Clock Genes, Metabolism, and Cardiovascular Risk

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KEYWORDS

• Clock • Gene • Circadian • Rhythm • Metabolism • Cardiovascular

KEY POINTS

- The biological clock rules periodic adjustments of biochemical processes controlling lipid and glucose metabolism, and the circadian timing system coordinates behavioral cycles and metabolic pathways with environmental cues.
- Metabolism, bile acid signaling, autophagy, and immunity/inflammation are driven by the clock gene machinery, which in turn is modulated by gut microbiota. Appropriate synchronization of these processes with behavioral cycles is required to thwart metabolism alteration.
- Derangements of the molecular clockwork or misalignment of the circadian timing system with respect to environmental cues causes chronodisruption and dysmetabolism, leading to cardiometabolic disease.

INTRODUCTION

The continued existence of living beings on planet Earth is taunted, especially in the wild, by environmental challenges as well as changes of ecological niches and life conditions, such as temperature swinging, food availability, and predation risk, which in turn impact processes and activities crucial for individual and species survival, such as feeding, mating, and hunting, among others. Survival advantage is warranted by proper physiologic and behavioral modifications anticipating periodic and predictable variations of the environment and cycling in a huge frequency range.[1] The periodicity interval may span from the hourly variations of heart rate to the monthly and seasonal fluctuations of hormone secretion and even to the circadecennial rhythm of oscillation of umbilical cord blood parameters.[2,3] The most frequent and explored biological rhythms are hallmarked by a 24-hour period of oscillation resonating with the daily transition from darkness to solar illumination dictated by Earth's rotation on its axis. This potent environmental cue is perceived by the retina via melanopsin-containing ganglion cells and transferred to the suprachiasmatic nuclei (SCN) of the hypothalamus through the glutamatergic fibers of the retino-hypothalamic tract. In mammals, 24-hour rhythmicity is driven by the circadian timing system, a hierarchical multilevel organization

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composed of the SCN, composed of approximately 15,000 to 20,000 neurons in rodents ad 80,000 to 100,000 neurons in humans, working as the principal oscillator synchronizing self-sustained oscillators in the peripheral tissues by means of neural fibers of the autonomic nervous system or by humoral factors (melatonin, cortisol).[4,5] Anatomic links connect the SCN to other brain regions, such as arcuate nucleus, ventromedial, dorsomedial, and lateral hypothalamic nuclei, controlling appetite, energy expenditure, and behavioral activity.[6,7] Environmental lighting is the prevailing entraining factor for SCN and sequentially for other brain areas and peripheral tissues, anyway alternative cues can overcome SCN control on peripheral clocks. In particular, feeding time is capable of disengaging peripheral oscillators and central oscillators; if experimental animals are fed only during the subjective day, when nocturnal animals usually are not active (restricted feeding), central and peripheral clocks tick in opposite phases.[8,9]

THE MOLECULAR CLOCKWORK

Neurons in SCN and interplaying brain areas as well as each cell in nearly all peripheral tissues harbor endowed biological clocks ticking through transcriptional-translational feedback loops (TTFLs) operated by a set of so-called clock genes and their coded proteins and revolving rhythmically with a roughly 24-hour period.[10–12] The positive limb of the TTFL in mammals, such as rodents and humans, is operated by the Period-Arnt-Single-minded and basic helix-loop-helix (PAS-bHLH) proteins circadian locomotor output cycles kaput (CLOCK), and its paralog neuronal PAS domain protein 2 (NPAS2), and by brain and muscle aryl-hydrocarbon receptor nuclear translocatorlike/aryl-hydrocarbon receptor nuclear translocatorlike (BMAL1/ARNTL1) or its homolog BMAL2/ARNTL2.[13] These transcription factors heterodimerize and bind to canonical E-box (5'-CACGTG-3') *cis*-regulatory enhancer sequences of their target genes Period (*Per1-3*) and Cryptochrome (*Cry 1-2*). The negative limb of the TTFL is operated by PER and CRY proteins, which in turn dimerize and form a repressor complex that translocates back to the nucleus and hinders CLOCK or NPAS2/BMAL1-2 transcriptional activity.[14,15] In *Drosophila melanogaster* as well as in other flies and insects, a cog of the molecular clockwork is represented by Timeless, which in mammals is conserved and collaborates with TIMELESS interacting protein in biological processes comprising embryonic development, cell cycle progression, DNA replication, and DNA damage response.[16]

