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Review article

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GUT CLOCK: IMPLICATION OF CIRCADIAN RHYTHMS IN THE GASTOINTESTINAL TRACT

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Circadian and seasonal rhythms are a fundamental feature of all living organisms and their organelles. Biological rhythms are responsible for daily food intake; the period of hunger and satiety is controlled by the central pacemaker, which resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, and communicates with tissues *via* bidirectional neuronal and humoral pathways. The molecular basis for circadian timing in the gastrointestinal tract (GIT) involves interlocking transcriptional/translational feedback loops which culminate in the rhythmic expression and activity of a set of clock genes and related hormones. Interestingly, it has been found that clocks in the GIT are responsible for the periodic activity (PA) of its various segments and transit along the GIT; they are localized in special interstitial cells, with unstable membrane potentials located between the longitudinal and circular muscle layers. The rhythm of slow waves is controlled in various segments of the GIT: in the stomach (about 3 cycles per min), in the duodenum (12 cycle per min), in the jejunum and ileum (from 7 to 10 cycles per min), and in the colon (12 cycles per min). The migrating motor complex (MMC) starts in the stomach and moves along the gut causing peristaltic contractions when the electrical activity spikes are superimposed on the slow waves. GIT hormones, such as motilin and ghrelin, are involved in the generation of MMCs, while others (gastrin, ghrelin, cholecystokinin, serotonin) are involved in the generation of spikes upon the slow waves, resulting in peristaltic or segmental contractions in the small (duodenum, jejunum ileum) and large bowel (colon). Additionally, melatonin, produced by neuro-endocrine cells of the GIT mucosa, plays an important role in the internal biological clock, related to food intake (hunger and satiety) and the myoelectric rhythm (produced primarily by the pineal gland during the dark period of the light-dark cycle). This appears to be an endocrine encoding of the environmental light-dark cycle, conveying photic information which is used by organisms for both circadian and seasonal organization. Motor and secretory activity, as well as the rhythm of cell proliferation in the GIT and liver, are subject to many circadian rhythms, mediated by autonomic cells and some enterohormones (gastrin, ghrelin and somatostatin). Disruption of circadian physiology, due to sleep disturbance or shift work, may result in various gastrointestinal diseases, such as irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD) or peptic ulcer disease. In addition, circadian disruption accelerates aging, and promotes tumorigenesis in the liver and GIT. Identification of the molecular basis and role of melatonin in the regulation of circadian rhythm allows researchers and clinicians to approach gastrointestinal diseases from a chronobiological perspective. Clinical studies have demonstrated that the administration of melatonin improves symptoms in patients with IBS and GERD. Moreover, our own studies indicate that melatonin significantly protects gastrointestinal mucosa, and has strong protective effects on the liver in patients with non-alcoholic steatohepatitis (NASH). Recently, it has been postulated that disruption of circadian regulation may lead to obesity by shifting food intake schedules. Future research should focus on the role of clock genes in the pathophysiology of the GIT and liver.

Key words: *cancerogenesis, circadian rhythms, food intake, gut clock, irritable bowel syndrome, light-dark cycle, melatonin, migrating motor complex, periodic motor activity*

INTRODUCTION

Rotation of the earth around the sun, and on its own axis, imparts light and dark cycles of 24 hours, which is the dominant environmental factor affecting living organisms. Organisms have developed the ability to predict these cycles, and have evolved to restrict their activity to the day or night-diurnal or nocturnal, respectively. Circadian rhythms are endogenously generated rhythms that occur with a periodicity of approximately 24 hours, and play a fundamental role in the survival and evolution of life. The circadian timing system regulates daily rhythms of physiology and behaviour, enabling organisms to anticipate periodic changes in the environment and develop important adaptive mechanisms. Moreover, circadian rhythms enable optimal energy utilization and reproduction (1). Virtually all aspects of human physiology (sleep-wake cycles, body temperature, hormone secretion *etc*.) are mapped onto 24-hour rhythms. However, modern life styles may frequently disrupt circadian rhythm. This circadian dysfunction is considered to be an important contributory factor

to the incidence of a wide range of clinical conditions including sleep disorders, gastrointestinal diseases, metabolic syndrome, inflammation, and even cancer (2).

The present review is focused on the role of the circadian system in the GIT, and the clinical consequences disrupting this system.

CENTRAL AND PERIPHERAL CLOCK OSCILLATORS

The clock system is complex, and consists of the central circadian clock ("master clock") located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus in the brain, and peripheral oscillators, which are not entirely autonomous (*Fig. 1*). Surgical ablation and transplantation experiments established that the SCN, coordinates most of the daily rhythms. The master SCN clock is composed of multiple, single-cell circadian oscillators, which, when synchronized, generate coordinated circadian outputs that regulate rhythms. In addition, in peripheral tissues such as GIT, liver, muscle or adipose tissue, similar clock oscillators have been found. The SCN sends signals to peripheral oscillators to prevent the dampening of the circadian rhythms in peripheral tissues. This task can be accomplished *via* neuronal connections or circulating humoral factors (3).

The SCN does not oscillate exactly over 24 hours. Therefore, it is necessary to entrain this circadian pacemaker to daily external light-dark cycle. Light is the most powerful signal regulating SCN. It is perceived by the retina, and the signal is transmitted *via* the retinohypothalamic tract (RHT) to the SCN. In addition to light, feeding occurring *via* GIT, and the availability of metabolites, represents another important and potent synchronizer for central and peripheral clocks (4).

MOLECULAR CLOCK

The key breakthrough in understanding the circadian system was discovery of the molecular mechanisms of the circadian clock ("molecular clock"). Molecular mechanisms underlying circadian rhythms are conceived as a series of interlocking molecular loops, involving rhythmic transcription of specific "clock genes", and interactions of the proteins they encode (*Fig. 2*). In a simplified model, these clock genes comprise "positive elements" such as clock and Bmail1, whose protein products dimerize, enter the nucleus, and stimulate transcription of negative elements period 1, 2, and 3 (Per 1-3) and cryptochrome (Cry 1-2). The protein products of these genes (*per 1-3*, *cry 1-2*) in turn oligomerize, enter the nucleus, and suppress the activity of the clock/Bmail1 complex.

In recent years, multiple molecular feedback loops (molecular clock machinery) were identified, which further fine-tune generation of intracellular circadian rhythms. A number of important nuclear receptors such as REV-ERB, ROR or PPAR, interact closely with circadian feedback loops. The regulation of clock genes, by nuclear receptors, renders the clock responsive to numerous circulating hormones (*e.g.* cortisol, estrogen), nutrients signals (*e.g.* fatty acids derivatives) and cellular redox status (NADH/NAD^+ratio). Nuclear receptors are now recognized as a key intermediaries between the molecular clock machinery and a wide array of physiological processes (5-7).

