Chinese Journal of Physiology 61(6): 325-340, 2018
DOI: 10.4077/CJP.2018.BAH641

Review Article

Host-Microbiota Interaction and Intestinal Epithelial Functions under Circadian Control: Implications in Colitis and Metabolic Disorders

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Abstract

Commensal microbes are involved in intestinal homeostasis, and the dysregulation of host-microbe interactions may lead to the development of local and systemic disorders. Recent evidence indicated that microbiota dysbiosis plays a key role in the pathogenesis of inflammatory bowel disease and metabolism-related disorders. The circadian clock system originally identified in the brain was later found in the gastrointestinal tract. Although the light-controlled central clock in the brain is responsible for the synchronization of peripheral clocks, the timing of meal consumption serves as another cue for the rhythmic setting of gastrointestinal digestion, absorption, and epithelial renewal and barrier functions. Multiple lines of evidence have indicated that in addition to daylight and food intake, microbiota (as an environmental factor) are involved in the circadian control of gut homeostasis. Recent studies demonstrated that microbial metabolites and innate signaling orchestrate the host circadian rhythm, revealing unforeseen molecular mechanisms underlying the regulatory role of microbiota in intestinal physiology and systemic metabolism. In this review, we discuss the host-microbe interplay that contributes to the regulation of intestinal clock signals and physiological functions and explore how microbiota dysbiosis may cause misalignment of circadian systems leading to the development of chronic inflammatory and metabolic diseases.

Key Words: circadian clock, epithelial barrier function, gastrointestinal physiology, microbiota dysbiosis, inflammatory bowel disease, metabolic disorders

Introduction

Physiological functions of the gastrointestinal (GI) tract are essential for supplying metabolic energy and maintaining cellular survival in organisms. Accumulating evidence indicates that commensal bacteria in the gut lumen (termed intestinal microbiota) are involved in GI homeostasis, and the dysregulation of host-microbe interaction may lead to local and systemic disorders. Circadian rhythmicity has long been observed in many visceral functions, including the digestive and absorptive functions of intestinal epithelia (57, 108). There is growing evidence that epithelial turnover and regeneration, gut barrier fortification, and microbiota composition are also controlled by circadian clocks. While an internal timing system based in the brain provides the uppermost signal for synchronizing visceral functions according to daylight cycles, food intake serves as another key trigger independently regulating intestinal circadian rhythm to optimize metabolic functions (46, 162). Recent studies have further

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Received: September 28, 2018; Revised: November 30, 2018; Accepted: December 4, 2018
 ISSN : 0304-4920. http://www.cps.org.tw

Table 1 Commensal microbiota is involved in various aspects of intestinal homeostasis.

Functions	Detailed Mechanisms	References
Metabolic	Fermentation of non-digestible dietary fibers to short chain fatty acids for salvage of energy source	31, 130
	Synthesis of essential vitamins, such as Vit K, Vit B3 (niacin), Vit B7 (biotin), Vit B9 (folate), and Vit B12 (cobalamin)	62, 78
Structural	Regulation of epithelial renewal and repair	148, 183
	Promotion of epithelial differentiation	69, 105
	Fortification of epithelial barrier and tight junctions	2, 199
Protective	Prevention of pathogen colonization by nutrient and anchoring competition	24, 39, 60, 77
	Induction of anti-microbial factors	4, 35
Immune	Production of secretory IgA	90, 146
	Regulation of Th17 differentiation	17, 58, 197
	Induction of oral tolerance	155, 181

shown that the interactions between host and microbiota are involved in the regulation of circadian rhythmicity to maintain gut homeostasis. In this review, we will discuss evidence of circadian clocks in the gastrointestinal tract, explore the cross-talk between host and microbes that contributes to the regulation of clock signals and how microbiota dysbiosis may disrupt the clock system leading to the development of intestinal and metabolic diseases.

Host-Microbe Interaction

Commensal microbes are found in various organs in the human body, including skin, mouth, and the GI tract. The fetal gut is germ-free, but rapidly after birth, microbes populate the GI tract, and the microbial community reaches equilibrium toward an adult-like microbiota approximately 3-5 years of age (129). Variations in microbial density exist along the longitudinal axis from the proximal to distal segments of the GI tract. From a radial viewpoint, the microbes are mixed with food chyme, feces, and loose mucus in the gut lumen, but are spatially segregated from the epithelial layer by a firm mucus layer (193). The highest amount of bacteria is found in the colonic segment with 10^{11}-10^{12} bacteria per gram of luminal content; it is estimated that approximately 100 trillion (10^{14}) bacteria occupy the whole GI tract (88, 138). The total gut bacterial numbers exceeds that of human cells, which is approximately 3.72 x 10^{13} cells (13, 143). When comparing gene numbers, bacterial gene numbers are 100-times higher than that of host genes, making *homo sapiens* only 1% human (122, 143, 144). Along with the massive amount of bacteria, other microorganisms, including viruses, archaea, and fungi, also colonize the GI tract and are collectively defined as the intestinal microbiota (170, 195).