SIRTUINS AND THE BIOLOGICAL CLOCK

The oscillation amplitude of numerous clock genes depends on the activity of SIRT1, a type III nicotinamide (NAM) adenine dinucleotide (NAD^+)-dependent histone/protein deacetylase, which rhythmically deacetylates BMAL1, histone H3, and PER2, decreasing PER2 stability in a circadian manner.[17,18] SIRT1 cofactor is de novo and cyclically synthesized from tryptophan through NAM phosphoribosyltransferase (NAMPT), the rate-limiting enzyme in the NAD^+ salvage pathway, whose expression is driven directly by BMAL1 with 24-hour periodicity and in the circulating form is defined as visfatin/pre-B-cell colony–enhancing factor.[19–22] High NAD+ and low adenosine triphosphate levels specify low-energy status in the cell, and high adenosine monophosphate (AMP) triggers AMP-activated kinase (AMPK), which activates NAMPT and modulates the NAD+/NADH balance,[23,24] working as a nutrient sensor prompted to reestablish energy balance in case of exercise, fasting, or hypoxia.[25,26] SIRT1 activity is obstructed through protein-protein interaction by deleted in breast cancer–1 (DBC1), which controls SIRT1 activity in metabolically active tissues, especially in the liver.[27] DBC1 was shown in animal models fed a high-calorie diet to bind SIRT1 and impede its deacetylase activity, whereas in animals starved or fed a low-calorie diet, DBC1 remains unbound and SIRT1 activity bolsters.[28] Experiments performed in vitro using cultured cells synchronized using different protocols show mitochondrial respiratory activity oscillation depends on BMAL1 levels and takes place independently from the cell type tested, the protocol of synchronization used, and the carbon source in the medium. Fluctuation in cellular NAD+ content and clock-genes–dependent expression of NAMPT and Sirtuins 1/3 dictate the rhythmic respiratory activity and is related to the acetylation/deacetylation cycle of a single subunit of the mitochondrial respiratory chain complex I, suggesting a molecular interplay between cellular bioenergetics and the molecular clockwork operated by a dedicated interlocked transcriptional-enzymatic feedback loop.[29,30]

POSTTRANSLATIONAL AND EPIGENETIC MODIFICATIONS

The functioning of the molecular clockwork crucially depends on posttranslational modification of the circadian proteins, comprising phosphorylation, acetylation, sumoylation, and ubiquitination, which modulates their transcriptional activity and intracellular localization.[31,32]

Mainly, casein kinases 1-δ and 1-ε (CK1δ and CK1ε) target Bmal1 as well as the PER and CRY proteins, tagging the latter for polyubiquitination by the E3 ubiquitin ligase complex β-transducin repeat containing protein 1 and SCF/Fbxl3 ubiquitin ligase complex (Skp1, Cullin1, F-box, and leucine-rich repeat protein 3), respectively.[33,34] The AKT-GSK3β system phosphorylates BMAL1,[35] and AMPK targets the CRY proteins tagging them for degradation through the 26S proteasome via the SCF/Fbxl3 ubiquitin ligase complex.[36] Another layer of regulation of the biological clock depends on cyclic epigenetic modifications. CLOCK is a histone acetyltransferase (HAT) and prompts protein acetylation and chromatin remodeling holding up gene transcription. Clock joins to E-boxes in the company of cyclic adenosine monophosphate (cAMP) response element–binding protein (CBP)/p300 and acetylates histones H3 and H4 and BMAL1, particularly the following: (1) the transcriptional coactivators and HAT p300/CBP, PCAF, and ACTR associate with CLOCK and NPAS2 to regulate trigger clock gene expression; (2) Cry2-mediated hindrance of NPAS2:BMAL1 transcriptional activity is surmounted by p300 overexpression; (3) p300 shows a 24-hour periodic association with NPAS2 in the vasculature heralding target genes expression climax; (4) a rhythm in core histone H3 acetylation on the mPer1 promoter in vivo correlates with mRNAs cyclical expression.[37–39] Besides, 24-hour rhythms of gene transcription at the level of the whole genome are driven by cycles of histone methylation catalyzed by methyltransferase MLL3 with alternation of activating (H3K4me3) and inhibitory (H3K9me3) chromatin marks[40] and cycles of histone lysine demethylation driven by the histone lysine demethylase JARID1a, which stimulates CLOCK-BMAL1 heterodimer transcriptional activity.[41]