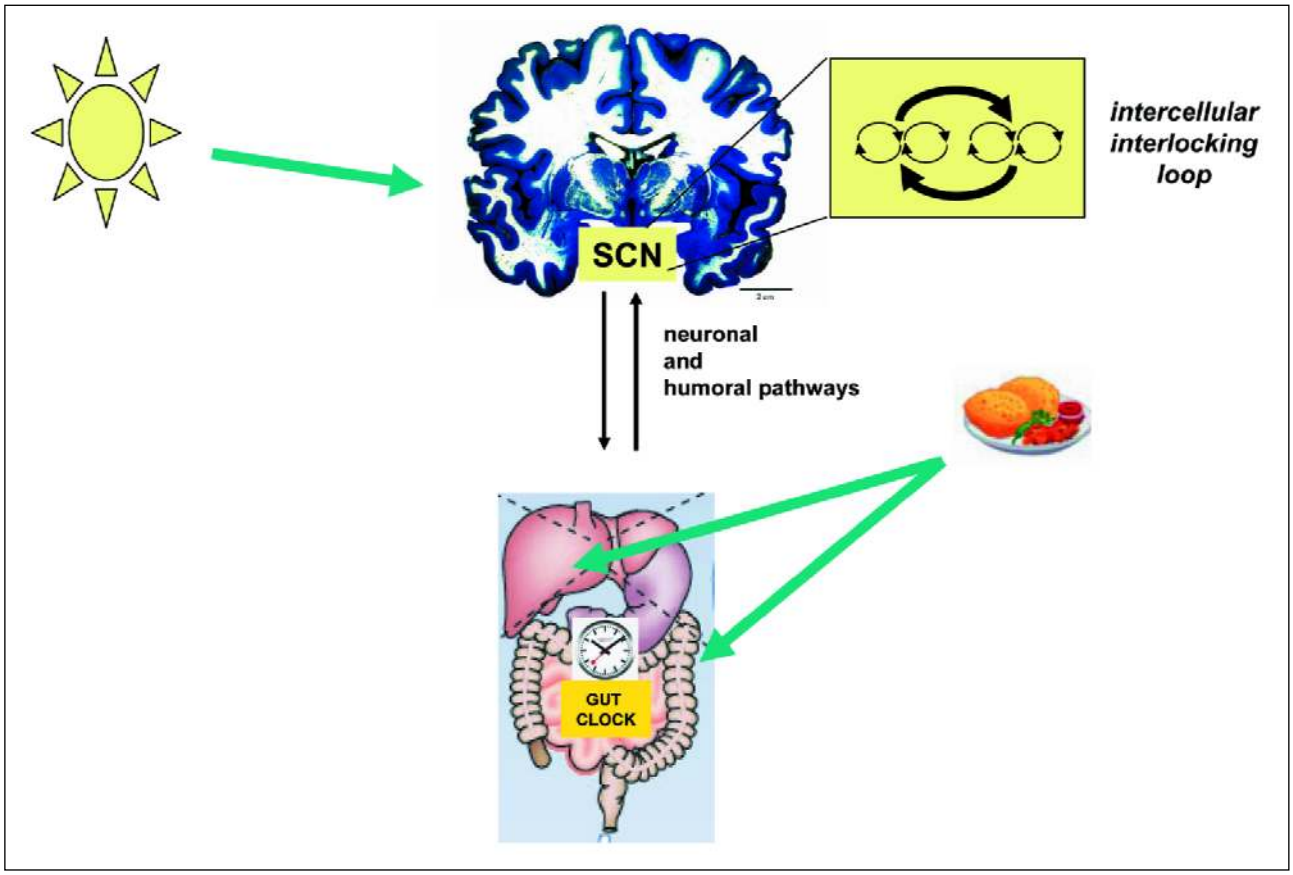

Fig. 1. Schematic presentation of central clock located in suprachiasmatic nucleus of hypothalamus and peripheral clocks, The circadian clocks are entrained by light (→ light entrainable oscillators) and food (→ food entrainable oscillators).

FOOD ENTRAINABLE OSCILLATORS

In all mammals, daily fluctuations of CNS arousal and activity in anticipation of a meal, termed food anticipatory activity (FAA), are observed. This activity depends on an endogenous circadian timing system termed food entrainable oscillators (FEO). The activity of FEO precedes the mealtime, and promotes eating behaviour (8, 9). The localization of FEO is still a matter of debate, but recent studies suggest the localization of FEO in the stomach (10). Oxyntic gland cells co-express ghrelin (important orexigenic peptide stimulating food intake) and circadian clock proteins such as PER1 and PER2. The expression of PER1, PER2 and ghrelin is rhythmic in light dark cycles, but remains constant in darkness, with *ad libitum* food. Interestingly, in the absence of the circadian clock genes *per1* and *per2*, ghrelin is no longer rhythmically expressed. These results point out an important role of the stomach in regulating the timing of meals, promoting anticipatory arousal, and inducing eating behaviour. Some forms of obesity have been associated with dysregulation of food intake, including night eating syndrome (NES) and compulsive overeating (11). Furthermore, the central timing system that regulates melatonin rhythms, was also phase delayed, similar to that observed for leptin. Thus, NES may have its roots in abnormalities of the peripheral (*e.g.*, stomach, liver) and/or central (*e.g.*, suprachiasmatic nuclei) circadian timing system.

REGULATION OF CENTRAL AND PERIPHERAL OSCILLATORS BY MELATONIN

Melatonin, produced in the pineal body, resynchronizes the SCN by providing information about light/darkness from the retinohypothalamic tract. Melatonin is also essential for regulation of rhythmic functions in peripheral target tissues of the clock. Previous studies strongly suggest that melatonin plays not only an important role in resynchronization between the central master clock and peripheral clocks, but also possesses very strong anti-oxidant properties (*Fig. 4*). On the other hand, SCN drives nocturnal melatonin synthesis *via* the sympathetic nervous system. Plasma melatonin concentration follows a daily rhythm, with high levels during the night. This is why it is called the hormone of darkness" (12-13).

Humans are unique in that they voluntarily shift their activity period to abnormal times of day, effectively forcing the misalignment between their activity period and their internal circadian clock. This is why disruption of circadian rhythms may have negative effects in both the short (fatigue, insomnia,