Intestinal Microbiota Composition

Four main phyla of bacteria have been identified in the human GI tract, including Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. More than 1000 species of bacteria have been found in a cohort study; each person harbors approximately 160 species (34, 83, 122). A high inter-individual variability was observed in healthy subjects, whereas only 30-40 species are shared among individuals (34, 83, 122). Throughout the life span of an individual, the gut microbial composition also changes with environment, diet, life style, infection, and age (5, 19).

The commensal microbiota are involved in the maintenance of intestinal homeostasis, including metabolic and structural functions, pathogen-competition and immunomodulatory effects (Table 1). The presence of gut commensals confer direct protection against pathogen colonization by competing for nutrients and receptors and by induction of antimicrobial factors (4, 24, 35, 39, 60, 77), and indirectly *via* immune-enhancing effects through the shaping of the mucosal immune system (17, 58, 197). Early references have shown aberrant lymphocyte homing patterns, altered phenotypes of intraepithelial lymphocytes, and lack of Peyer's patches-associated IgA synthesis in germ-free intestines (48, 90, 95, 175). Others documented that

germ-free mice failed to develop or exhibited a delayed tolerance toward dietary antigens, suggesting that commensals are involved in oral tolerance (155, 181). The metabolic roles of gut bacteria involve production of essential vitamins, degradation of non-digestible dietary substances, and generation of short chain fatty acids as an energy source (21, 62, 78, 130). It was documented that germ-free mice consume 10-30% more food to maintain the same body weight as conventionally raised mice (6, 31). Lastly, the microbes play an active role in regulating intestinal epithelial renewal and differentiation, as well as fortifying epithelial repair and barrier integrity (20, 39, 69, 89, 105, 146). Aside from the GI tract, commensal bacteria are also involved in functions of distant visceral organs, such as hepatic glucose homeostasis and brain development (11, 12, 29, 106, 154). The various health-promoting characteristics have justified the use of the term 'symbionts' for our co-evolved microbial partners in life.

Microbial Regulation of Intestinal Epithelial Functions

Gut microbiota are involved in the control of epithelial turnover by regulating crypt proliferation, cellular apoptosis, and differentiation status. The crypt-villus axis of intestinal epithelia is governed by the pace of stem cell proliferation in the crypts and the rate of cell shedding on the villous surface (Figure 1). Newly divided progenitor cells in the transit amplifying zone undergo differentiation into absorptive epithelial cells, which are highly expressed with digestive enzymes and nutrient and ion transporters. Other secretory progenitors turn into specialized cell types such as mucus-secreting goblet cells, hormone-producing enteroendocrine cells, chemosensory Tuft cells, and Paneth cells with antimicrobial peptide synthesis (8, 50). For details on stemness and differentiation, please refer to other review articles (96, 141, 166). The majority of epithelial cells migrate upward to the villous apex or surface area where cell apoptosis and detachment occurs at the so-called 'extrusion zone', except that Paneth cells migrate downward to the bottom of the crypt (Fig. 1). Paneth cells that produce antimicrobial peptide and Wnt (a molecule to promote stem cell renewal) are located only in small intestinal crypts, but are not found in colonic crypts. Of note, deep crypt secretory cells with Paneth-like functions have been identified in the large intestine (131, 137). Subcellular structures of brush borders and tight junctions are formed on small and large intestinal epithelial cells (i.e. enterocytes and colonocytes) during the differentiation process and are responsible for establishing the gut barrier functions (55, 191). Longer and more densely packed microvilli were observed on enterocytes compared to those on colonocytes. The brush borders prevented physical contact between gut microbes and cellular soma, whereas the tight junctional proteins sealed off the paracellular gaps between adjacent epithelial cells and halted the entry of macromolecules and bacterial particles (79, 87, 188, 189, 192).

A germ-free model of piglets displayed aberrant small intestinal morphology with longer villi and shorter crypts, associated with lesser epithelial apoptosis, compared with their conventionally raised counterparts (26, 183). Others showed that oral inoculation of fecal commensal bacteria or administration of nonpathogenic *Escherichia coli* to gnotobiotic pigs stimulated epithelial apoptosis, but increased crypt depth and proliferation, and elevated brush border enzyme activities in the small intestine compared to those raised in a germ-free environment (72, 148, 183). Furthermore, germ-free animals or mice with bacterial disturbance by antibiotic treatments were more susceptible to chemically induced colitis, and showed impaired epithelial regenerative ability and more extensive denudation of the colonic surface epithelium (49, 121, 125). These findings supported that intestinal microbiota are critical for promotion of epithelial turnover and restitution in a healthy state.

