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Review

Time for Food: The Intimate Interplay between Nutrition, Metabolism, and the Circadian Clock

Gad Asher[1,*] and Paolo Sassone-Corsi[2,*]
[1]Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 7610001, Israel
[2]Center for Epigenetics and Metabolism, Department of Biological Chemistry, U904 INSERM, University of California, Irvine, Irvine, CA 92697, USA
*Correspondence: gad.asher@weizmann.ac.il (G.A.), psc@uci.edu (P.S.-C.)
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The circadian clock, a highly specialized, hierarchical network of biological pacemakers, directs and maintains proper rhythms in endocrine and metabolic pathways required for organism homeostasis. The clock adapts to environmental changes, specifically daily light-dark cycles, as well as rhythmic food intake. Nutritional challenges reprogram the clock, while time-specific food intake has been shown to have profound consequences on physiology. Importantly, a critical role in the clock-nutrition interplay appears to be played by the microbiota. The circadian clock appears to operate as a critical interface between nutrition and homeostasis, calling for more attention on the beneficial effects of chrono-nutrition.

The approach to healthful eating proposed by Maimonides (1135–1204), a medieval Jewish philosopher and doctor also known as Rambam, has garnered followers from well beyond the grave. In his writings, the Rambam gave clear instructions regarding what, when, and how much people should eat in order to lead a healthy life. One of his well-known quotes is: ''Eat like a king in the morning, a prince at noon, and a peasant at dinner.''

Feeding behavior is a principal factor that plays a role in the organism's nutritional status. Eating schedules are predominantly dictated by an inherent timing mechanism, but in addition, are affected by food availability, hunger, and satiety and also by social habits and convenience. A large body of nutritional studies has extensively examined the effect of the quantity and quality of food ingested on the organism's well-being. Nowadays, it is widely accepted that these parameters are critical and that their alteration is associated with morbidity and mortality (e.g., high-fat diet). Evidence accumulated during recent years suggests that meal timing can affect a wide variety of physiological processes, including sleep/wake cycle, core body temperature, performance, and alertness. Moreover, it appears that feeding time has a dramatic effect on health and can be employed to prevent obesity and various other metabolic pathologies. Hence, ''chrono-nutrition'' refers to food administration in coordination with the body's daily rhythms. This concept reflects the basic idea that, in addition to the amount and content of food, the time of ingestion is also critical for the well-being of an organism.

Hitherto, as detailed in this Review, the vast majority of studies have focused on the effect of scheduled meals on metabolic pathologies such as obesity and diabetes. However, one can envision that the ''optimal'' feeding schedule might harbor wide medical benefits beyond metabolic syndrome. Future studies are expected to shed more light on the prospects of feeding timing in preventing morbidity and reducing mortality in relation to other pathologies such as aging.

Circadian Clocks and Metabolism

A wide array of physiological and metabolic variations depends on the time of the day, including sleep-wake cycles, feeding behavior, body temperature, and hormonal levels. The past two decades have witnessed a remarkable increase in our knowledge of how circadian (from the Latin words, *circa diem*, about a day) biology is controlled, both from physiological and molecular standpoints. We refer the interested reader to several detailed review articles on the subject (Asher and Schibler, 2011; Eckel-Mahan and Sassone-Corsi, 2013; Feng and Lazar, 2012). Briefly, circadian rhythms are controlled by molecular clocks, whose key features are (1) an input pathway that includes receivers for environmental cues and subsequently transmits them to the central oscillator; (2) a central oscillator that keeps circadian time and generates rhythm; and (3) output pathways through which the rhythms are conveyed and control various metabolic, physiological, and behavioral processes. Circadian clocks are uniquely characterized as entrainable, self-sustained, and temperature-compensated oscillators (Brown et al., 2012; Buhr and Takahashi, 2013; Dibner et al., 2010). The master or ''central'' clock is located in the hypothalamus, within a paired structure so-called the suprachiasmatic nucleus (SCN). The SCN contains 15–20,000 neurons, which have the remarkable feature of oscillating with a 24 hr based rhythm. Indeed, the SCN clock can function autonomously, without any external input, and can be reset in response to environmental cues (zeitgebers, or time givers) such as light. The SCN functions as an ''orchestra director'' for the ''peripheral clocks,'' thought to be present in all other tissues and cells in the body. Synchronization of peripheral clocks is essential to ensure temporally coordinated physiology and is achieved through yet-ill-defined pathways controlled by the master clock (Saini et al., 2011).