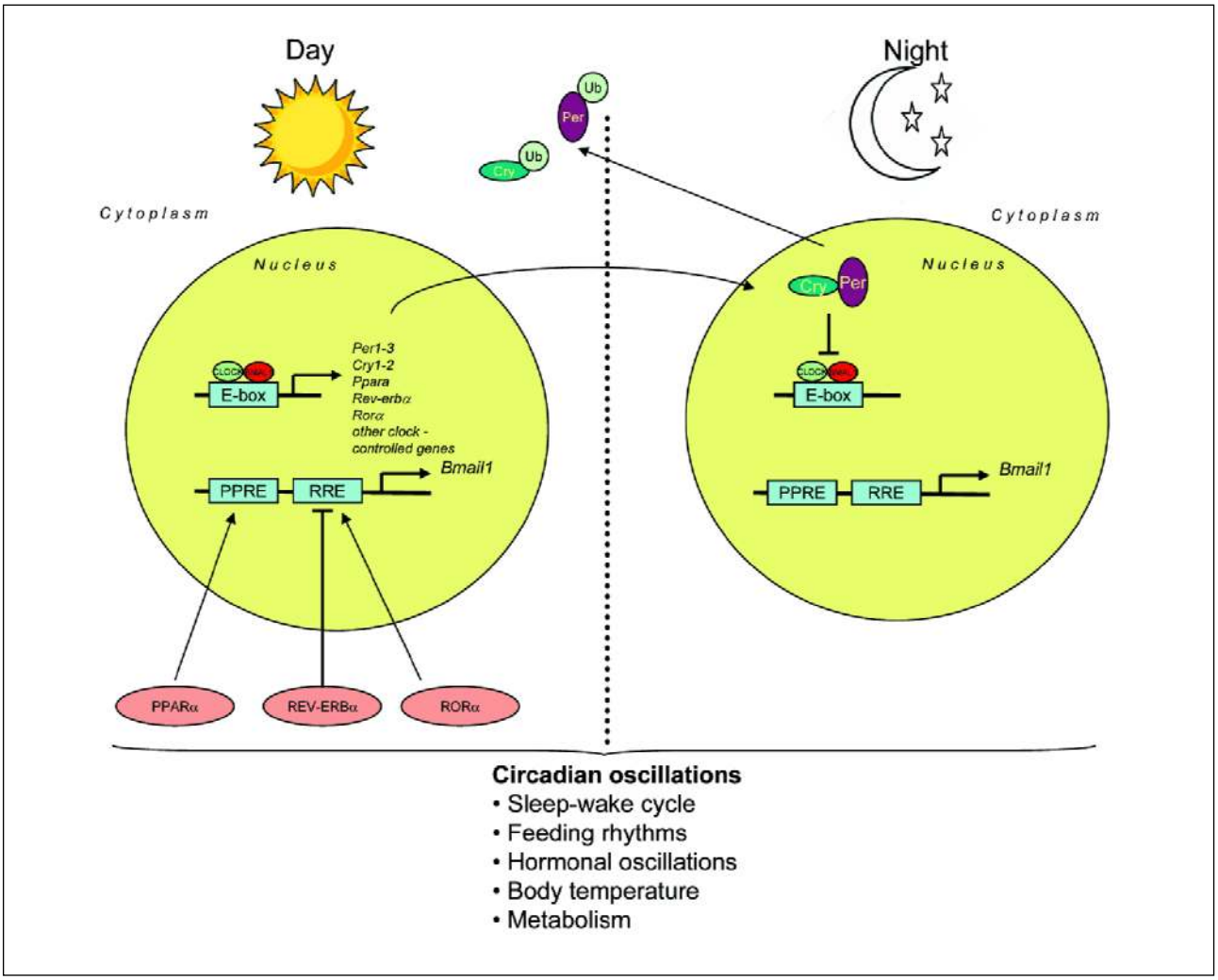

Fig. 2. Simplified model of molecular clock. Note that nuclear receptors (RORα, PPARα, REV-ERBα) play an important modulatory role on circadian system.

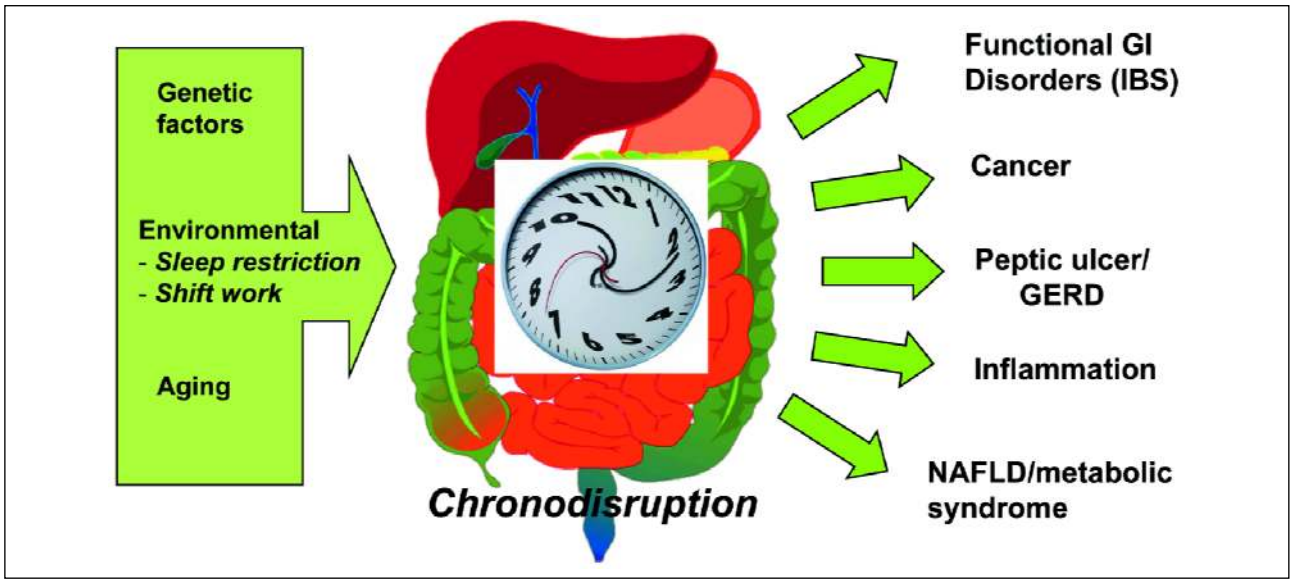

Fig. 3. Clinical consequences of the disruption of the gut clock.

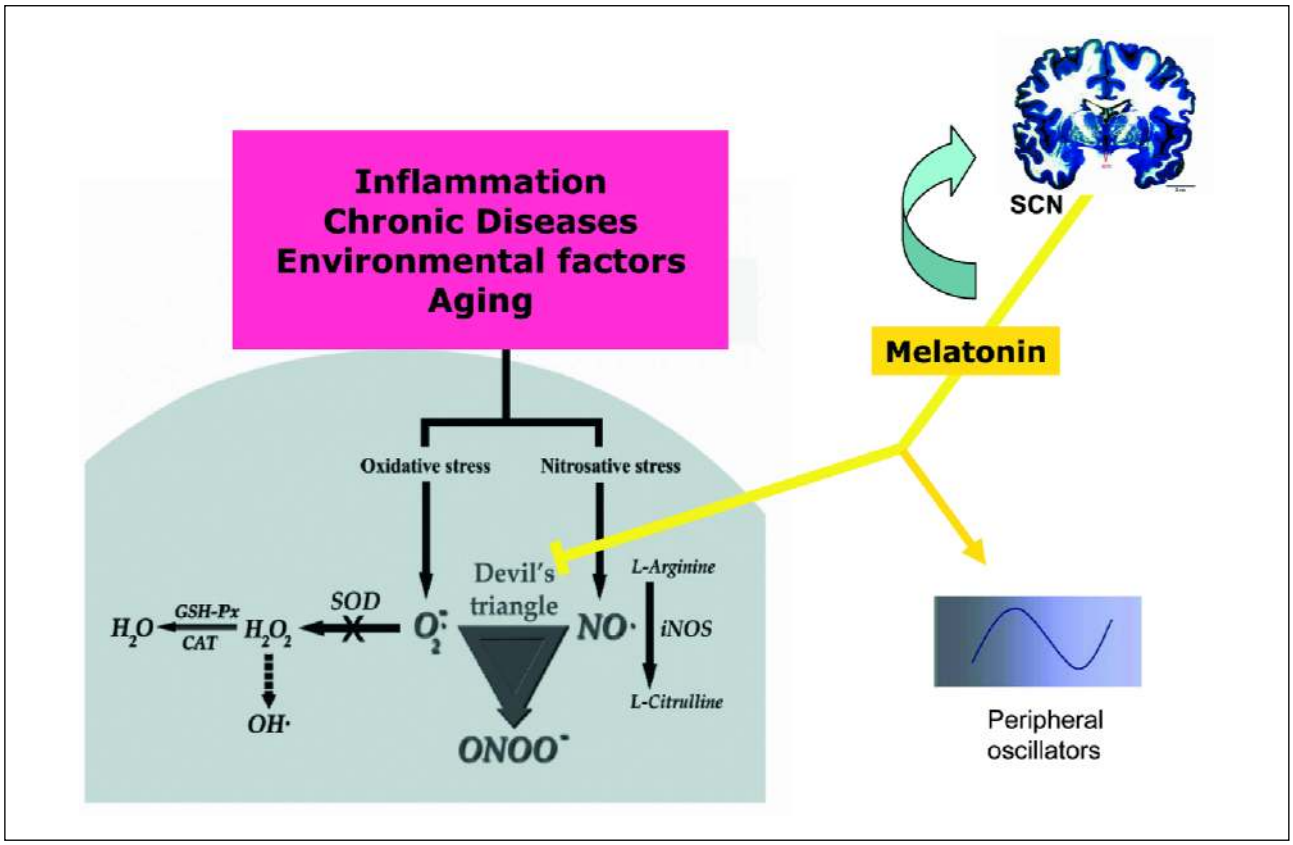

Fig. 4. Role of melatonin as important anti-oxidant molecule and important regulator of central and peripheral clocks.

disorientation) and long term (acceleration of aging, cancer development, functional and inflammatory diseases).