Evidence of commensal bacteria in the fortification of intestinal barrier integrity was also found in studies of germ-free animals or by inoculation of specific bacteria to experimental models of colitis. A thinner mucus layer with lower concentrations of acid and sulphated mucins was documented in the colon of germ-free rats compared to conventionalized counterparts (161). Another study demonstrated variable mucin compositions associated with different microbiota community in the large intestine of two colonies of C57BL/6 mice raised in separate rooms. Despite similar thickness of the colon mucus between the two mouse colonies, the penetrable properties of the inner mucus layer were different and were transmissible to germ-free mice by transfer of caecal microbiota (59).

Abundant studies have showed that several strains of commensal bacteria with known health benefits also strengthen gut epithelial barriers (15, 98). Many known probiotics were isolated from healthy human guts, including *E. coli* Nissle (EcN) 1917, and *Lactobacillus rhamnosus GG* (32, 151). *EcN* 1917 was originally isolated from the stool sample of a healthy German soldier by Alfred Nissle during the First World War in 1917. To date, it is the only probiotic recommended in European Crohn's & Colitis Organization (ECCO) guidelines as effective alternatives to mesalazine in the main-

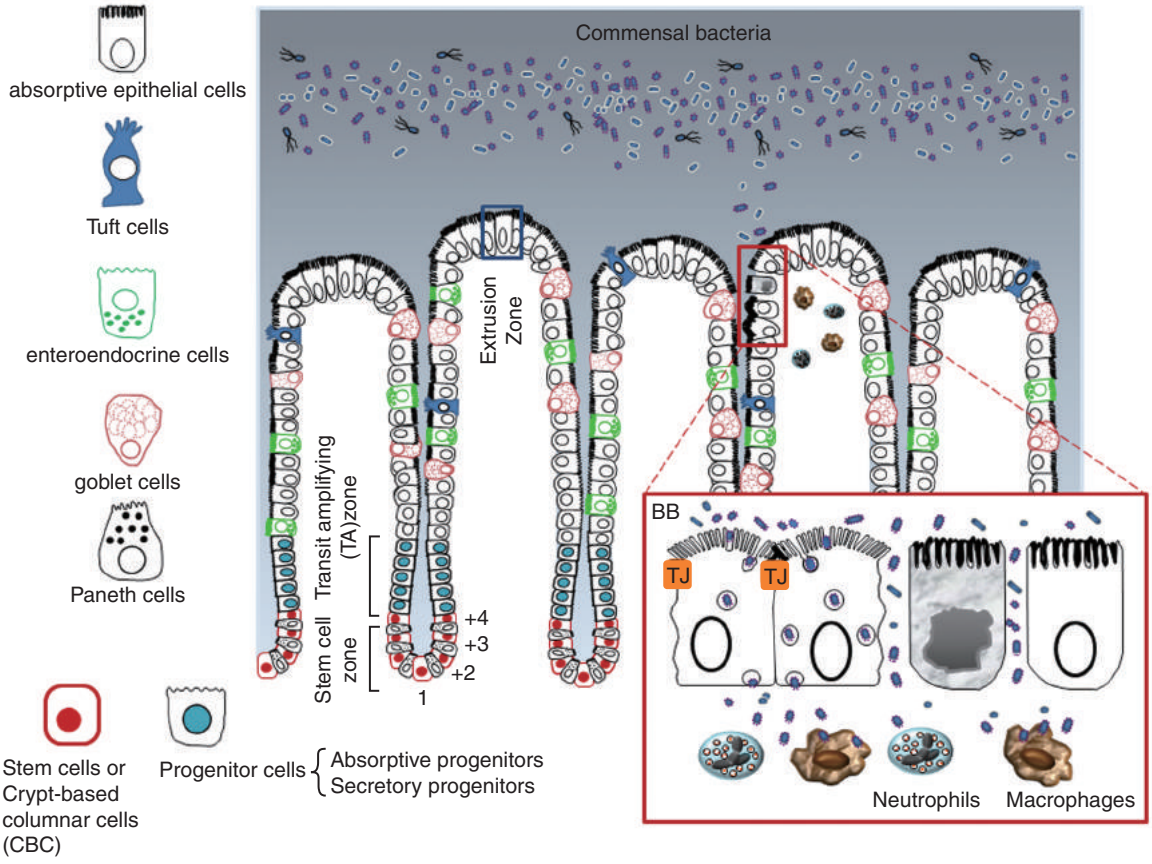

Fig. 1 Dynamic turnover of epithelial cells for maintenance of gut homeostasis. At the bottom of intestinal crypts, highly proliferative crypt-based columnar cells (CBC) divide to +4 stem cells and daughter cells (transit amplifying zone) that are progenitors of either absorptive or secretory types. Five cell lineages with specialized functions differentiate from the progenitors in small intestine (four cell lineages in large intestine) – Paneth cells, goblet cells, enteroendocrine cells, tuft cells, and absorptive epithelial cells. The majority of newly divided cells migrate upward to the villous tip or surface area within 5-7 days, whereby cell apoptosis and detachment occurs at the so-called 'extrusion zone'. An exception is the Paneth cells which migrate downward and intermingle with the CBCs. Although Paneth cells are localized only in small intestinal crypts, Paneth-like cells are present in the colon. The secretory progenitors differentiate into mucus-producing goblet cells or antimicrobial peptide-producing Paneth cells, and into hormone-releasing enteroendocrine cells. The origin of tuft cells which express chemosensory receptor similar to that of enteroendocrine cells remain unclear. The absorptive progenitors differentiate to absorptive epithelial cells (enterocytes and colonocytes) with brush borders (BB) and tight junctions (TJ). The BB on epithelial cells not only constitutes a large surface area for digestive and absorptive activity, it also serves as a barrier ultrastructure against bacterial penetration. The TJ complex linking adjacent cells forms another key ultrastructure for preventing intercellular leakiness. In pathological conditions, transcellular and paracellular barrier disruption and/or epithelial cell death leads to bacterial translocation, followed by activation of phagocytes such as neutrophils and macrophages (Inset). Despite the defensive nature of the host to fend off bacterial invasion by forming an epithelial barrier, the gut microbiota is involved in intestinal physiology. The epithelial barrier integrity is crucial for the maintenance of this interdependent symbiotic relationship which is established under an immunotolerance state.

