the microbiota has on host metabolism and associated metabolic pathologies.[18-22] Notably, high fat diet-induced microbiota disturbances were shown to be responsible for endotoxemia, impaired gut barrier function, low-grade inflammation, and metabolic disease characteristics in mice.[23] The observation that perturbances to the gut microbiota may preclude the onset of chronic diseases, such as diabetes, has important clinical implications. For example, clinical analysis of a patient's microbiota from noninvasive stool samples may provide insights to a patient's risk of developing diabetes prior to the onset of traditional clinical diagnostic measures such as hyperglycemia or elevated glycated hemoglobin (e.g. HbA1c). Therefore, the microbiome may one day be used as an early marker of diabetic pathogenesis, and thus enable more timely lifestyle or therapeutic intervention to prevent or delay disease onset.

Circadian Rhythms and Immune Function

Perhaps the most interesting aspect of the aforementioned findings is the realization of the complex role that microbiota regulation may have on the predisposition to human health and numerous diseases. The demonstration that arrhythmic feeding schedules can induce microbiota associated metabolic disease brings with it exciting new questions to the subject of nutrition. The technological advancements and city lifestyle have increased the prevalence of perturbed human diets due to a combination of artificial light, irregular sleep schedules, shift work, and jet lag associated with travel.[24,25] These insults on circadian rhythmicity appear to challenge the human body's ability to prepare for rest, activity, and feeding. It is interesting to consider what states of human health and diseases may be mechanistically linked to rhythmic circadian cellular functioning. Currently it is not known how or why certain members of the microbiota are more susceptible to arrhythmic insults than others. Future studies could utilize total parenteral diets to empirically demonstrate that dietary changes are the sole factor responsible for circadian dysbioses of the microbiota.

Many studies have shown that microbes are crucial for immune system development and maturation. Furthermore, the immune system has been shown to respond in a circadian manner in response to stimulation by the gastrointestinal microbes.[13,14] Therefore, it is possible that host-microbe circadian rhythm-mediated disturbances to the immune system could influence immunopathologies such as Crohn's disease, inflammatory bowel disease, systemic autoimmune diseases, and cancer risk. It is also worth investigating whether rhythmic regulation of the microbiota-immune system axis is necessary and/or sufficient for "healthy" immune development. The circadian clock has also been demonstrated as an important indicator of its host's ability to prevent opportunistic infections.[26] However, it is currently unknown how circadian fluctuations in the microbiota influence bacterial competition in the gastrointestinal niche. The close interplay between the organisms and immune system is still in its infancy.

Unanswered Questions About the Gastrointestinal Microbiota Circadian Fluctuations

Interestingly, not all species of the microbiome have been shown to fluctuate rhythmically.[12] In particular, high fat diet was shown to decrease the microbiota cycling of the Bacteroidetes phylum. This finding could be correlated with the study's observation of a shift in microbiota dominance towards bacterial members of the Firmicutes phylum.[12] This could be due to a combination of nutritional availability and interspecies competition. In this model, species of bacteria that compete for commonly utilized energy sources may be prone to drastic fluctuations in growth and activity. In contrast, bacteria able to metabolize nutrients that other species cannot, may exhibit stable phenotypes due to the plentiful availability of nutritional resources. An experiment using only parenteral/intravenous nutrition in an animal model would provide powerful insight to the cause and effect between diet and microbiota circadian rhythms. Alternatively, the microbiome circadian rhythms may simply reflect the fluctuations in gastrointestinal immunity against the microbiota.[14] Microbes more resistant or less susceptible to mucosal immune responses would be less likely to undergo cyclical fluctuations. The knowledge is now in place for many of these theorized mechanisms to be evaluated *in silico* and be complemented by animal studies using arrhythmic environmental stimuli, variable diets, germ-free, and/or circadian clock mutants.

Conclusion

Ongoing research is revealing the important relationship between the human and animal body and the colonizing microbes. A new concept worth considering is the importance of crosstalk between the circadian rhythms of the host and its resident microbiota. Experimental evidence suggests that maintenance of this mutualistic relationship appears to be

important for host health.[9-12] Since most tissues are subject to circadian rhythm regulation,[7] it will be interesting to see what future host physiological processes are modulated by host-microbe circadian crosstalk. Due to its targetable nature through diet, pre- and probiotics and antibiotics, the circadian rhythmicity of the microbiome likely will reveal exciting findings that could influence clinical diagnostics, therapeutics, and transcontinental or space travel in the near future.

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