At the heart of the molecular network that constitutes the circadian clock are the core transcription factors CLOCK and

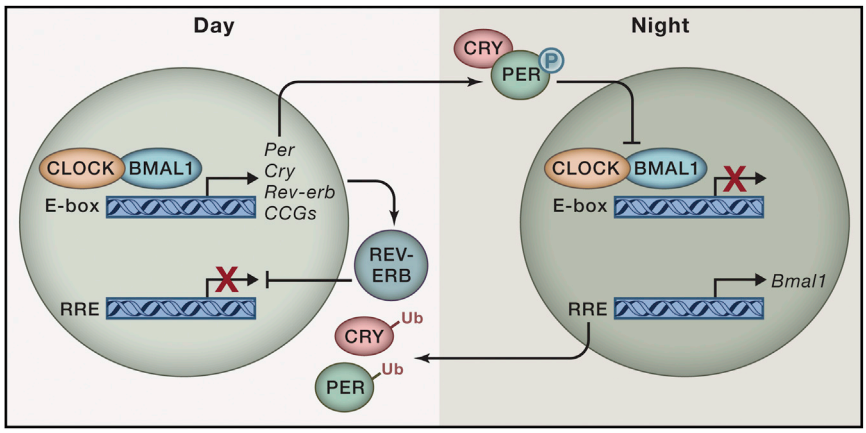

Figure 1. The Molecular Organization of the Circadian Clock

The transcriptional activators CLOCK and BMAL1 dimerize to stimulate the expression of many CCGs with E-box promoter elements in their promoters. CLOCK:BMAL1 also activate the expression of the *Period* (Per) and *Cryptochromes* (Cry) gene families. PERs and CRYs protein levels become high during the night, after which they dimerize and translocate to the nucleus to repress CLOCK:BMAL1-mediated transcription. PERs and CRYs undergo a number of post-translational modifications that induce their degradation, required to start off a new circadian cycle. Another loop involves the proteins REV-ERBα/β, whose levels increase during the day and bind specific responsive promoter elements (RRE) and thus inhibit *Bmal1* transcription. At night, REV-ERBα protein amounts are low, allowing *Bmal1* transcription to take place. These transcriptional-translational regulatory loops are operating in most cells and control a remarkable fraction of the mammalian genome.

BMAL1 (Figure 1) (Crane and Young, 2014). They heterodimerize and drive the transcription of a large number of clock-controlled genes (CCGs) by binding to E-box sites within their promoters. CLOCK and BMAL1 also direct the transcription of their own repressors, period (PER) and cryptochrome (CRY) family members, generating a tightly self-regulated feedback loop. During the day, the increase in transcription of *per* and *cry* genes results in the accumulation of the PER and CRY circadian repressors. These, in turn, inhibit CLOCK:BMAL1-driven transcription of *per*, *cry*, and CCGs. The highly controlled degradation of PER and CRY alleviates transcriptional repression and allows CLOCK:BMAL1-mediated transcription to proceed again, establishing cycles in circadian gene expression. Additional levels of circadian regulation exist with the orphan nuclear receptors ROR and REV-ERB that activate and repress transcription of the *Bmal1* gene, respectively. Furthermore, clock proteins are modified in a post-translational manner by phosphorylation, acetylation, ubiquitination, and SUMOylation, adding multiple layers of regulation to the core clock machinery (Crane and Young, 2014; Robles and Mann, 2013).

The CLOCK:BMAL1-driven activation of CCGs deserves special attention in the context of this Review. First, it allows for the circadian regulation of cellular, metabolic, and physiological output functions. Second, transcriptome studies have shown that the overlap of CCGs in different tissues is relatively marginal, questioning the possible contribution of tissue-specific factors to clock control. Finally, it reveals that a large fraction of the genome is potentially under clock control (Masri and Sassone-Corsi, 2010). The intrinsic plasticity of the circadian system could thereby be provided, at least in part, by the potential of expanding or restricting the regulation of CCGs depending on the nutritional, metabolic, and epigenetic state.