tenance of remission in ulcerative colitis patients (139). Another example is the *Lactobacillus rhamnosus GG* which was also isolated from the intestinal tract of a healthy human by Gorbach and Goldin in 1983. Pretreatment with *EcN* 1917 or *Lactobacillus species* inhibited intestinal hyperpermeability and prevented colonic cell apoptosis in the mouse models of colitis (91, 101, 172, 198). Others reported that *EcN* 1917 and *Lactobacillus* species upregulated tight junctional proteins and reorganized the paracellular junctional structures in *in vitro* human epithelial cell models (2, 67, 140, 142, 199), suggesting a direct effect of gut microbes in restoring epithelial barriers.

While critical for gut physiology, intestinal microbes could pose a threat to the host upon barrier breach and induce local inflammation, bloodstream infection, and septicemia. A detrimental role of commensals was found in colonic inflammation and colitis-associated cancer formation. Previous studies showed that IL-10(-/-) mice that had developed spontaneous colitis in conventionalized housing showed no signs of inflammation when raised in germ-free environments (63, 65). Similarly, germ-free carcinogen-treated IL-10(-/-) mice were devoid of tumors in the distal colon (173). Moreover, growing evidence indicate that altered microbial compositions (termed microbial dysbiosis) and conversion of commensal-derived opportunistic pathogens (termed pathobionts) are responsible for intestinal disorders. This may account for the Janus-like behavior of gut microbiota between symbiotic and pathobiotic bacteria. For information on intestinal dysbiosis and pathobionts in diseases, please refer to review articles elsewhere (194, 195). Overall, gut microbiota are involved in the maintenance of epithelial homeostasis. However, in dysbiotic conditions, gut microbiota may be culprits in disease development.

Circadian Rhythmicity

In the 18th century, Jean-Jacques d'Ortous de Mairan started to investigate daily rhythms in nature. He observed Mimosa leaves as a model to measure their response to alternating night and day periods and found that the leaves opened only in the presence of sunlight (92). Because the opening and closing patterns followed a 24-hour period, the phenomenon was named "circadian rhythms". Since then, the circadian clock was identified in many other organisms, including bacteria, mammals and humans (47, 70, 102).

Circadian rhythmicity is defined as an instinctive adaptive ability for responding to external stimuli that follows a daily cycle and is essential for organism survival. The alignment of the inner clock (including central and peripheral clocks) to an external rhythm is called entrainment (127). As the central clock is located in the suprachiasmatic nucleus (SCN) of the ventral periventricular zone of hypothalamus, it is controlled by light–dark cycles through input stimuli from the optic nerve of the eye into the primary circadian oscillator (18, 126). The SCN signals the pineal gland to secrete melatonin to drive daily rhythmicity in target tissues. The SCN also synchronizes the peripheral clocks in other neuronal or non-neuronal tissues through temporal sympathetic and parasympathetic signals (117, 182). In addition to daylight, patterns of dietary intake also regulate rhythmicity in the human body at cellular, organ, and system levels or even behaviors (42, 53, 134, 147).