AN AUXILIARY LOOP IN THE MOLECULAR CLOCKWORK

The oscillation of the starting cog of the TTFL-positive limb depends on the cycling of the reverse transcript of the erythroblastosis gene (REV-ERB) α/β and the retinoic acid–related (RAR) orphan receptor (ROR) α, β/δ, γ, whose expression is driven by the molecular clockwork and hard-wires an additional regulatory loop controlling BMAL1 expression. REV-ERBα/β, not capable of engaging coactivators and trigger target gene transcription, binds ROR-specific response elements (RORE) in *Bmal1*, *Clock*, and *Cry1* promoters, impeding binding and activation of transcription by RORα.[42–44] RORα cooperates with the transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α), which engages chromatin-remodeling complexes to the proximal *Bmal1* promoter and prompts *Bmal1* transcription. Conversely, REV-ERBα cooperates with histone deacetylase 3 (HDAC3) and nuclear receptor corepressor 1 (NCOR1), operates as an HDAC3 activating subunit and induces repression of transcription.[45] As a result, the 24-hour rhythm of the RORα/PGC-1α activator complex and REV-ERBα/NCOR1-HDAC3 repressor complex recruitment manages the 24-hour rhythmicity of *Bmal1* expression. Experiments performed in mouse models showed that HDAC3 binds to the liver genome with circadian periodicity and drives the expression of gene-enriching pathways involved in lipid metabolism, whose alteration induces in vivo hepatic steatosis.[46] Heme is a physiologic ligand of REV-ERBα/β, binds to the ligand-binding domain with a 1:1 stoichiometry, and enhances thermal stability of these nuclear receptors. Heme synthesis is catalyzed by the rate-limiting enzyme delta-aminolevulinate synthase 1, whose expression is driven by the molecular clockwork; its binding to REV-ERBs induces corepressor NCOR1 recruitment, with repression of target genes (*Bmal1* included), whereas heme dissociation prompts the expression of target genes according to modifications in intracellular redox balance.[47,48] REV-ERBs are also highly responsive to the redox state and gases. The addition of nitric oxide pulls out transcription repression induced by heme-bound REV-ERBs.[49] In addition, a thiol-disulfide redox switch modulates heme and REV-ERBβ interaction; the reduced dithiol state of REV-ERBβ binds heme 5-fold more tightly than the oxidized disulfide state. However, changes in the iron redox state do not impact heme binding to the ligand binding domain.[50] Moreover, heme influences BMAL1-NPAS2 transcription activity in vitro through a NPAS2 heme-binding motif via inhibition of DNA binding in response to carbon monoxide.[51]

THE CLOCK-CONTROLLED GENES

The molecular clockwork drives the expression of so-called clock-controlled genes, some in common among the different peripheral tissues and others specific to particular tissues and defined output genes, which manage biological processes at the cell level and physiologic functions at the tissue and organ level.[52] Principally, CLOCK:BMAL1 heterodimer drives the expression of the proline- and acidic amino acid–rich domain basic leucine zipper transcription factors albumin gene D-site binding protein (DBP), thyrotroph embryonic

factor, and hepatic leukemia factor (HLF); these sequentially drive the transcription of thousands of genes.[53] In turn, DBP triggers *Per1* transcription feeding back on the molecular clockwork,[54] whereas REV-ERBs bind to RORE and trigger the expression of the nuclear factor interleukin 3 regulated protein (defined adenoviral E4 protein–binding protein, [E4BP4] as well), which fluctuates in antiphase regarding DBP, in order that these transcription factors manage the expression of downstream genes peaking in an opposite phase.[55] Another cog of the machinery is represented by the bHLH transcription factors differentially expressed in chondrocytes protein 1 (DEC1) and 2 (DEC2), which manage transcriptional repression/regulation of multiple circadian genes and feed back to the molecular clockwork.[56,57]