The circadian clock is intimately connected to metabolism (Asher and Schibler, 2011; Bass, 2012; Eckel-Mahan and Sassone-Corsi, 2013; Green et al., 2008). Direct evidence is provided by targeted mutations of clock genes in the mouse that yield animals with a variety of metabolic disorders (Sahar and Sassone-Corsi, 2012). From a molecular point of view, it has been shown that the clock machinery controls the expression of essential genes within numerous metabolic pathways. A paradigmatic example is the control by CLOCK:BMAL1 of *Nampt* (nicotinamide phosphorybosil transferase) gene expression (Nakahata et al., 2009; Ramsey et al., 2009). The product of this gene, namely the enzyme NAMPT, functions as the rate-limiting step in the NAD^+-salvage pathway. By controlling *Nampt* cyclic transcription, the clock directs the circadian synthesis of NAD^+ and thereby the potentially cyclic activity of NAD^+-consuming enzymes. This is indeed the case of the NAD-dependent deacetylase SIRT1, an enzyme involved in the control of cellular metabolism, inflammation, and aging (Guarente, 2011). Importantly, SIRT1 had been shown to contribute to CLOCK:BMAL1 function by physically interacting with these circadian regulators (Asher et al., 2008; Nakahata et al., 2008). Recent work has shown that a similar deacetylase, SIRT6, also contributes to circadian control, though acting on a different group of cyclic genes than SIRT1 (Masri et al., 2014). Intriguingly, SIRT6 is chromatin bound, controls lipid metabolism, and is enzymatically activated by fatty acids. SIRT1 and SIRT6 partition the circadian epigenome, leading to segregated control of cellular metabolism (Figure 2), a finding that could be relevant with respect to different nutritional regimes (Eckel-Mahan et al., 2013). In addition, SIRT3, a mitochondrial NAD^+-dependent deacetylase, has been implicated in circadian control of mitochondrial function (Peek et al., 2013), and PARP-1, an NAD^+-dependent ADP-ribosyltransferase, was shown to participate in the phase entrainment of circadian clocks to feeding (Asher et al., 2010). Thus, changes in food composition/feeding time may lead to differential activation of epigenetic and transcriptional control systems through harnessing specialized enzymatic pathways and circadian metabolic sensors.

"Chrono-Nutrition" from Rodents to Humans

Evidence from Studies in Mice

Several studies in mice indicate that changes in feeding schedule carry clear metabolic implications. When mice are housed in constant bright/dim light, they consume more food during the subjective light phase. These mice exhibit significantly increased body mass and reduced glucose tolerance compared

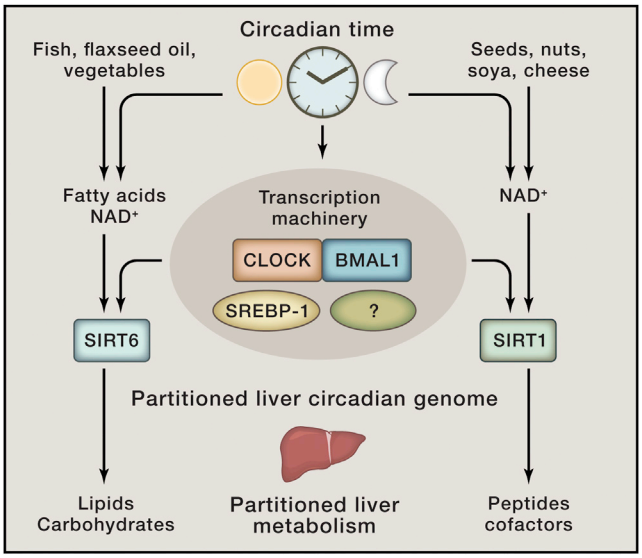

Figure 2. Interplay between Nutrition, the Circadian Clock, and Metabolism

A large body of evidence demonstrates that the circadian clock and cellular metabolism are intimately interconnected. A paradigmatic example is the one represented by two chromatin remodelers, the deacetylases SIRT1 and SIRT6 in the liver (Masri et al., 2014). These two enzymes contribute in partitioning the circadian epigenome as they control distinct groups of genes through different molecular mechanisms, including the activation of alternative transcriptional pathways, such as SREBP-1. The combination of these regulatory events results in segregation of circadian metabolism. SIRT1 participates predominantly in the cyclic control of cofactors and peptides, whereas SIRT6 seems dedicated to cyclic synthesis of lipids and carbohydrates (Masri et al., 2014). As SIRT1 consumes NAD^+ (Guarente, 2011), whereas SIRT6 appears to be activated by free fatty acids (Feldman et al., 2013), these may reflect differential responses to distinct nutritional intakes.