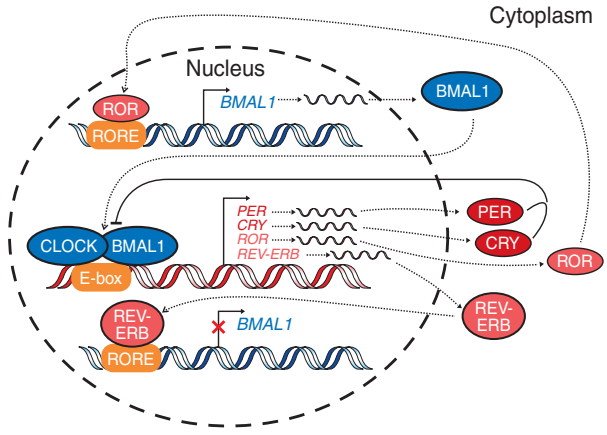

Fig. 2 Clock-related gene expression that exhibits a 24-hour cycling pattern. There are two core circadian clock genes, including circadian locomotor output cycles kaput (*CLOCK*) and brain and muscle aryl hydrocarbon receptor nuclear translocator-protein 1 (*BMAL1*). The CLOCK-BMAL1 heterodimers interact with E-box elements in the promoter regions of downstream target genes, such as period (*PER)* and cryptochrome (*CRY)*. The accumulating PER and CRY proteins subsequently constitute heterodimers and relocate to the nucleus to suppress the transcriptional activity of CLOCK and BMAL1, forming a negative feedback loop as the primary autoregulatory pathway. In addition, the CLOCK-BMAL1 heterodimers also increase the expression of nuclear receptors retinoic acid-related orphan receptor alpha (RORα) and reverse erythroblastosis virus alpha (REV-ERB) as the secondary autoregulatory pathway. The RORα serves as the activator whereas REV-ERB acts as the repressor for control of BMAL1 production. The REV-ERB protein competes with RORα for binding to ROR response elements (RORE) for the transcription of *BMAL1*. The two autoregulatory pathways regulates the clockwork to operate in daily periodicity.

The central clockwork in the SCN neurons is controlled by a set of genes known as clock genes and clock-controlled genes. This molecular clock is an interlocking transcription/translation feedback loop that regulates various gene expression at different time points to exhibit an endogenous 24-hour pattern (Fig. 2). The two core circadian clocks, i.e., circadian locomotor output cycles kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator-protein 1 (BMAL1), contain basic helix-loop-helix (bHLH)–PER-ARNT-SIM (PAS) domains to form heterodimeric transcription factors (51, 182). The CLOCK-BMAL1 dimer binds to E-

box motifs of the promoter regions of two groups of genes, period (*PER)* and cryptochrome (*CRY)* for transcriptional activity (73, 176). The accumulation and dimerization of PER and CRY proteins subsequently constitute multimers and relocate to the nucleus, where they directly interact with CLOCK and BMAL1 to inhibit downstream transcription and thus, form a negative feedback loop. After a period of time, the degradation of PER and CRY *via* ubiquitination leads to the activation of a new cycle (145, 196). A second autoregulatory pathway, consisting of a positive regulator, nuclear receptor retinoic acid-related orphan receptor alpha (RORα), and a negative regulator, reverse erythroblastosis virus alpha (REV-ERB), is involved in the control of circadian oscillation. RORα binds to ROR response elements (ROREs) for the transcription of *BMAL1*, whereas REV-ERB competes with RORα for binding to the specific response element (44). Recent studies have also demonstrated that RORγ directly regulates the circadian expression of *Clock* gene and a neuronal paralog of *Clock* (*Npas2*) (163). Overall, the RORα/REV-ERB system stabilizes the clock loops and helps to maintain accurate circadian timing (Fig. 2).

Ablation of the SCN does not annihilate the peripheral rhythms, but results in a desynchronization of the two clockworks (45, 190). This observation coincides with theories that circadian rhythms endogenously exist but external stimuli, such as daylight, help synchronize the inner clocks. The peripheral clock in the GI tract communicates with the central clock *via* direct neurohormonal inputs. This clock also adapts to external cues, such as meal consumption timing and diet composition, to optimize metabolic functions (94, 97). In the following section, circadian rhythms of the GI tract will be discussed.

Circadian Clocks in Gastrointestinal Physiology

A number of GI functions, including digestion, absorption, epithelial renewal and barrier, are linked to circadian regulation. The presence of circadian clock genes, including *Clock, Bmal1, Per1/2/3, Cry1/2* and *Rev-erbA,* has been documented in all regions of the rodent GI tract (38, 52, 149). Gradual increases in the expression levels of clock genes have been observed from the duodenum to colon; the expression levels are more abundant in epithelial cells compared with other mucosal cells (52, 113, 149). Although circadian controls for the regulation of gastric and small intestinal functions have been more extensively studied (see below), the highest levels of clock genes were found in the colonic epithelia, suggesting unknown circadian regulation mechanisms for colon-specific functions other than nutrient uptake.

The time of eating is known as a strong environmental synchronizer for intestinal rhythms. However, anticipation of feeding is a key trigger for GI-related hormone fluctuation (116). A number of hormones oscillate in a daily cycle in anticipation of feeding, including ghrelin, leptin, gastrin, corticosterone, insulin, glucagon, and glucagon-like peptide 1 (17, 76, 81). Early studies in mouse models documented food-related clocks in oxyntic gland cells that produce ghrelin, Per1, and Per2 in a rhythmic fashion (75, 81). The absence of *Per1* and *Per2* removed the rhythmic release of ghrelin. However, ghrelin also acts directly on SCN, which was identified by the expression of ghrelin receptors, implying an interplay between food oscillators and the circadian rhythm (75). Other studies have shown circadian variations in serum gastrin concentration and in gastrin receptor expression in the fundus in both freely fed and fasted rats (132). Gastrin-releasing peptide produced by enteroendocrine cells may induce phase-resetting in the SCN neurons (40, 61), supporting peripheral entrainment to the master clock.

