Dancing to circadian rhythms: microbes and metabolism

Since the turn of the 21st century, two advances in biology have revolutionized our thinking of human metabolism. First, is the in-depth characterization of a previously recognized, but hitherto poorly defined organ system, the gut microbiome. This microbial organ exquisitely interacts with the diet which greatly influences its metabolic functions to impact host metabolism through the production of small bioactive molecules that continuously enter the bloodstream to act at local and distant organ tissues. Equally important, the host metabolism can also modulate the gut microbiome setting up the two as intricate and well-suited partners. Second, is the recognition that most of the cells and organs of the body are dependent on circadian rhythms, systemic timekeepers that play a major role in regulating behavioural and physiological functions to manage energy balance. In conditions of metabolic health, circadian rhythms are the beat to which the two well-matched partners, microbes and metabolism, dance. When these partners are out of step or mismatched, negative consequences may develop that promote metabolic disturbances and disease. Thus, unravelling this complex choreography becomes key to understanding how to maintain metabolic health and to correct missteps that may lead to the development of conditions like diet-induced obesity.

Two to tango: gut microbes and host metabolism

With the advent of pyrosequencing in combination with advances in data science, we have begun to unravel the mysteries enshrouding the gut microbiome. This organ system is comprised of a trillion or more bacteria, fungi, archaea, protists and viruses belonging to thousands of species which colonize the lumen and mucosal lining of the gastrointestinal tract. Collectively, these groups have 100-times more genes than the human genome and many of these genes are essential for the host’s well-being. These organisms affect a myriad of host functions and, to mention a few, they range from early immune and metabolic development, to deterrence of pathogens, and to the regulation of gene expression and function of many organ systems. Like other critical organ systems, loss or dysfunction (dysbiosis) of the gut microbial organ, as seen in response to broad-spectrum antibiotics can have severe, life-threatening consequences. Clostridium difficile colitis, a common, sometimes lethal, hospital-derived infection is a good example of this. Although the field is still nascent, the role that gut microbes have in regulating host metabolism is becoming increasingly recognized. Thus, transplantation of a microbial ‘organ’ from a healthy donor, as seen with faecal microbiota transplants, remarkably reverses C. difficile infections and restores health. Germ-free mice (devoid of any microbiota) are highly resistant to the development of diet-induced obesity, despite the fact that they consume more chow than their counterparts with a normal gut microbiota, just to maintain body weight. This simple observation strongly suggests that the gut microbiome plays an essential role in determining the energy balance of the host. In addition, colonization of germ-free mice with faecal microbiota from obese mice or humans exhibit increased adiposity. In humans, the transfer of gut microbes from lean subjects to overweight recipients has also been shown to improve glucose homeostasis over a six-week period. Thus, it appears that the gut microbiome has considerable influence on host metabolic networks and vice versa. However, it should be noted that it is not healthy for humans to be completely germ free. When host and microbiome...
are in step, host metabolic functions are optimal, but when out of step due to disturbances on either the host or microbiome side (or both), metabolic balance can become disrupted to promote diet-induced obesity.

**Dancing to circadian rhythms**

Nearly all life forms, whether human, animal, plant or photosynthetic microbe, possess a network of biological clocks, driven by a circadian transcriptional-translational feedback loop of proteins, which depend on the proper expression of a full complement of a dozen or so circadian ‘clock’ genes. Circadian clocks are master regulators and integrators of host energy balance, behaviour (e.g. sleep/wake cycles) and metabolic homeostasis. Not surprisingly, perturbations to circadian rhythms are accompanied by changes in metabolism, behaviour and physiological functions. For instance, disruption of circadian function in humans by sleep disorders, shift-work, jetlag and high-fat ‘Western-type’ diets can be associated with metabolic consequences including obesity. Similarly, genetic disruption of circadian clock function in animal models is associated with a range of metabolic and physiological perturbations. Up to 20% of the gut microbes exhibit diurnal variations, wherein the levels of individual taxa oscillate over a 24-hour period such that the composition of the day and night gut microbiota can be quite different. The main driver of this appears to be the exquisite sensitivity of the microbiota to host dietary cues. Synchronous with this diurnal variation, the microbes release bioactive molecules that provide important cues to host metabolic networks necessary for the maintenance of energy balance. It is now becoming apparent that circadian rhythms are a key step by which the gut microbiome and host metabolism are inextricably linked. The circadian links are bi-directional and it is essential for these systems to be synchronized, but, at the same time, have some fluidity to respond to variations in environmental and dietary stimuli. The master regulatory centre for circadian rhythms, located in the suprachiasmatic nucleus (SCN) of the brain, receives light and dark cues from ocular photoreceptors that are important for setting the circadian clock signal. The clock signal, in turn, entrains peripheral circadian networks such as that of the liver and also affects the diurnal variation of the gut microbiome. Conversely, the oscillations of the gut microbiome affect both central and some peripheral clock functions. For example, these
networks are disturbed in the germ-free mouse with some circadian genes showing diminished amplitude and phasic properties, despite mice being in the same light: dark conditions as their non-germ free counterparts. These findings underscore the importance of gut microbes in maintaining host circadian rhythms and metabolic functions, presumably by providing input on when, what and how much nutrients are consumed. This information is integrated with other circadian cues so that the host can adjust energy expenditure relative to activity and food consumption. As with any highly tuned system, perturbations of any component of these networks can have adverse consequences. The consumption of high-fat, high-caloric, ‘Western-type’ diets, for example, creates a gut dysbiosis that is characterized by a drastic change and loss of diurnal variation in microbial membership, a shift in the metabolome to an obesogenic profile and not surprisingly a disruption of rhythmicity and function of both central and peripheral circadian clock genes. Key microbe-derived signalling molecules such as short-chain fatty acids and tryptophan derivatives that may be critical inputs for host circadian networks are severely attenuated. Without these oscillator signals, host circadian networks are disrupted, shifting energy balance and promoting the development of obesity.