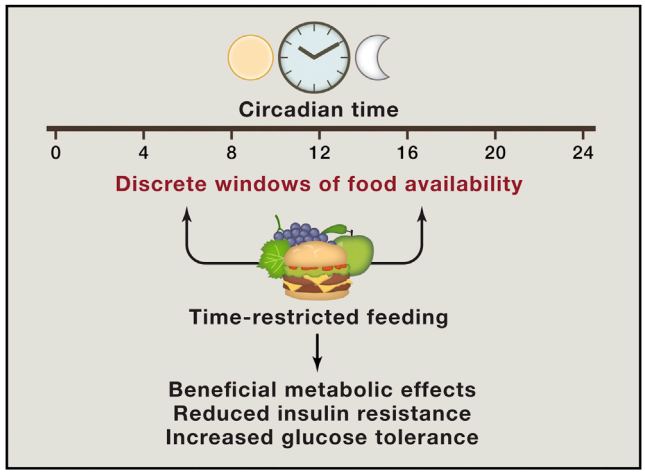

Figure 3. Time-Restricted Feeding and Its Beneficial Effects

Food can be made available only at discrete windows of time within the daily cycle. A large body of evidence indicates that time-restricted feeding is beneficial for a number of metabolic responses, reducing insulin resistance and increasing glucose tolerance (Adamovich et al., 2014b; Hatori et al., 2012; Sherman et al., 2012; Chaix et al., 2014). Although further studies in humans are needed, the beneficial effects of chrono-nutrition should be taken into serious consideration.

with mice under standard light/dark cycles, despite similar caloric intakes and total motor activity (Fonken et al., 2010). Additional support emerges from analysis of genetically modified mouse models. Altered feeding rhythms in these mice further correlated feeding schedule with obesity and metabolic syndrome. In particular, *Clock* mutant mice exhibit greatly attenuated diurnal feeding rhythm. These mice are obese and develop a metabolic syndrome (Turek et al., 2005). Likewise, adipocyte-specific *Bmal1* null mice display increased food intake during the light phase and elevated body weight. Disruption of the clock in the adipocytes seems to modify the circulating concentration of polyunsaturated fatty acids in the hypothalamus, resulting in altered feeding behavior (Paschos et al., 2012).

Time-restricted feeding experiments further highlighted the metabolic effects of feeding schedule. Upon nighttime restricted feeding of regular chow, hepatic triglycerides content in wild-type mice decreases by 50%, whereas the total daily caloric consumption is unaffected (Adamovich et al., 2014b). Similar studies with high-fat diet demonstrated compelling effects on the propensity to develop obesity and metabolic syndrome. Mice under time-restricted high-fat diet consume equivalent calories as those with ad libitum access yet are protected against obesity, hyperinsulinemia, hepatic steatosis, and inflammation (Figure 3). Time-restricted feeding regimen improves CREB, mTOR, and AMPK pathway function and oscillations in expression of circadian core clock and output genes (Hatori et al., 2012; Sherman et al., 2012). It should be noted that both studies applied time-restricted high-fat diet, yet at complete different times throughout the day. The former limited the food availability to 8 hr during the dark phase, whereas, in the latter, food was provided for 4 hr during the light phase. Thus, it is conceivable that the key factor is the time restriction from food per se, rather than its occurrence at a specific circadian time. A recent study from Panda and colleagues further characterized the different requirements for time-restricted feeding to be beneficial (Chaix et al., 2014). Time-restricted feeding appears to be effective against preexisting obesity, there is an after effect upon cessation, and it remains effective even when applied only 5 days per week. This comprehensive study highlighted the effectiveness of time-restricted feeding against different nutritional challenges, including high-fat, high-fructose, and high-fat combined with high-fructose diets, all of which are known to cause dysmetabolism.

Even changes in food availability solely throughout the active phase (e.g., breakfast versus dinner) have been reported to affect body weight. Shibata and co-workers have compared metabolic parameters in mice that consumed only a single large meal at the beginning of the active phase with mice that had an additional relatively small meal at end of the active phase. Although mice in each group consumed an equal amount of food per day, mice on two meals exhibited reduced body weight gain and improved metabolic parameters compared with those on a single meal or freely fed animals (Fuse et al., 2012). Additional studies in rodents demonstrated that early nocturnal fasting increases body weight, whereas late nocturnal fasting reduces weight gain (Wu et al., 2011; Yoshida et al., 2012).

Evidence from Studies in Humans

Although there is a widespread belief that eating late at night carries high risk of developing obesity, the supporting evidence is relatively scarce. In a recent study, the comparison of two isocaloric weight-loss groups revealed greater improvement of metabolic markers in the group given a bigger breakfast and a smaller dinner than vice versa (Jakubowicz et al., 2013). Another study showed that early mealtimes significantly decrease serum lipid levels (Yoshizaki et al., 2013). Moreover, several human epidemiological studies identified a correlation between eating pattern and obesity. For example, breakfast consumption among adolescents was inversely associated with weight gain in a large cohort study (Timlin et al., 2008). Several studies have demonstrated a correlation between short sleep duration (<5 hr) or late sleepers (midpoint of sleep > 5:30 AM) and eating late dinners/consuming more calories late in the evening with significantly higher risk for developing obesity and diabetes (Baron et al., 2011; Hsieh et al., 2011). Moreover, night eating syndrome characterized by a time-delayed eating pattern is positively associated with elevated body mass index (BMI), (Colles et al., 2007). Interestingly, studies both in humans and rats have shown selected food predilections for higher fat composition at dinnertime than at breakfast time (Lax et al., 1998; Westerterp-Plantenga et al., 1996), suggesting a nutritional preference that might be related to obesity associated with late-night feeding. These studies indicate that late dinners carry the risk of obesity in humans; however, they should be cautiously interpreted.