THE BIOLOGICAL CLOCK AND THE NUCLEAR RECEPTORS

The clock gene machinery drives rhythmic oscillations of several biological processes whose output feeds back in the biological clock (Fig. 1). A crucial role in this interplay is played by the nuclear receptors, ligand-dependent transcription factors capable of binding lipophilic ligands and interact directly with promoters of specific DNA sequences to modulate target gene expression. The intracellular level of several nuclear receptors oscillate with circadian rhythmicity in several metabolically active tissues, such as liver, muscle, and adipose tissue and in particular include constitutive androstane receptor (CAR); estrogen-related receptor (ERR) α, β, and γ; farnesoid receptor (FXR) α and β; glucocorticoid receptor (GR); Nur-related protein 1; PPAR α, δ/β, and γ; RAR α, β, and γ; retinoid X receptors (RXR) α, β, and γ; small heterodimeric partner (SHP); and thyroid hormone receptor α.[58,59] The rhythmic assembly of metabolites capable of binding nuclear receptors and the interplay among molecular clockworks and signaling pathways activated on ligand binding to nuclear receptors sustain the circadian regulation of metabolism. The biological clock drives the oscillation of nuclear receptors as well as their ligands; sequentially, the nuclear receptors gauge the metabolic status and feed back in the clock gene machinery binding to response elements on definite clock genes, specifying transcriptional networks that convey time-related and feeding-related cues to the metabolic pathways.[60] For instance, oxysterols and bile acids are produced with 24-hour periodicity, bind liver X receptor (LXR) and FXR, respectively, and in turn oxysterols binding LXRs trigger, whereas bile acids binding FXRs hinder the expression of *Cyp7a1*, which encodes CYP7A1, the rate-limiting enzyme in bile acid synthesis whose expression oscillates with circadian rhythmicity driven by the alternate binding of LXR and FXR on response elements at the gene promoter level.[61,62]

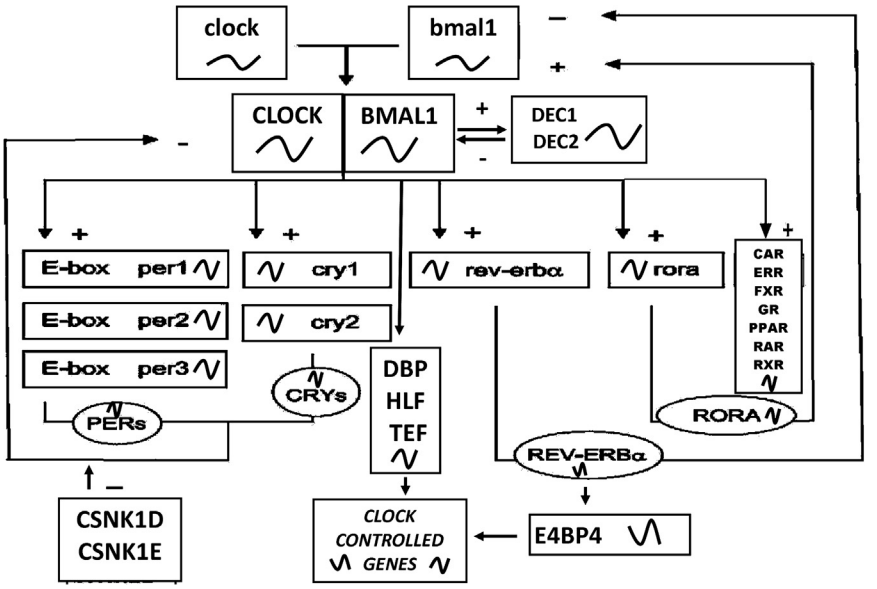

Fig. 1. The functioning of the clock gene machinery. Plus signs indicate activation; minus signs indicate inhibition; arrow-ended continuous lines indicate molecular interaction. CAR, constitutive androstane receptor; ERR, estrogen-related receptor; FXR, farnesoid receptor; GR, glucocorticoid receptor; RXR, retinoid X receptors.