The circadian clock genes are also involved in rhythmic cycles of ion, sugar, drug and peptide transporter expression in small intestinal epithelial cells. Diurnal rhythmicity of *Sglt1*, *Glut2,* and *Glut5* were documented in rat and mouse enterocytes, with high levels of mRNA expression observed in the afternoon and evening (7, 128, 165). Peptide absorption *via* H+-peptide cotransporter Pept1 also displayed diurnal rhythmicity as a peak signal; L-histidine absorption coincided with the highest levels of Pept1 expression during the dark phase in nocturnal animals (111). Diurnal variations for lipid absorption were also found in mouse small intestine and in primary enterocytes (112, 113). Recent studies using murine *Clock* mutant models demonstrated higher rates of monosaccharide and lipid absorption associated with increased basal expression of glucose transporters (Sglt1, Glut2 and Glut5), compared with the wild type. However, peptide absorption by Pept1 was much lower compared with wild-type mice (113). The detailed mechanisms of clock-related transcriptional activity of nutrient transporters remain unclear and warrant further studies.

Gastrointestinal epithelial proliferation is controlled by the circadian clock in crypt cells directly or indirectly by following rhythmic hormonal fluctuations. The circadian levels of gastrin impact its mucosal trophic functions (132, 184). Additionally, glucocorticoids with peak levels in the early morning in diurnal species (or in the early night in noc-

turnal animals) also modify the rates of crypt cell proliferation through the upregulation of p21 and c-Myc (30, 36, 93). A study of the human colon demonstrated the circadian rhythmicity of cyclins D1 and E and cyclin-dependent kinase inhibitors p16 and p21 (43). Moreover, the rhythmic expressions of clock genes *Per1/2*, *Bmal1* and *Rev-Erbα* and a clock-driven gene *Wee1* (a cell cycle checkpoint kinase) in epithelial cells were observed to synchronize with a phase delay in the cranio-caudal axis from the duodenum to distal colon in rats (118). A recent study showed that rhythmic proliferation and regeneration of intestine epithelia were disrupted in the absence of Bmal1 by using mouse mutant *in vivo* and intestinal organoid cultures *in vitro* (153). Another report demonstrated the loss of Paneth and goblet cells, impaired crypt proliferation, and enhanced necrotic cell death in small intestines of *Per1/2* mutant mice, suggesting a role of circadian genes in regulating epithelial cell turnover and differentiation (109).

A growing body of evidence indicated that gut barrier functions are under circadian control. Expression levels of tight junctions, such as occludin and claudin-1, exhibited circadian variations in the colonic epithelia that correlated with altered intestinal permeability in wild-type mice (107). Mice with *Clock* mutant genes displayed increased baseline intestinal permeability as demonstrated by heightened urinary excretion of sucralose in a sugar drinking test (157). Additionally, *Per1/2* mutant mice showed mucosal barrier disruption due to enhanced necroptotic cell death in the epithelium (109). Overall, self-sustained and entrained clock signals in epithelial cells are essential for the temporal control of GI functions. Environmental factors, including daylight, food, and microbiota, all play regulatory roles in intestinal epithelial homeostasis.

Circadian Dysrhythmia in Intestinal and Metabolic Disorders

Epidemiology studies have demonstrated that shift workers and human subjects with sleep interruption exhibited higher risks for inflammatory bowel disease (IBD) (1, 82), diabetes, obesity and metabolic syndrome (10, 179). The observation suggested that circadian disturbance may be involved in the pathogenesis of IBD and metabolism-related disorders (MRDs). However, the causal relationship has been challenged due to difficulties in differentiating the order of sleep disturbances and early subclinical lesions in patients (114, 158, 159). Evidence linking circadian dysrhythmia with IBD and MRDs in patients and experimental models will be highlighted here.

Inflammatory Bowel Disease

IBD is characterized by chronic inflammation in the gut which arises as a result of the interaction of genetic, environmental, barrier and microbial factors. A link between circadian disruption and inflammatory disease activity has been suggested in IBD (150). A report using genome-wide cDNA microarrays demonstrated the upregulation of BMAL1 and RORα and downregulation of PER1/3 in mucosal biopsies of Crohn's patients when compared with healthy colonic mucosa (110). In biopsies of ulcerative colitis, the clock genes *BMAL1, RORα,* and *CRY1* were upregulated whereas *PER3* was downregulated (110). Reductions in mRNA expressions of all circadian genes (*BMAL1, CLOCK, PER1/2,* and *CRY1/2*) were observed in intestinal biopsies and peripheral blood mononuclear cells in IBD patients compared with healthy controls (85). However, the single time point for biopsy collection in individuals may have caused variations in data interpretation. Experimental models with chemically induced colitis and clock gene disruption have provided further information on a causal relationship.