**Can microbiome-based interventions be used to prevent or treat human obesity?**

Obesity is no longer a problem restricted to rich nations but is affecting both developed and developing countries worldwide. The ready availability of inexpensive, processed, fatty foods is the ‘enemy’. In Western societies, poor food choices combined with less physical activity and overindulgence have dramatically contributed to the increased incidence in obesity. In low- and middle-income countries, while infectious diseases and poor child nutrition prevail in many settings, the food that is available at low cost is high in fat, sugars and calories and low in essential micronutrients. Many of these factors are ingrained in Western societies and difficult to change, yet they contribute to shifts in the metabolic set-points of individuals that defines the trajectory of their metabolic outcomes. Few things can change this trajectory, although bariatric surgery has been successful in achieving sustained weight loss in the morbidly obese. Originally thought to work solely through reducing caloric uptake, bariatric surgery may in fact work through altered energy balance, i.e. changing the metabolic set-point of the individual so calorie conversion to fat is reduced. While the underlying mechanisms for this shift are not well understood, a role of the gut microbiome has been implicated. Understanding if and how gut microbes can restore metabolic homeostasis therefore represents an emerging area for discovery and development of novel non-surgical alternatives for the prevention and treatment of diet-induced obesity. In this regard, there has been considerable attention paid to the use of prebiotics, probiotics and their combination, synbiotics, to restore ‘healthy’ gut microbiota and their functions. More recently, faecal microbial transplant therapy has gained credence. However, the jury is still out on these approaches of ‘restoring gut microbiota’ since our understanding of microbial gut ecology is still in its infancy. At issue is whether gut microbial ‘oscillators’, including defined microbial strains and/or their metabolic products, can be used to restore missing microbe-derived signals to a broken circadian clock even with continued consumption of Western-type diets. In essence, is it possible to have your cake and eat it too? There are some emerging experimental data that support this notion. Restricting feeding of mice on a high-fat diet to a certain time of the day has been shown to restore some of the oscillatory properties of the gut microbiome, to restore host circadian rhythms and to prevent obesity. Additionally, we have found that supplementation of high-fat ‘Western-type’ diets with the short-chain fatty acid butyrate can partially restore host circadian rhythm and reduce adiposity. This observation provides compelling evidence that timed delivery of microbiome-based interventions for diet-induced obesity is realistic and feasible.

**Is there a finale?**

There are a few key take-home messages from watching the two partners, the microbiome and metabolism, dance to the rhythm of the circadian clocks as summarized in Figure 1. The orchestration of three complex dynamic systems, the microbiome, host metabolism and circadian rhythms is not governed by a single musical score. Their functions are closely linked and are integrated with other organ systems to balance caloric intake and energy utilization. Tweaking any one component undoubtedly affects the other two with drastic consequences. Thus, when there is a mismatched partner, such as when fermentative bacteria are replaced with obesogenic microbes, cues like short-chain fatty acids are lost, resulting in misdirection of host circadian networks and the loss of specific clock gene oscillations. Regarding metabolism, an example could be the switch from fatty acid oxidation to lipogenesis. Asynchrony among the microbial, peripheral and
Eugene B. Chang is the Martin Boyer Professor of Medicine and Director, Inflammatory Bowel Disease Research Center at the University of Chicago. His research focuses on intestinal host-microbial interactions, particularly in defining communication signals that are critical for maintaining homeostasis and unraveling how perturbations lead to complex immune and metabolic diseases. His novel findings on the role of epithelial stress proteins, diet, and the circadian clock, in host-immune and metabolic diseases. His research focuses on understanding the molecular mechanism(s) underlying neurohumoral regulation of ion transport across epithelia. Recent work has focused on discerning how bile acids cause diarrhea. This is of relevance to understanding chronic diseases such as Inflammatory Bowel Syndrome. She has also worked on diversity in the gut microbiota, with the preponderance of readily available processed foods, social and behavioral changes will be slow. Further, while considerable progress has been made with ‘biotics’, the scientific community is still far from completely unravelling the mysteries of microbial ecology and host–microbe interactions. Therefore, we posit that a feasible in-hand solution may lay in defining a cocktail of key microbial ‘oscillators’, consisting of microbes and/or their metabolites, given at the right time of day that can restore host circadian rhythms to which microbe and metabolic partners can again harmoniously dance.