CLINICAL CONSEQUENCES OF DISRUPTING THE GUT CLOCK

Dampened circadian rhythms, due to chronodisruption, may result from gene polymorphism, desynchronisation of environment (shift work, sleep restriction, behavioural desynchronization) or physiological aging. Recent studies indicate that disruption of the circadian system is an important contributory factor in a number of important pathological conditions including: sleep disorders, cancer, gastrointestinal and liver diseases (functional and inflammatory), and even metabolic syndrome (14). There is evidence that chronodisruption affects the brain-gut axis, contributing to the pathogenesis of a number of important diseases in the GIT such as: gastroesophageal reflux disease (GERD), gastric dyspepsia, peptic ulcers, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and other

functional bowel disorder, non-alcoholic steatosis hepatis (NASH), and even cancer of the GIT (15).

The GIT displays various biological rhythms (*Table 1*). Circadian oscillations affect important core functions of the GIT such as motility, maintenance and replacement of the protective barrier, and immunology and production of digestive enzymes. Changes in circadian rhythm due to shift work or transmeridian flights have been associated with gastrointestinal symptoms such as abdominal pain, constipation and diarrhea. All of these symptoms resemble those observed in patients with functional bowel disorders (dyspepsia, irritable bowel syndrome *etc.*). Recently, Knutson *et al*. analyzed the epidemiological link between chronodisruption due to shift work and gastrointestinal dysfunction (16). This epidemiological analysis demonstrated a significantly increased risk for GIT symptoms in individuals performing shift work. However, not all studies confirmed this link (16).

In terms of the association between circadian rhythms and colonic motility the following conclusions can be drawn from previous studies: 1) colonic motility is under the influence of circadian control (maximal motility during the day, minimal during the night); 2) components of the molecular clock have been identified in the GIT; 3) neurotransmitters expressed in the myenteric plexus (*e.g*., VIP) were also identified in the neurons of SCN master clock; 4) there is strong epidemiological evidence that alterations in colonic motility, due to the disruption of the molecular clock, (shift work, transmeridian flights) may have a potent impact on GI functions (17).

Over the past two decades, many components of the molecular clock were identified in the GIT (18, 19). Prior to the identification of the molecular clock, various rhythmic processes were already characterized within the GIT, including motility, cell proliferation, absorption rates, and enzyme secretion rhythms. The identification of a molecular clock in the GIT, especially the myenteric plexus, initiated a number of important studies in that field. Hoogerwerf *et al*. found a rhythmic expression of clock genes, such as *per2,* in myenteric plexus and epithelial cells (20). Therefore, they proposed a model of circadian regulation of colonic motility. According to this model, the rhythmic expression of clock genes within neurons of the myenteric plexus modulates colonic motility through direct and indirect clock-controlled transcription of genes such as acetylcholine transferase and neuronal nitric oxide synthase. Transcription of these enzymes leads to rhythmic release of acetylcholine and nitric oxide, which may, in turn, enhance colonic motility. Direct clock-controlled transcription can be mediated through an E-box element (short DNA element that binds to transcription factors CLOCL-BMAL1) (20).

Table 1. Important gastrointestinal functions under control of circadian system.

Major gastrointestinal functions affected by circadian oscillations
➢ **Gut motility**
➢ **Gastric acid secretion**
➢ **Maintenance and restoration of the protective mucosal barrier**
➢ **Production of digestive enzymes**
➢ **Nutrient transport in small intestine**
➢ **Immunologic system of GIT**

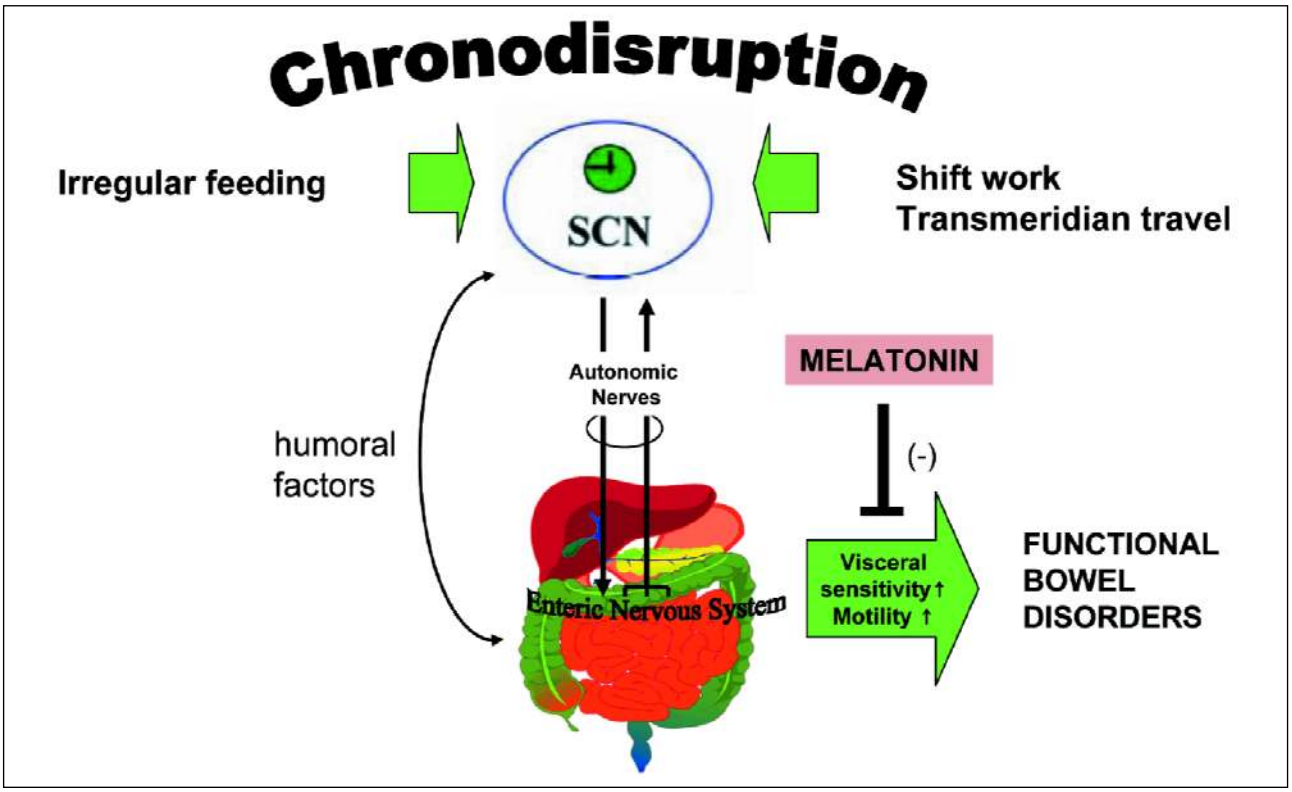

Fig. 5. Impact of chronodisruption on functional bowel disorders.

The importance of the link between the gut clock and colonic motility is supported by clinical studies showing an increased prevalence of functional colonic motility disorder due to disruption of circadian rhythms (21). Recently, Nojkov *et al*. investigated the association between shift work and the prevalence of functional bowel disorders; 399 nurses engaged in patient care were included in this study. The following groups were investigated: 214 nurses from the day shift, 110 from he night shift and 75 from rotating shifts. This study demonstrated that rotating shift may have a significant impact on functional bowel disorders, especially abdominal pain. Notably, the association between functional bowel disorders and rotating shift work was independent of sleep quality (22).