THE MOLECULAR CLOCKWORK AND INTERMEDIATE METABOLISM

The biological clock drives the expression of many genes enriching the metabolic pathways that manage intermediate metabolism and coordinates the enzymatic cascades implicated in lipid and glucose metabolism.[63]

Lipid Metabolism

The circulating levels of lipids and the activity of enzymes catalyzing their synthesis and lysis, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, fatty acid synthase, fatty acyl-CoA synthetase 1, as well as the carriers involved in their transport, such as apolipoprotein A-IV and C-III, fatty acid transport protein 1, or the receptors mediating their turnover, such as low-density lipoprotein receptor, oscillate with circadian rhythmicity in mammals.[63] The 24-hour cycle of *Cyp7a1* expression is additionally controlled by REV-ERBα, DBP/E4BP4, and DEC2, which manage correct ruling of the circadian pattern, working through Rev-ROR response elements, DBP/E4BP4-binding elements, and E-boxes, respectively.[64] RORα is capable of binding cholesterol and its metabolites, for example, 7-oxygenated sterol, with transcriptional activity modulation,[65] and triggers the expression of apoC-III, a very low-density lipoprotein component,[66] whose transcription is thwarted by REV-ERBα.[67] On its side, REV-ERBα drives sterol regulatory element binding protein (SREBP) activity controlling the 24-hour rhythm of oscillation of the insulin-induced gene (INSIG) 2, which encodes an enzyme that seizes at the level of the endoplasmic reticulum membranes, the SREB-cleavage activating protein–INSIG-SREBP complex, which gauges cholesterol accessibility; in addition, driving the rhythmic nuclear accrual of SREBP, REV-ERBα also drives the time-related expression of *Hmgcr*, encoding HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway, involved in the biosynthesis of cholesterol and other isoprenoids.[68] Besides, REV-ERBα manages bile acid metabolism via fluctuations of oxysterol synthesis and LXR activity and interplays with FXR to regulate SHP, hinders the expression of SHP and E4BP4 expression and triggers the expression of *Cyp7a1* in the liver.[69] REV-ERBα manages lipid metabolism also through epigenetic changes brought on via HDAC3-NCOR1 complex recruitment at the level of genes involved in lipid metabolism, causing chromatin remodeling and histone modification: during the activity/feeding time, small REV-ERBα levels decrease HDAC3 binding to the liver genome and allow lipid buildup; however, during the resting/fasting time, high REV-ERBα levels augment HDAC3 recruitment to liver metabolic genes, hampering lipid biosynthesis.[46]

Glucose Metabolism

Glucose levels must be accurately gauged to provide a vital energy supply for cells in the different tissues of the organism and the equilibrium between glycogen anabolism and catabolism in metabolically active tissues helps to maintain roughly stable concentrations in the peripheral blood during the 24-hour day. On food intake, increasing plasma glucose levels prompts insulin secretion by Langerhans islets β cells in the pancreas, insulin signaling pathway triggering, glucose uptake, and polymerization into glycogen stores. However, glycogenolysis and/or gluconeogenesis in the liver during fasting generates glucose and the increase of *GLUT2* expression induces GLUT2-mediated transport of glucose in the peripheral blood. Circadian changes ruled by central and peripheral biological oscillators drive the transcription of genes encoding enzymes and carriers implicated in glucose metabolism, comprising glycogen synthase 2 (GYS2), glycogen phosphorylase, phosphoenolpyruvate carboxykinase (PEPCK), glucokinase, glucose-6-phosphatase (GLC-6-Pase), and the glucose transporter GLUT2, among the others.[70,71] The biological clock drives glucose metabolism and in particular CRY1 and CRY2 control gluconeogenesis in the liver decreasing cAMP signaling in response to G protein–coupled receptor activation,[72] CLOCK drives glycogen synthesis in the liver triggering *Gys2* transcription,[73] KLF10, a transcription factor encoded by a clock-controlled gene, hinders hepatic glucose production decreasing *Pepck* expression.[74] Glucocorticoids control glucose homeostasis and circadian rhythmicity modulating the expression of core clock genes, specifically *Per1* and *Per2*, through binding via GRs to glucocorticoid response elements in their promoters.[75,76] GR levels oscillate with 24-hour periodicity in metabolically active tissues, in particular in white and brown adipose tissue, linking time-related and feeding-related cues to manage synchronicity between metabolic adjustments and clock gene machinery as well as activity and feeding.[76] Furthermore, Rev-erbα inhibits gluconeogenic gene expression in the liver and modulates hepatic glucose production in response to heme,[77] whereas RORα induces the expression of GLC-6-Pase and adjusts glycogen metabolism in the liver.[78] Specifically, the SWI/SNF chromatin-remodeling complex subunit