In conclusion, the effects of time-restricted feeding have not yet been thoroughly examined in humans. The current evidence emerging from studies performed in humans is indirect and correlative and calls for additional well-controlled analyses.

Feeding-Fasting Cycles

Feeding time and circadian clocks are tightly intertwined, as feeding schedule has a prominent effect on circadian clocks in peripheral organs. As expected for nocturnal animals, mice mostly consume food during the night. When food is provided exclusively during the day, the phase of peripheral clocks is gradually inverted within several days (Damiola et al., 2000). By contrast, inverted feeding regimen has very little impact on the phase of the master clock in the brain. Therefore, feeding-fasting cycles appear to function as potent timing cues for peripheral clocks, even bypassing the otherwise-dominating synchronization signals emitted by the master clock in the brain. The prominent effect of feeding on circadian rhythmicity is also evident from studies comparing circadian gene expression in mice fed ad libitum with mice under time-restricted feeding regimen. Feeding time had a profound effect on the repertoire, phase, and amplitude of rhythmic gene expression. Specifically, feeding cycles have been shown to rescue the 24 hr rhythmicity in gene expression of numerous transcripts in mice with a genetically disrupted clock (i.e., *Cry1/2* null mice) (Vollmers et al., 2009). Moreover, the diet composition seems to have an impact on the rhythmic feeding behavior in mice and rats (Hariri and Thibault, 2011; Kohsaka et al., 2007). Upon being high-fat fed, mice exhibit altered feeding-fasting cycles very similar to clock-deficient mice (e.g., *Cry1/2* and *Per1/2* double-null mice), as they consume a higher percentage of their daily food intake during the light phase. Concomitantly, circadian gene expression is attenuated (Kohsaka et al., 2007). Interestingly, when mice are fed with high-fat diet exclusively during the night, circadian rhythmicity in gene expression is restored (Hatori et al., 2012).

Molecular Basis for Reprogramming and "Chrono-Nutrition"

Nutritional challenge does not simply disrupt normal circadian rhythmicity. When mice are subjected to high-fat diet ad libitum, the liver clock undergoes a rewiring program that involves a number of molecular mechanisms (Eckel-Mahan et al., 2013). Although many circadian genes lose their cyclic expression due to impaired chromatin recruitment of the CLOCK:BMAL1 activator complex at their promoters, many other genes whose expression is normally non-cyclic become circadian through the cyclic activation of surrogate transcription pathways. These include both PPARγ and SREBP, which begin to activate a large number of target genes by cyclic chromatin recruiting. These findings reveal the remarkable plasticity of the clock system in response to nutritional challenges and indicate that more genes than previously thought have the potential to become circadian, depending on the nutritional, metabolic, and epigenetic state of the cell.

The finding that meal timing has major effects on metabolic and physiological parameters has led to the conviction that, in choosing food, it is not only important to consider its nutritional value but also its timing. So is there an "optimal time" to ingest food? The aforementioned studies delineate the benefits in time-restricted feeding; however, it is still unclear whether there is an "optimal time" for food intake. In some studies, food accessibility was limited to the dark phase, whereas in others, it was limited to the light phase, yet the outcome was very similar. This apparent discrepancy can be resolved by centering on the direct consequences of time restricted feeding. When mice are fed regular chow, they mostly ingest food during the dark phase (Adamovich et al., 2014b). Upon high-fat diet, the situation is aggravated as they consume an almost equal amount of food throughout the day (Hatori et al., 2012). Conceivably, time-restricted feeding generates sharp feeding-fasting cycles, which consolidate circadian rhythmicity in gene expression and circadian activation of various metabolic pathways. This is because clocks in most peripheral organs readily respond to feeding cycles, and feeding time can shift their phase. Upon several days of time-restricted feeding, food availability and the endogenous clocks are aligned, irrespectively on whether the food is provided during the dark or the light phase. Hence, high-fat diet disrupts circadian rhythmicity through dampening of feeding-fasting cycles that serve as an extremely potent zeitgeber for peripheral clocks. It is yet unclear whether this effect of time-restricted feeding by high-fat diet is operating through molecular mechanisms analogous to those involved in the reprogramming of the circadian clock induced by nutritional challenge when food is available ad libitum (Eckel-Mahan et al., 2013).