Since melatonin is not only the hormone regulating sleep-wake cycles but shows many positive effects on sensitized or minimally inflamed gastrointestinal nervous system, it represents an interesting candidate substance for the treatment of IBS (23) (*Fig. 5*). Additionally, animal studies have shown that melatonin demonstrates regulatory effect on GI motility. From the clinical standpoint, there is a strong evidence for a modulatory effect of melatonin on GI motility in humans. The hypothesis that melatonin could be beneficial in patients with IBS has been investigated by several groups. Recently, Lu *et al*. (24) demonstrated that an 8-week course of oral melatonin, at a dose of 3 mg/day, was effective in improving bowel symptoms in female patients with IBS. The beneficial effects of melatonin were successful in alleviating abdominal pain, abdominal distention, and abnormal sensations during defecation. This was a strong study with a randomized, double-blind, placebo controlled design (24). Previous studies have suggested that the regulatory effect of melatonin on colonic motility counterbalances that of its precursor, 5-HT. It is also possible that melatonin exerts its effects through the central sympathetic and parasympathetic nervous system. In other words, melatonin, as a regulator of the sleep-wake cycle, could be a promising therapeutic agent for the treatment of IBS in the future.

One of the strongest impacts on the circadian system is the aging process. In summary, the following alterations of circadian system were observed: 1) age-related changes in the master clock, such as decreased neuropeptide expression of VIP and AVP, resulting in reduction of the amplitude of circadian electrical activity and loss of responsiveness to melatonin in SCN; 2) age-related changes in circadian entrainment (decreased sensitivity to light and food entrainment, reduction in melatonin levels in circulating blood); 3) alterations in the clock genes due to aging (reduced expression of some important clock genes in the SCN and peripheral clocks); 4) occurrence of functional GI disorders due to aging and neurodegeneration of the myenteric plexus (cholinergic degeneration) (25, 26).

As mentioned above, aging processes are determined by differentiated neurodegeneration of the myenteric plexus (cholinergic degeneration) *via* reactive oxygen and nitrogen species as well as alteration of protective and regenerative processes (27). The consequence of this neurodegeneration could be disruption of circadian oscillators in the GIT, leading to the development of functional bowel disorders. Recently, Frieling *et al*. (28) reported the increased prevalence of functional GI symptoms in the aging population. Since oxidative stress plays a key role in the initiation and promotion of age-related impairment of colonic motility, melatonin, as a strong anti-oxidant may represent a promising candidate for the prevention and treatment of these motility disorders (29).

In addition to aging, there is an evidence from the experimental model of diabetes mellitus that this metabolic disease may lead to alterations in GI motility, due to changes in expression of the per clock gene (30). The interplay between clock genes and the GIT of diabetic patients is of great clinical importance, because *diabetes mellitus* is known to delay gastric emptying and impair colonic transit time. Moreover, other studies indicate that *diabetes mellitus* alters not only the gut clock, but also liver and central clocks (31, 32). Further studies are needed to clarify changes to the gut

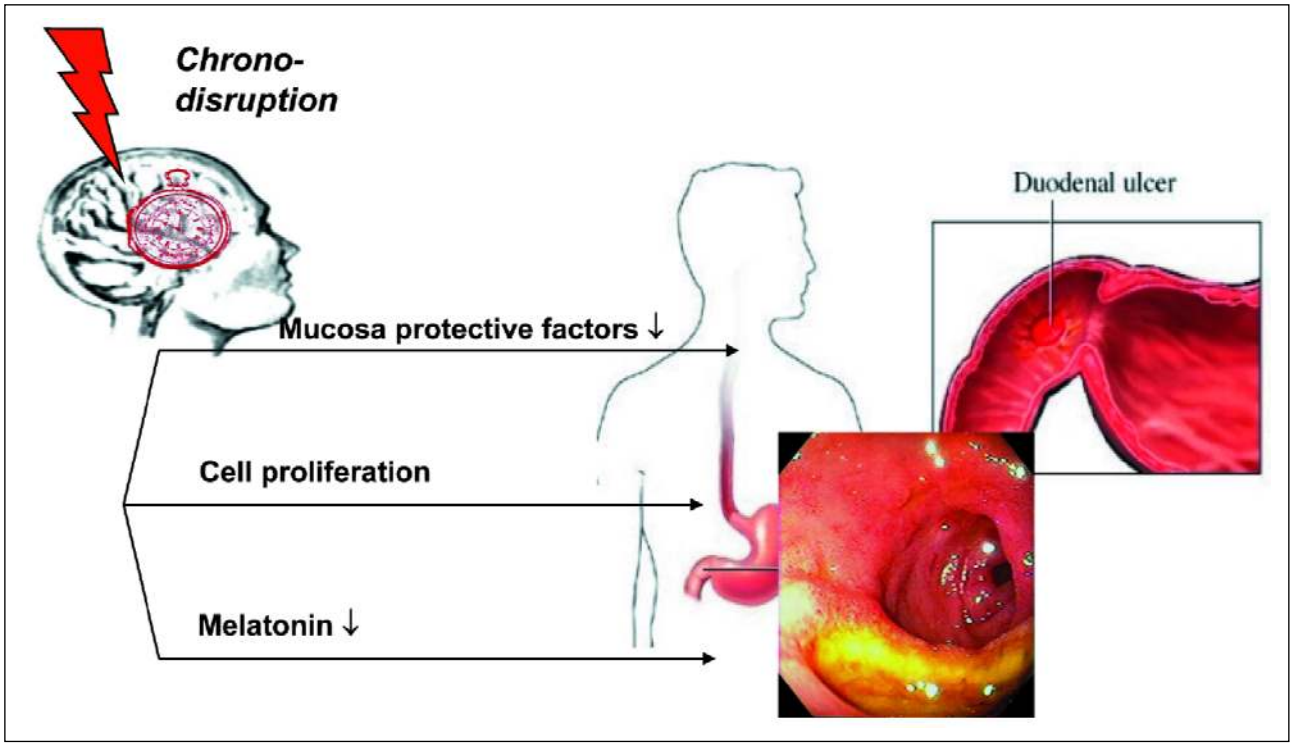

Fig. 6. Possible links between disruption of circadian rhythms and integrity of gastrointestinal mucosa.

clock in patients with diabetes mellitus, the most common metabolic disease.

Alterations in the circadian gut clock may have effects on the integrity of GI mucosa. There is evidence that the disruption of circadian oscillators could have a direct and indirect (*via* melatonin and other humoral factors) impact on protective factors of the GI mucosa and cell proliferation (*Fig. 6*) (33, 34).

Recently, Pietroiusti *et al*. postulated a possible association between shift work and development of duodenal ulcers. The authors reported that shift workers, compared to day-time workers, more frequently develop duodenal ulcers (35). However, the mechanisms responsible for this phenomenon are still poorly understood. One of the contributing factors responsible for this phenomenon could be a decrease of circulating melatonin, due to the shift work in the night. Our previous studies demonstrated that melatonin plays a central role in gastric protection (*via* effects on angiogenesis, NOS system, COX-2, and gastric mucosal blood flow), and that melatonin deficiency could be an important promoting factor in the development of peptic ulceration (*Fig. 7*) (36). This is supported by our animal studies, which show strong healing effect of melatonin and its precursor, L-tryptophan in experimentally-induced gastric ulceration (37). Melatonin also shows a potent protective and ulcer healing effect, not only in animal models of induced-ulcerations, but also in humans. Our group recently reported that oral melatonin and its precursor, L-tryptophan, significantly reduce gastric lesions induced by aspirin in healthy volunteers (38). In an additional study we demonstrated that the addition of melatonin to proton pump inhibitor (PPI) therapy significantly accelerated ulcer-healing in humans (39). In addition to melatonin, ghrelin can contribute to healing of gastro-duodenal ulcers. Our recent studies in rats also showed that treatment with ghrelin accelerates healing of chronic gastroduodenal ulcers and that this may be mediated by growth hormone and IGF-1 (40).