BAF60a, expressed in mouse liver with circadian rhythmicity, is bound at ROR response elements on the proximal *Bmal1* and *G6Pc* promoters, prompts their transcription through coactivation of RORα, and impacts the harmonized regulation of circadian clock, glucose metabolism, and energy homeostasis in the liver.[79] Moreover, experiments performed in primary hepatocytes showed that PGC-1α physically interacts with CK1δ and is phosphorylated at multiple sites within its arginine/serine-rich domain and sequentially degraded through the proteasome system, with the decrease of transcription of genes enriching pathways involved in hepatic gluconeogenesis and glucose secretion.[80]

THE BIOLOGICAL CLOCK AND DERANGED METABOLISM

The several cogs of the biological clock manage signaling pathways and biochemical reactions crucially involved in metabolic regulation and alteration of the molecular clockwork severely impacts lipid and glucose homeostasis. A comprehensive revision of the scientific literature regarding the role played by the altered functioning of the molecular clockwork in the derangement of lipid metabolism and in particular in liver steatosis, the most important anatomopathological manifestation of metabolic syndrome, is provided by a previous review article.[81] Regarding the role played by the molecular clockwork in glucose metabolism, its crucial involvement is corroborated by the reduced rhythmicity of clock genes expression found in peripheral leukocytes of patients affected by type 2 diabetes[82] and by the increased risk of impaired fasting glucose and type 2 diabetes highlighted by genome-wide association studies in subjects carrying a *Cry2* variant allele.[83,84] Accordingly, experiments performed in mouse models showed that *Clock* and *Bmal1* mutation induces disrupted glucose homeostasis.[85] $Clock^{\Delta 19/\Delta 19}$ and pancreas-specific $Bmal1^{-/-}$ mutant mice are hallmarked by altered glucose tolerance, decreased insulin secretion, and reduced size and proliferation of pancreatic islets deteriorating in the course of time.[86,87] Furthermore, Langerhans islets β cells harbor a self-sustained and autonomous molecular clockwork,[88] and disruption of the biological clock induced altered transcription of genes enriching pathways involved in insulin secretion (GNAQ, ATP1A1, ATP5G2, KCNJ11) as well as granule maturation and release (VAMP3, STX6, SLC30A8) and caused altered circadian pattern of basal insulin secretion by human islet cells synchronized in vitro.[89] Besides, mice with specific *Bmal1* disruption in the liver are hallmarked by deranged hepatocyte molecular clockwork; GLUT2 expression is stably low; these animal models show undue glucose clearance, altered rhythmic patterns of hepatic glucose regulatory genes expression, and hypoglycemia expression during the fasting/resting phase of the nychthemeral period.[90]

THE CLOCK GENE MACHINERY, LIVER STEATOSIS, AND CARDIOVASCULAR DISEASE

The altered functioning of the biological clock is critically involved in the pathogenesis of nonalcoholic fatty liver disease (NAFLD), which is associated with increased risk of cardiovascular disease.[91] NAFLD is the most frequent hepatic pathology in the Western world[92] and in one-fifth of all cases may progress to chronic hepatic inflammation (nonalcoholic steatohepatitis [NASH]) associated with cirrhosis, portal hypertension, and hepatocellular carcinoma.[93] Diseases related to dysmetabolism represent public health problems and pose a huge economic and social burden on national health systems worldwide. Obesity, a distinctive metabolic syndrome trait, augments the risk for diabetes and cardiovascular diseases; NAFLD is considered the hepatic manifestation of metabolic syndrome.[94] Impressive modifications occurred throughout the previous decades in dietary macronutrient intake, such as overconsumption of energy-dense foods, particularly high-fat and high-sugar diets, which, in addition to reduced physical activity, determine energy imbalance leading to obesity and impacted metabolic diseases prevalence.[95] Nutrient-sensing information is exchanged among organs to preserve systemic energy homeostasis, and the liver plays a key role in the integration and processing of signals derived from other tissues, such as intestine, pancreas, and adipose tissue. Interorgan communication is conveyed by humoral factors, such as insulin, adipocytokines, and glucocorticoids; by the autonomic nervous system[96]; and by dietary signals, such as fatty acids, glucose, and other metabolites: these factors are sensed by nuclear receptors that consecutively control nutrient signaling pathways. Bile acids, chiefly identified as important detergents necessary for lipid absorption in the intestine, are able to turn on nuclear receptor signaling pathways and come out as crucial metabolism regulators.[60] In the past decade, an ever-increasing bulk of evidence has highlighted a critical role played by the intestine in molecular regulation of diet-related diseases that exceeds its function in nutrient digestion and extraction to maintain body metabolic homeostasis. The intestine secretes enteroendocrine hormones as well,

and the harbored gut microbial flora is ever more regarded as an essential player in the modulation of metabolic processes. Consequently, innovative preventive and therapeutic approaches, for instance, drugs targeting nuclear receptors, bile acid signaling, or gut microbiota modulation, are investigated in addition to conventional strategies, including diet and physical activity, which have been ineffective in diminishing metabolic disease prevalence.[97]