A Role for the Microbiota

The contribution of the microbiota in regulation of physiology is vast and complex (Henao-Mejia et al., 2013; Tremaroli and Bäckhed, 2012). The impact of the microbiota is determined,

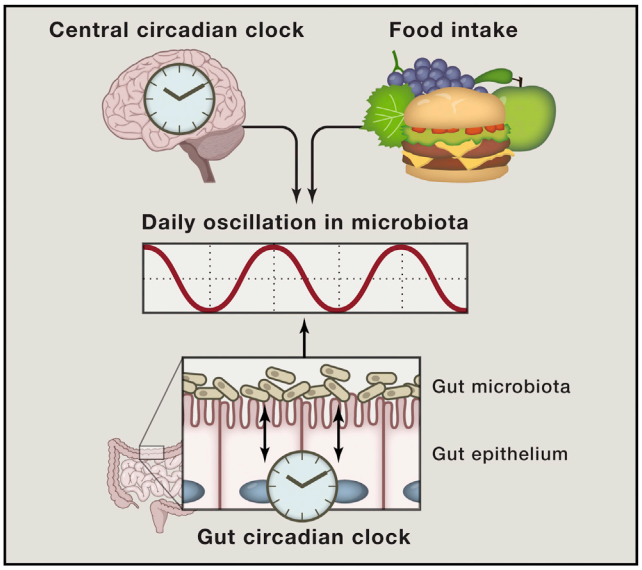

Figure 4. Interplay between the Microbiota and the Gut Circadian Clock

Intestinal cells contain a powerful circadian clock that is connected through yet-ill-defined physiological pathways to the central clock in the SCN. Nutrition is also cyclic, and its processing occurs through the rhythmic metabolism of the gut clock. Here, microbiota have been shown to participate in food processing and to interact with the intestinal clock. This interplay goes both ways, as the gut clock needs the microbiota to function properly, and the microbiota levels oscillate following the gut cycles (Mukherji et al., 2013; Thaiss et al., 2014; Voigt et al., 2014; Zarrinpar et al., 2014). Thus, the circadian clock is a critical component in the relationship between food and the gut.

among various factors, by its diverse composition that includes bacteria, archaea, and fungi. These populate a remarkable variety of sites in the organism of their multicellular hosts (Lozupone et al., 2012). In these locations, they contribute to local organ homeostasis, as well as to system-wide physiology through a number of specialized metabolic and signaling pathways (Lee and Hase, 2014; Sharon et al., 2014).

A large fraction of the microbiota is located in the gastrointestinal tract, a site that also has a powerful circadian clock (Bellet et al., 2013; Hussain and Pan, 2012). The clock in the gut has been shown to participate in the daily cycles of food digestion (Hussain, 2014; Tahara and Shibata, 2013), as well as in coping with the pathogenic condition of *Salmonella* infection (Bellet et al., 2013) and regulation of interleukin-17-producing $CD4^+$ T helper (T_H17) cells that protect against bacterial and fungal infections at mucosal surfaces (Yu et al., 2013). As the gut microbial population regulates the energy derived from food and modulates the levels of host- and diet-derived products, the question of how the gut clock interplays with metabolic pathways and the microbiota is critical (Sharon et al., 2014).

Is the microbiota controlling the gut clock or vice versa? The first answer to this question came from Chambon and colleagues through the analysis of the large intestine, which has the highest concentration of microbiota in mammals (Mukherji et al., 2013). The circadian clock within intestinal epithelial cells (IECs) is responsible for cyclic glucocorticoid production and is under endocrine control from the pituitary-adrenal axis. Remarkably, the IECs clock is profoundly disrupted by depletion of the microbiota, leading to altered corticosteroids levels and consequent metabolic disorders. Thus, the microbiota determines the appropriate function of the gut clock, most likely contributing to additional, system-wide cyclic homeostasis. This control operates through the innate immune receptors TLRs and NOD2, whose expression in IECs is cyclic and under clock regulation (Mukherji et al., 2013; Silver et al., 2012). Thus, changes in the microbiota levels and composition induced by different types of nutritional regimens could differentially regulate the gut clock and thereby influence the organism's homeostasis. This is indeed the case. The composition of the microbiota undergoes diurnal oscillations in both mice and humans (Thaiss et al., 2014), and these oscillations are disturbed in mice with a genetic mutation in the clock system, as well as in jet-lag experiments (Thaiss et al., 2014; Voigt et al., 2014). It appears thereby that the relationship between the gut epithelium of the host and the microbiota goes both ways, where both cell populations influence each other's physiology (Figure 4).