Variable fluctuating patterns of clock gene expression were observed at different Zeitgeber times (ZT) in colitic mice after giving dextran sodium sulfate (DSS) in drinking water (85). Conversely, chronic intermittent but not acute sleep deprivation worsened the histological and clinical manifestation of DSS-induced colitis in wild-type mice (120, 164). Genetic ablation of *Per1/2* in mice increased susceptibility to DSS-induced intestinal inflammation partly by clock gene-associated epithelial necroptosis and reduction of secretory cells (i.e., Paneth and goblet cells) (109). Other studies showed that circadian disruption aggravated alcohol-induced intestinal hyperpermeability with the use of environmental factors by altering the daily light/dark cycle in humans (160) and in genetic models of *Clock*-mutant mice (157). Taken together, the findings suggested that circadian dysrhythmia impaired gut barrier function and exacerbated chronic inflammation in the intestine.

Metabolism-Related Disorders

Circadian disruption was documented in patients with diabetes and obesity. Based on genome-wide association studies, single-nucleotide polymorphisms in clock genes, such as *CLOCK* and *CRY1/2*, were identified in cohorts with metabolic syndrome (28, 71). Gene mutations in *BMAL1* and *CLOCK* were associated with the incidence of type 2 diabetes (23, 185). Moreover, genetic variants of

CLOCK were also linked with susceptibility to being overweight and obese (41, 152).

Experimental models of *Clock*-mutant mice showed various symptoms of MRD with increased levels of triglycerides, glucose, and leptin and were associated with decreased insulin secretion when maintained under a high-fat diet (133, 169). Systemic and liver-specific Bmal1-deficient mice displayed impaired gluconeogenesis, glucose tolerance, and insulin sensitivity (74, 133). Moreover, Cry1/2-deficient animals showed increased insulin secretion and lipid storage and are more susceptible to gaining weight than wild-type animals (9). In addition to gene mutant models, altering the feeding period to a rest phase caused a 12-hour shift of the clock genes associated with hypoinsulinemia and increased free fatty acids and glucagon levels during the active phase (103). Long term high-fat diets alter the expression of circadian genes in the intestine, adipose tissues, liver, kidneys and brains in animal models (54, 66). These findings indicated bidirectional effects between circadian dysrhythmia and metabolic syndrome.

A Novel Concept: Microbiota-Dependent Circadian Setting and Dysrhythmia

Intestinal dysbiosis characterized by reduced taxonomic richness and bacterial population changes in fecal samples were observed in patients with IBD (14, 68, 136) and MRDs (*e.g.*, diabetes and obesity) (3, 37, 123). Accumulating evidence has indicated that dysbiotic bacteria play critical roles in disease pathogenesis, including IBD and MRD, as shown in fecal transplantation and monoassociation experiments in germ-free models (22, 56, 64, 86, 115, 156, 168, 171, 187). Mucosa-associated bacteria with adherent and invasive characteristics, which were found enriched in Crohn's patients, harbored colitogenic ability (16, 27, 33). A wide array of mechanisms has been proposed to explain bacteria-associated pathogenesis along with the rapidly growing field of research on microbiota in gut physiology. Gut microbes have been implicated in circadian clock setting. Furthermore, accumulating evidence has indicated the possibility of dysbiosis-driven circadian disruption for driving chronic disease progression.

Previous reports documented that circadian disruption and sleep deprivation caused changes in the intestinal microbiota composition. Weekly phase reversals of light/dark cycles altered the composition of gut microbiota in mice given high-fat, high-sugar diet but not in those fed standard chow (177). Another study demonstrated that chronic sleep deprivation caused gut microbiota changes in mouse fecal samples with higher levels of *Lachnospiraceae* and *Ruminococcaceae* and lower levels of *Lactobacillaceae* families (119). Others showed that animals kept in constant darkness lost rhythmic oscillations in intestines associated with overabundant Clostridia in the microbial population (186). Furthermore, reduced microbial diversity and altered bacterial composition were documented in *Clock*-mutant, *Per1*-mutant and Bmal1-deficient mice fed with regular chow diet (84, 168, 178). Overall, circadian disorganization alters microbiota community. However, the responsible mechanism, whether by a direct effect on bacteria or indirectly through immune changes, remains unknown.