THE BIOLOGICAL CLOCK AT THE CROSSROAD OF AUTOPHAGY, GUT MICROBIOTA, INFLAMMATION, BILE ACID SIGNALING, AND INTERMEDIATE METABOLISM

Multifaceted interactions occur among metabolic pathways of lipids, glucose, bile acids and autophagy, inflammation, and their regulation by the biological clock in response to nutrients, bile acids, hormones, nuclear receptors, or gut microbiota.[98–103] This interplay is supported by strong evidence: (1) the metabolic derangements underlying NAFLD and the progression from NAFLD to NASH hint of a key role played by nutrients and bile acids acting as ligands of nuclear receptors, which manage the metabolic pathways, and as signaling molecules in metabolism and inflammation. Bile acids influence macrophage function, energy homeostasis, and gastrointestinal insulinotropic hormones secretion and are metabolized by the gut microbiota, which may change the bile acids binding capacity of their receptors and influence the intestinal immune system and the metabolic processes[104–109]; (2) NLRP6 and NLRP3 inflammasomes negatively regulate NAFLD to NASH progression through changes of the gut microbiota configuration and entry of Toll-like receptor (TLR) 4 and TLR9 agonists into the portal circulation, with modulation of hepatic tumor-necrosis factor (TNF)-α expression driving NASH progression, and TLR7 impacts NAFLD pathogenesis as well[110,111]; (3) recent studies have pinpointed the role played by autophagy in NAFLD pathogenesis, suggesting the therapeutic potential of its regulation.[112] Remarkably, hepatic metabolic pathways and bile acid synthesis as well as autophagic and immune/inflammatory processes are driven by the biological clock. Besides, gut microbiota impact the biological clock[113]; appropriate timing of circadian patterns of hormone secretion, metabolism, bile acid turnover, autophagy, and inflammation with behavioral cycles is necessary to avoid hepatic dysfunction and metabolic disorders.[114–123] Furthermore, experiments performed in aryl hydrocarbon receptor (AHR, a xenobiotic receptor for exogenous toxicants) liver-specific and inducible transgenic mice fed an obesogenic diet showed that AHR signaling pathway activation worsens liver steatosis and in contradiction avoids obesity and systemic insulin resistance interplaying with the biological clock.[124,125] Interestingly, fibroblast growth factor 21, which interacting with β-Klotho coordinates a change to oxidative metabolism during fasting and starvation and has been involved as a mediator joining nutrition, growth, reproduction, and longevity,[126] was recognized as a direct AHR target in the liver and as the inducer of the systemic metabolic benefits in addition to liver steatosis observed in AHR transgenic mice.[124,125]

SUMMARY

The biological clock controls the molecular signaling pathways involved in metabolism regulation; the circadian timing system drives sleep/wake, rest/activity, and fasting/feeding rhythmicity, harmonizing behavioral cycles with energy flux and expenditure and synchronizing the timing of anabolic/catabolic processes with environmental cues, predominantly light/dark alternation and temperature fluctuations. The cogs of the molecular clockwork drive the periodic oscillation of biochemical processes; the nuclear receptors sense nutrient levels and the cellular redox state, guiding the recruitment of coactivators, corepressors, HATs, and HDACs to DNA sequences. These molecular events prompt chromatin remodeling and histone modifications and trigger rhythms of epigenetic modification, transcriptional activity, and gene expression, coordinating metabolic pathways with nychthemeral rhythmicity of behavior. Alteration of the biological clock as well as misalignment of body 24-hour rhythmicity with respect to environmental cues lead to chronodisruption with internal synchronization failure and metabolic derangements, ultimately causing liver steatosis, obesity, metabolic syndrome, and diabetes mellitus, with increased risk of cardiovascular diseases.

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