The circadian clock is also linked to the diurnal rhythm of cytoprotective factors in the GI mucosa such as human trefoil protein TFF2. Trefoil protein (TFF2) protects GI mucosa from damage, and supports its repair. However, the diurnal rhythm of TFF2 is significantly attenuated in older people, suggesting that chronodisruption due to aging, may have a strong impact on the protective mechanisms of GI mucosa. Interestingly, chronic *H. pylori* infection causes a similar reduction in the TFF2 diurnal rhythm. This observation indicates that disruption of the circadian clock results in similar deleterious effects on the integrity of GI mucosa as those of chronic inflammatory responses to *H. pylori* infection. The exact link between clock genes and cytoprotective factors in GI tract is still poorly understood and awaits further clarification (41).

The gut clock also modulates proliferative changes in GI mucosa. However, diurnal changes in amplitude, phasing, and average level of DNA synthesis, vary with dependence on the region of the GIT. The explanation of this phenomenon is not fully understood, but it was demonstrated that cells progressing through the cell cycle are regulated by circadian proteins. The circadian clock regulates the activity and expression of several

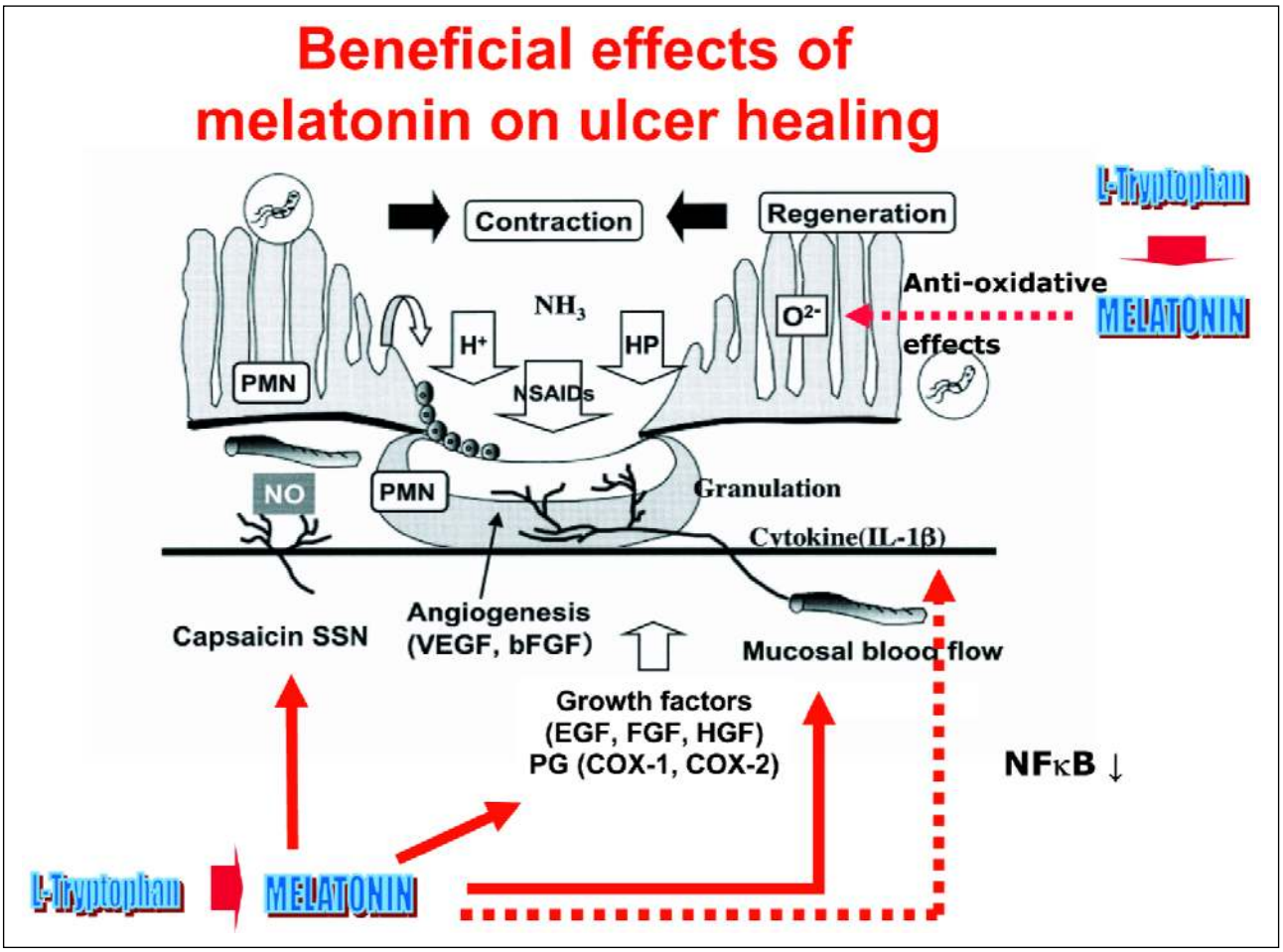

Fig. 7. Effect of melatonin on healing of experimentally induced ulcers.

critical cell cycle and cell cycle check-point-related proteins. In turn, the cell cycle-associated proteins regulate circadian clock proteins (42, 43).

Additionally, circadian rhythms affect secretory changes in the GIT, especially changes in gastric acid secretion. One of the most common disorders associated with gastric hypersecretion is gastroesophageal reflux disease (GERD). Typically, GERD patients complain about frequent night reflux symptoms. Night-time GERD can profoundly impair the quality of life by causing pain and disturbance of sleep, thereby interfering with mental and physical functioning the next day. Additionally, for some GERD patients taking anti-secretory drugs such as PPIs, the phenomenon of nocturnal acid breakthrough has been described (44-46). The cause of night-time complaints due to GERD is not fully understood. The fact that during the night, melatonin, is released, reveals its potential role in the development of GERD (47). However, the exact role of melatonin in this disorder remains inconclusive and is not fully understood. Klupinska *et al*. (48) reported that in patients with the erosive form of GERD, circulating melatonin levels are significantly depressed, compared to patients with non-erosive reflux form (NERD). Our own studies suggest the possible dose-dependent protective role of melatonin in preventing reflux-induced changes in the esophageal mucosa, and reducing the incidence of experimentally induced esophagitis (49). However, there is currently no information available on the association between circadian proteins and protective factors in the esophageal mucosa.

The circadian clock may play a fundamental role in liver physiology *via* regulation of fatty acid and carbohydrate metabolism, and thus represent an important link between chronodisrutpion and metabolic disorders such as obesity and fatty liver disease. This aspect is still a matter of intensive research, the details of which would be beyond the scope of this article. Briefly, it may be summarized that: 1) microarray studies have revealed a large number of rhythmically expressed genes in the liver which are involved in the maintenance of metabolic homeostasis; 2) important nuclear receptors such as PPAR and thyroid hormone receptors, exhibit circadian expression in the liver, providing a possible explanation for diurnal variations in glucose and lipid metabolism; 3) clock-mutant mice develop metabolic syndrome, hyperlipidemia, hyperglycemia and fatty liver disease; 4) clock gene expression is strongly attenuated in the livers of mice with experimentally induced diabetes mellitus; 5) lack of clock gene *mper2* promotes liver cirrhosis in experimental models of acute injury; 6) feeding cycles can entrain the liver independently of the master SCN clock; 7) clock genes play a role in lipid metabolism in the liver, and circadian disregulation can contribute to non-alcoholic fatty liver disease (NAFLD) (50-54).