Another key component in the equation is food, whose intake is also cyclic, following an intricate system of neuronal and endocrine control. Different nutritional challenges alter the composition of the microbiota, and specific members of the intestinal microbiota have been linked with metabolic disease (Zarrinpar et al., 2014). Yet, how does time-specific and diet-specific food intake alter in parallel the gut clock and the microbiota? High-throughput genomic and metabolomics analyses have revealed that specific "signatures" exist for each dietary condition, as well as for time-restricted feeding (Zarrinpar et al., 2014). These intriguing results reveal the complexity of the gut homeostasis, as well as the importance that key molecular pathways, such as TLRs and NOD2, must play. It remains to be established the role of the clock in IECs cells by using tissue-specific mutations of clock proteins in the mouse, as well as the critical effect that the circadian gut-microbiota interplay most likely has on other metabolic organs, such as the liver, fat, and muscle. Finally, the fascinating influence that the microbiota appears to have on neuronal functions (Mayer et al., 2014) begs the question of whether the central clock or other brain structures may be receiving "nutritional" information in a cyclic manner through the gut-microbiota interplay.

Circadian Metabolomics

In the last decade, numerous transcriptome profilings performed throughout the day in various peripheral organs have highlighted the pervasive circadian control of metabolism and physiology (Masri and Sassone-Corsi, 2010; Green et al., 2008). Circadian transcriptomes are considered a hallmark for circadian rhythmicity and shed light on metabolic pathways that are potentially under circadian control. In recent years, in view of the expansion of cutting-edge technologies, several research groups commenced employing high-throughput metabolomics approaches to study circadian rhythms. These recent advances evinced multiple layers of regulation and complexity in circadian control. Metabolomics enables the systematic study of the unique chemical fingerprints involved in biological processes. This technology instigated the progress from learning individual compounds to exploring a broad combination of well-defined

metabolites and even to the identification of novel, previously uncharacterized metabolites. Early studies by Ueda and coworkers established a reliable metabolite timetable method to determine internal body time by quantifying the spectra of hundreds of metabolites throughout the day both in mice (Minami et al., 2009) and human blood samples (Kasukawa et al., 2012). Hence, similar to transcriptomes, metabolomes can be employed as a signature for endogenous time.

Subsequent studies were designed to identify metabolic pathways that exhibit daily oscillations and to dissect their circadian control. Metabolomics study of plasma and saliva samples from humans revealed that ~15% of all identified metabolites oscillate in a circadian manner, independently of scheduled sleep and food intake (Dallmann et al., 2012). Notably, a high proportion of rhythmic metabolites in blood plasma were fatty acids. Likewise, daily oscillations in blood plasma metabolites have been observed in human individuals under normal conditions (Ang et al., 2012). Similar studies conducted with mouse liver samples from wild-type and clock mutant mice identified clock-controlled circadian oscillation of various groups of metabolites, including lipids and, more specifically, fatty acids (Eckel-Mahan et al., 2012). Circadian metabolomics, therefore, do not only serve as a reliable readout for internal time but also constitute a productive tool to study the interplay between clocks and metabolism.

Circadian Lipidomics

Lipid homeostasis appears to be under circadian control, and disruption of circadian rhythmicity is associated with dyslipidemia and obesity in various clock mutant mouse models (Gooley and Chua, 2014; Sahar and Sassone-Corsi, 2012). These include elevated VLDL triglyceride levels in *Rev-Erbα* null mice (Raspé et al., 2002); dampening of triglycerides oscillations in blood plasma of *Bmal1*$^{-/-}$ mice (Rudic et al., 2004); hyperlipidemia and hepatic steatosis in *Clock* mutant mice (Turek et al., 2005); and reduced whole-body fat, total triglycerides, and fatty acids in blood plasma of *Per2*$^{-/-}$ mice (Grimaldi et al., 2010). Concomitantly, lipids also appear to play a role in circadian control (Adamovich et al., 2014a). Lipidomics analysis on human blood plasma revealed that ~13% of lipid species exhibited circadian oscillations (Chua et al., 2013), with high prevalence of diglycerides, and triglycerides that peaked around the circadian time (CT) 8 (i.e., 8 hr after lights are turned on). This is in line with the phase observed for lipid accumulation in previous human plasma metabolomics (Dallmann et al., 2012). Daily changes in triglycerides were also observed in blood plasma of rats—total plasma triglyceride levels were elevated during the night (CT 18) (Pan and Hussain, 2007). Thus, triglyceride levels in blood plasma reach their zenith levels during the active phase, namely in human during the day and in rodents during the night.