Multiple lines of evidence have indicated that gut microbiota influence clock setting in the intestine and distal organs under physiological conditions and is involved in circadian dysrhythmia in MRD models. Gut commensals exhibited diurnal oscillations in terms of bacterial composition, metabolite synthesis, and mucosal adherence driven by feed intake patterns in healthy wild-type mice (80, 167). Recent findings have suggested that food consumption and gut microbiota are intricately involved in intestinal clock setting through interdependent or individual pathways. Antibiotic treatment caused microbial depletion and absence of bacterial adherence oscillation, leading to either loss or gain of transcriptional rhythmicity in colonic tissues (167). Despite regular feeding schedules, there were lost rhythms, including nucleotide replication and cell-cycle pathways, and added rhythms, such as pyruvate metabolism, tricarboxylic acid cycles and tight junctions in colonic tissues (167). This study showed that microbial depletion by antibiotics did not impact the core circadian clock machinery in mouse intestinal tissues but only influenced the promoter and enhancer activity to drive downstream rhythmic gene expression (167). However, others have reported that antibiotic treatment caused disruption in the clock genes of intestinal epithelial cells, including decreased *Bmal1* and *Cry1* and increased *Per1/2* (104). Moreover, intestinal microbial signaling through toll-like receptors was shown to orchestrate intestinal epithelial circadian clocks and regulate clock-dependent corticosterone synthesis in mouse studies (104). In addition to local tissues, gut microbiota also regulated the circadian oscillation in the liver and hypothalamus in studies comparing germ-free and antibiotic-treated animals with conventionally raised controls (80, 167).

Consumption of a high fat (HF) diet leading to MRDs, such as obesity and diabetes, is a long-known fact. Evidence of germ-free mice being resistant to HD-induced obesity and insu-

lin resistance indicated a role of gut microbes in energy metabolism (6, 124). Moreover, glucose intolerance and obesity were induced in germ-free mice upon transplantation of jet-lag- or HF diet-associated microbiota, suggesting that MRD-related fecal dysbiosis was transmissible (115, 156, 168, 171). A growing body of evidence further indicated that intestinal microbiota regulated body fat accumulation through circadian modulation in intestinal epithelia and distant organs (80, 180). Heightened expression and oscillation of epithelial Rev-Erbα was associated with suppressed levels of NFIL3 (also known as E4BP4, a protein related to lipid absorption and metabolism) in germ-free mice and antibiotic-treated animals (104, 180). Interestingly, the microbial impacts on epithelial Rev-Erbα and NFIL3 were mediated by MyD88 signaling of dendritic cell-group 3 innate lymphoid cells but not *via* direct activation on the epithelium (180). A recent study showed that a diurnal oscillation of gut microbiota and butyrate synthesis directly impacted expression of clock genes in the liver by using hepatic organoid cultures *in vitro* and a mouse model *in vivo* (80). However, different patterns of clock genes modulated by HF were observed in distant organs: suppressed *Bmal1* and *Clock*, *Per1*, and *Cry1* expressions were found in the liver but enhanced expressions were noted in the brain (80). Overall, the traditional concept of high fat promoting energy harvest and obesity is complemented with an additional environmental factor of intestinal microbiota *via* regulating circadian rhythm.

Although a clear role of intestinal pathobionts was established in IBD development, a link between microbiota dysbiosis and circadian dysrhythmia remains elusive. The mechanistic studies underlying gut dysbiosis in chronic inflammation has been limited to altered microbial metabolites and bacterial influx-induced mucosal immune hyperactivation. Considering that bacterial invasion to the interfacing epithelia occurs prior to exposure to immune cells, clock genes with abundant expression in colonic epithelial cells may be dominant players in intestinal homeostasis dysregulation and predisposition of disease onset. Previous studies demonstrated pathophysiological changes of intestinal epithelia in IBD patients, such as an abnormal expression of PEPT1 on colonocytes, leading to augmented neutrophil activation (99, 100). Colonic epithelial PEPT1 mediated the transport of bacterial peptides, including formylated methionyl-leucyl-phenylalanine, muramyl dipeptide, and tripeptide L-Ala-gamma-D-Glu-meso-DAP (25, 174). It remains unclear whether abnormal transporter expression in colonic epithelia is a consequence of circadian disturbance. Overall, more research is required to understand the cross-talk between microbiota dysbiosis and circadian dysrhythmia in colitis models.

Concluding Remarks

Significant progress has been made to understand the impact of gut microbiota on the well-being of a host. A growing body of evidence further places microbiota dysbiosis at the center of the pathogenesis of multiple chronic disorders. Irregular sleeping and meal intake schedules causing circadian dysrhythmia has long been suggested to increase the risk of developing metabolic and inflammatory diseases. Now, with evidence showing reciprocal actions between microbiota and clockwork, these two seemingly independent factors have merged into one pathway in the context of gastrointestinal functions. There is a need for more comprehensive understanding of host-microbe interplay for clock setting and how misalignment of gut microbiota and circadian rhythm may contribute toward the onset or aggravation of pathological lesions in intestines and distant organs.

Acknowledgments

The work was supported by Ministry of Science and Technology, Taiwan (MOST 107-2320-B-002-041-MY3), National Health Research Institute (NHRI-EX105-10520BI, NHRI-EX106-10520BI, NHRI-EX107-10520BI, NHRI-EX108-10823BI, NHRI-EX109-10823BI, NHRI-EX110-10823-BI), and National Taiwan University (NTU-CDP-105R7798, NTU-CCP-106R890504).

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