Recent studies have linked dysregulation of the circadian system with metabolic syndrome and non-alcoholic steatohepatitis (NASH), but the data on this topic is still very inconclusive. Some studies indicate the preservation of the biological clock against NASH, but other studies indicate that clock genes are dampened in NASH (52, 55). There is a strong need to better understand the interactions between the circadian timing system and development of NASH.

Since many studies postulate that impairment of the circadian system contributes to NASH (55), it is important to determine whether melatonin is a key resynchronizing factor of central and peripheral clocks as a strong anti-oxidant, has any impact on NASH. Our preliminary data indicates that melatonin treatment improves NASH, attenuates liver enzymes levels, and decreases the level of proinflammatory cytokines in humans with NASH (57). The liver protective mechanism of melatonin could be attributed directly to its anti-oxidative effect, and indirectly, to a regulatory effect on the liver clock.

Finally, circadian rhythms may affect physiological activity in the small intestine. There is evidence that some mucosal nutrient transporters in the small intestine appear to be under circadian control. Importantly, rhythmic patterns of

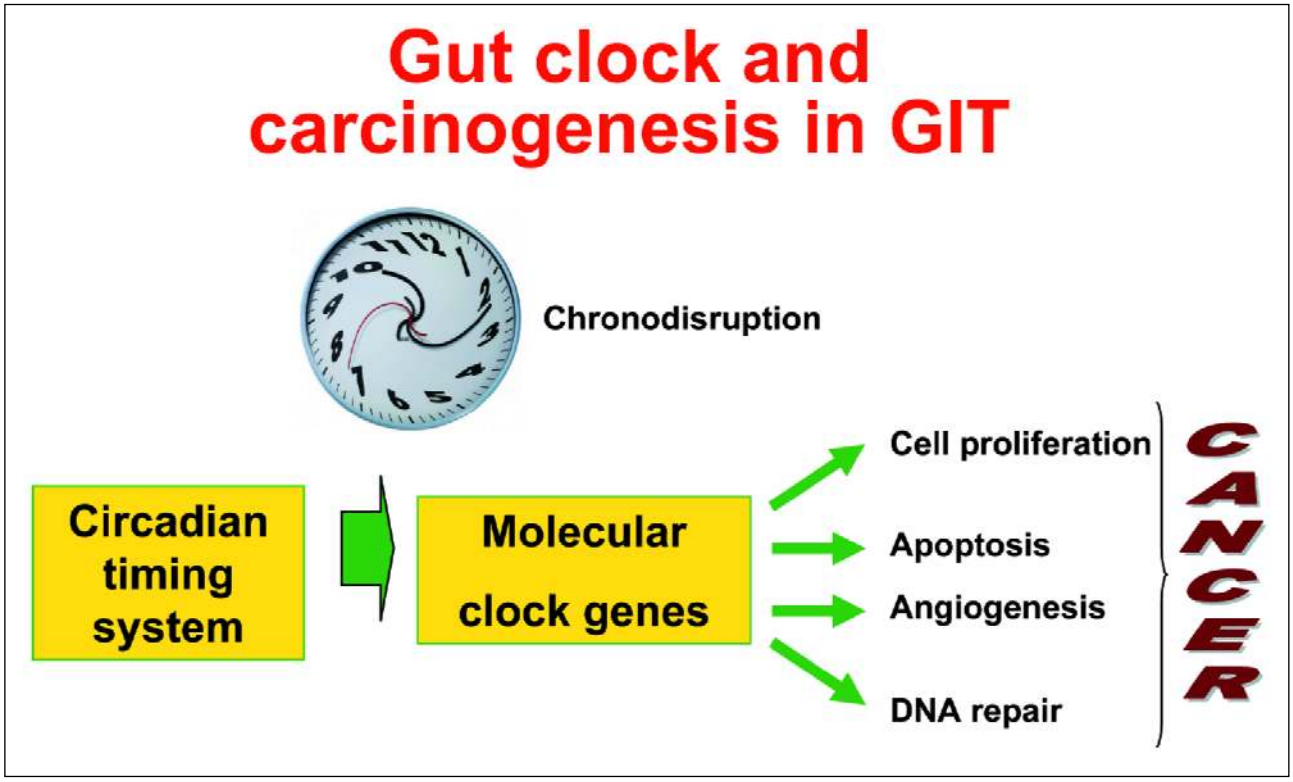

Fig. 8. Link between gut clock genes and carcinogenesis in gastrointestinal tract

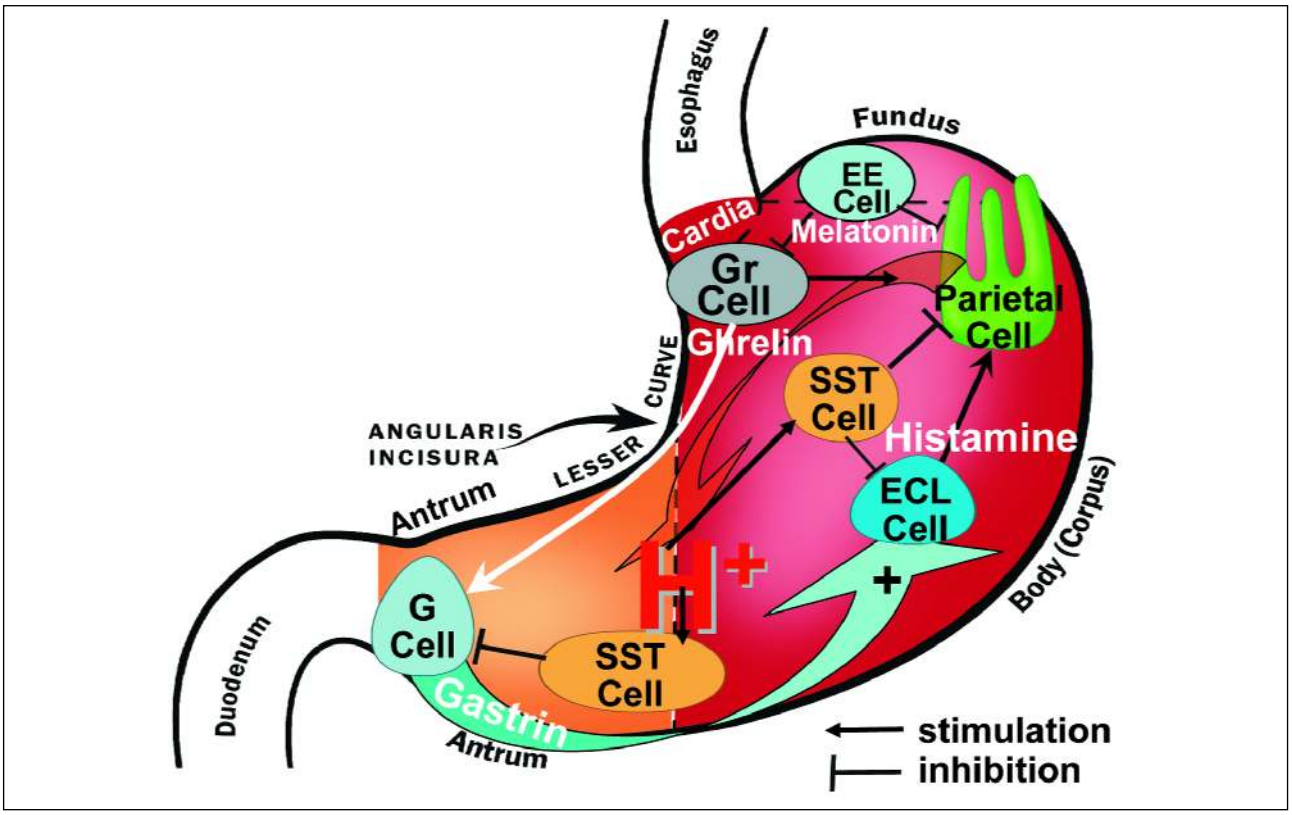

Fig. 9. Feedback control of gastric acid secretion involving gastrin, ghrelin, melatonin and somatostatin release in the stomach (adapted and modified from *J Physiol Pharmacol* 2008; 59(Suppl 2): 7-31).