A recent comprehensive circadian lipidomics analysis of mouse liver has identified that ~17% of quantified lipids display circadian rhythmicity (Adamovich et al., 2014b). Notably, the majority of the oscillating lipid species were triglycerides (~33%) and reached their peak levels in the liver during the subjective light phase (i.e., CT8). The findings that triglycerides accumulate in rodent plasma during the active phase and in liver during the rest phase may suggest that triglyceride levels build up in different phases in liver and blood in a manner that most likely depends on feeding-fasting cycles and circadian clocks. Because food is a major source for triglycerides, it is conceivable that these accumulate first in the blood upon food ingestion during the active phase and are subsequently deposited in peripheral organs such as the liver during the rest phase. Surprisingly, a similar fraction of lipids (~17%) was oscillating in both wild-type and clock-disrupted mice (i.e., *Per1/2* null), most notably triglycerides. However, they largely differed in their accumulation phase and composition (Adamovich et al., 2014b). These observations are intriguing, as mice lacking both PER1 and PER2 are behaviorally arrhythmic under constant darkness, and their circadian expression of core clock genes is largely abolished. Further studies should aim at identifying the molecular mechanisms that drive the circadian accumulation of triglyceride in the absence of a functional clock feedback loop.

Integrative Omics Analysis

The above-described studies emphasize the need for an integrative and comprehensive exploration of the data emerging from the different omics. Indeed, a database (CircadiOmics database; http://circadiomics.igb.uci.edu/) that integrates circadian genomics, transcriptomics, proteomics, and metabolomics has been generated (Eckel-Mahan et al., 2012; Patel et al., 2012). It facilitates the use of the current data for deciphering circadian control of various metabolic pathways. It also illustrates the coherence that specific metabolic pathways share with distinct circadian transcription nodes. More importantly, these studies shed some light regarding the complexity of circadian control. First, in contrast to the high-amplitude oscillations in transcript abundance derived from numerous transcriptome studies, daily changes in protein and metabolite levels appear to be significantly shallower. For example, metabolite oscillations in human blood samples ranged around 2-fold (Ang et al., 2012; Dallmann et al., 2012), and most lipid species in the liver oscillated with an amplitude of ~1.5-fold (Adamovich et al., 2014b). A similar trend was observed with circadian proteomics data sets in which, for most cases, the amplitude of protein oscillations is relatively shallow (Mauvoisin et al., 2014; Masri et al., 2013; Reddy et al., 2006; Robles et al., 2014). Second, comparison of circadian transcriptome, proteome, and metabolome data demonstrated that, in some cases, they do not overlap. For instance, the levels of many proteins encoded by rhythmically expressed mRNAs do not oscillate at significant levels, whereas some proteins produced by constantly expressed transcripts do cycle in abundance. Third, in contrast to the disparate expression phases of genes encoding enzymes that participate in hepatic triglyceride homeostasis, triglyceride accumulation in the liver is highly coordinated and peaks toward the end of the light phase (Adamovich et al., 2014b). The discrepancy between the phase and amplitude of oscillations in transcript levels in comparison with proteins and metabolites probably reflects the complexity involved in their accumulation. Specifically, control of metabolites concentration often requires multiple steps and relies on the activity of several enzymes, substrates, and cofactor availability.

Concluding Remarks

During the past decade, remarkable progress has been made in our understanding of the mammalian circadian timing system.

The exciting emerging era of proteomics, metabolomics/lipidomics, commence to deepen our understanding of circadian rhythms.

Several circadian transcriptome studies have been conducted in the last decade characterizing circadian gene expressing in different organs, mouse strains, and feeding conditions (as an example, see Zhang et al., 2014). By contrast, circadian metabolomics are still in their infancy and should be cautiously analyzed and interpreted. Indeed, as aforementioned, the amplitude of metabolites oscillations is often relatively shallow and, hence, more likely to be affected by the experimental design and quantification methods. Future studies are expected to shed more light and provide better tools to tackle these caveats. In any rate, these approaches promote the identification of pathways that couple the molecular clock to metabolism, uncovering new circadian outputs and dissecting the interplay between environmental cues (e.g., feeding) and clocks in circadian control. Concomitantly, the flare-up in nutritional studies that address the effect of feeding schedule on obesity and metabolic syndrome, together with the entry of new players such as microbiota into the circadian playground, reveals the true potential of operating on circadian clocks as strategy toward therapeutics for metabolic diseases and other pathologies.

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