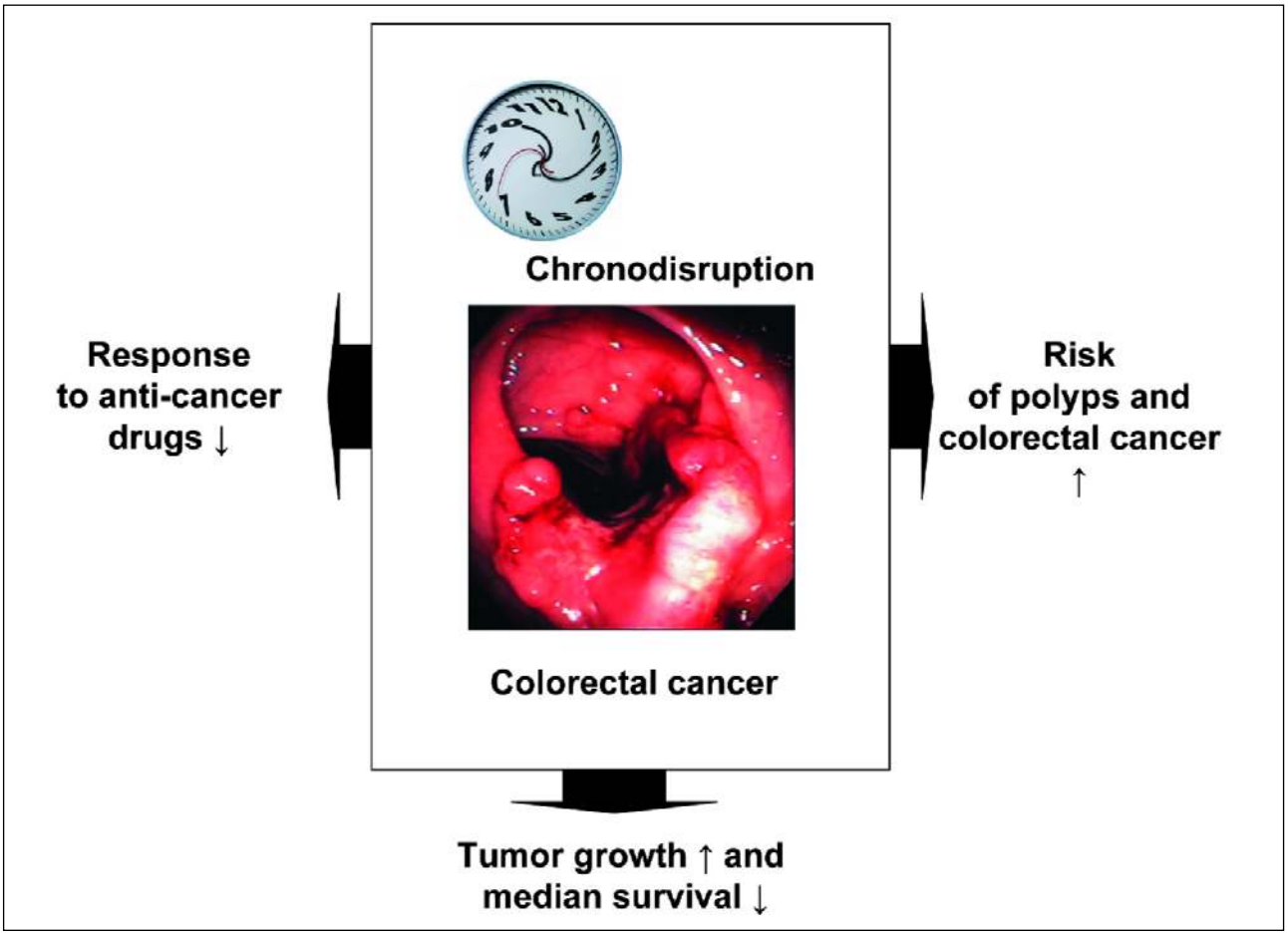

Fig. 10. Impact of chronodisruption on colorectal carcinogenesis.

expression of nutrient transporters is driven by the timing of food intake and appetite, so gut hormones regulating food intake may play an important regulatory role. The involvement of neuronal connections is supported by the fact that vagal innervation appears to mediate diurnal variations in the expression of mucosal nutrient-transporters in the small intestine. Finally, diurnal variations in the expression and function of mucosal transporters serve, presumably, to match the expected amounts of nutrients being delivered, diurnally, to the gut lumen (58, 58).

Interestingly, disruption of the circadian system may also have a strong impact on carcinogenesis. Scientists have demonstrated a link between the molecular clock machinery and some aspects of carcinogenesis such as angiogenesis, cell proliferation, apoptosis and DNA repair which seem to be under control of circadian timing system (59) (*Fig. 8*). Our recent studies showed that fasting plasma levels and postprandial responses of ghrelin and gastrin were inversely correlated in healthy subjects, but gastric cancer patients undergoing total or distal gastrectomy failed to show any significant decrease in plasma ghrelin after feeding, while exhibiting high fasting and postprandial gastrin levels (60). This study demonstrated the feedback relationship between gastric acid secretion, plasma ghrelin and gastrin levels that disappeared following gastrectomy in patients with advanced gastric cancer. In animals model such as gastric fistula rats, it was demonstrated that melatonin in the stomach is capable of inhibiting the release of ghrelin as well as to inhibit gastric acid secretion (61, 62) (*Fig. 9*). Discussion of this wide area of research would be beyond the scope of this article and is discussed elsewhere (63, 64).

Several important conclusions can be drawn about the link between the gut clock, and carcinogenesis in the GIT: 1) disruption of the gut clock due to shift work (working a rotating night shift at least 3 nights per month for 15 or more years) increases the risk of colorectal cancer; 2) down-regulation of clock genes like *bmal1* accelerates the development of tumors and may influence the response to anti-cancer drugs; 3) circadian disruption has been shown to be associated with faster tumor growth in experimental models and shorter survival in clinical studies; 4) circadian clock gene regulates tumor cell proliferation and Per1 provides an important link between the circadian system and the cell-cycle system; 5) clock gene polymorphisms are associated with an increased risk of cancer; 6) circadian based chemotherapy may optimize therapeutic efficacy (chronomodulated infusion) (*Fig. 10*).

In this review we discussed the role of the circadian system in GIT and liver functions. We have concluded that: 1) biological rhythms controlled by the master clock (SCN) and peripheral clocks seem to play an important role in physiological GI and liver functions; 2) chronodisruption associated with aging or environmental factors (shift work, frequent transmeridian flights) may result in several GIT and liver diseases such as alterations in colonic motility, peptic ulcer disease, NASH, and even obesity; 3) future research should focus on the role of clock genes in the pathophysiology of the GIT and liver.

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