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Review

Energy-Metabolism Oscillation in the Living Organisms with Circadian Rhythms

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Abstract: It has been generally accepted that the circadian rhythms are genetically controlled by circadian clocks consisting of a transcriptional/post-translational feedback-loop of circadian proteins. In contrast, yeast, which lacks circadian rhythms and clock protein homologues, shows an ultradian rhythm of energy-metabolism oscillation (EMO) with a periodic change of the predominantly anabolic and catabolic phases. EMO is comprised of a feedback loop of oxido-reductive reactions mediated by metabolites like NADH, ATP, and acetyl-CoA which periodically fluctuate in their intracellular levels in concert with the metabolic change; yeast synthesizes storage carbohydrates like glycogen and trehalose in the anabolic phase under reductive conditions and degrades them through cAMP production in the catabolic phase under predominantly oxidative conditions. So, to explore the possibility that EMO underlies the circadian rhythms, I reviewed findings in biochemical and behavioral studies of circadian rhythms of mammals and cyanobacteria. Many lines of evidence show that daily changes in the daily oscillation of energy metabolism between the anabolic and catabolic states are operated in both species in coupling with the metabolism of glycogen mainly through the cAMP-transduction pathway. Further, in mammals, the master pacemakers in the brain are engaged in the metabolic coordination among various peripheral tissues through hormonal and neural signal-transduction pathways which are in turn regulated by signals produced in the peripheral tissues like glucose, insulin, and catabolic hormones. Further, it seems likely that the circadian clock oscillates with a circadian periodicity by itself sensing the redox state of the cell and entrains the metabolic oscillator gives a time signal to a network of gene expression. Finally, I propose that all living organisms are autopoietic dissipative structures, that is, self-productive structures driven by the sustained oscillator EMO dissipating energy.

Key words: energy-metabolism oscillation, circadian rhythm, circadian clock, dissipative structure, autopoiesis

INTRODUCTION

Biological rhythms are undoubtedly essential for all living organisms and biological clocks involving clock proteins are considered to be ubiquitous in eukaryotic and prokaryotic organisms. Among various biological rhythms, the circadian rhythms have been most extensively studied and circadian clocks are thought to regulate a network of gene transcription to synchronize the circadian rhythms with the environmental light/dark cycles. However, yeast, which lacks circadian rhythms and molecular clocks, shows EMO with a periodicity of approximately 4 hours which arises spontaneously under glucose- and nitrogen-limited conditions in the chemostat culture[1-4)]. EMO sustains the periodic change in the respiro-fermentative and respiratory phase in which oxygen demand is low and high, respectively, keeping a high cell density in

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coupling with cell cycle division[1-6]. We reported that EMO is basically regulated by the periodic change in the reductive and oxidative states of NAD cofactor corresponding to the change in the respiro-fermentative and respiratory phases, respectively, thereby forming a feedback-loop of dehydrogenase reactions in energy metabolism[7] (Fig. 1). In brief, in the respiro-fermentative phase, glucose is anabolized into trehalose and glycogen at a rate comparable to that of catabolism and the intracellular level of NADH is kept high. Thus, this phase is relatively anabolic and reductive compared to the respiratory phase. Apparently, the attenuation of the early steps of glycolysis by the flux of glucose into storage carbohydrates suppresses the flux into the aerobic glycolysis preventing a premature shift to the respiratory phase, that is, an excess breakdown of glucose which may lead to a metabolic equilibrium. On the transition to the respiratory phase, cAMP levels increase triggering the breakdown of storage carbohydrates by activating degradative enzymes like neutral trehalases and glycogen phosphorylase, and the increased influx of glucose into the glycolytic pathway activates production of glycerol and ethanol consuming NADH as a cofactor. In the respiratory phase, the resulting increase in the NAD^+ level stimulates respiration by activating pyruvate dehydrogenase complex (PDC) in combination with a decrease in the level of ATP which is rapidly consumed in the formation of biomass leading to budding. Thus, the respiratory phase is catabolic and oxidative, and at the same time productive. In parallel with the activation of mitochondrial respiration, the ethanol and glycerol that accumulated are degraded by respiration via NAD^+- (and $NADP^+$-) dependent oxidation to acetyl-CoA[7]. Eventually, the biomass formation ceases leading to the recovery of NADH and ATP levels, and the synthesis of

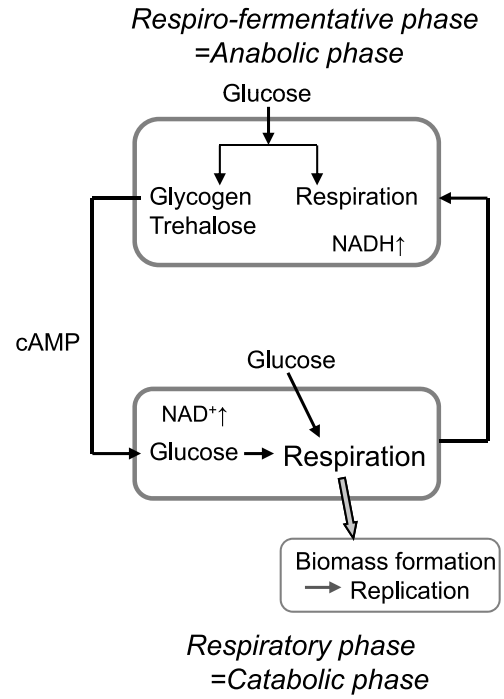

Fig. 1. A schematic presentation of EMO in yeast. EMO is a periodic change of the two phases which are relatively anabolic and catabolic forming a feedback-loop of dehydrogenase reactions in energy metabolism. In the respiro-fermentative phase, glucose is anabolized into trehalose and glycogen at a rate comparable to that of catabolism and the intracellular level of NADH is high. Thus, this phase is relatively anabolic and reductive. On the transition to the respiratory phase, cAMP levels increases triggering a breakdown of storage carbohydrates and the increased influx of glucose into the glycolytic pathway activates production of glycerol and ethanol consuming NADH as a cofactor. In the respiratory phase, the resulting increase in the NAD^+ level stimulates respiration by activating PDC in combination with a decrease in the level of ATP which was consumed in the formation of biomass leading to budding. Thus, this phase is oxidative and catabolic coupled with macromolecular synthesis for self-maintenance and replication. Eventually, the biomass formation ceases leading to a recovery of NADH and ATP levels, and the synthesis of storage carbohydrates resumes promoting a shift to the respiro-fermentative phase, thus making EMO a closed circular process, that is, an oscillator.

storage carbohydrates resumes promoting a shift to the respiro-fermentative phase, thus making EMO a closed circular process as a whole (Fig. 1).

So far, two studies using DNA microarray technology have been reported with the aim of demonstrating that a network of gene expression primarily regulates EMO in yeast[8,9]. The results indicate that a fairly large number of the genes were expressed in the phase when their specified proteins are required to function raising the possibility that a network of gene transcription primarily regulates EMO[9]. However, the genes encoding the synthetic and degradative enzymes of storage carbohydrates are all expressed in the late respiro-fermentative phase suggesting that post-transcriptional regulation is required for the enzymes to function properly[7,9], ruling out the possibility that the network of gene expression exclusively regulates EMO. Further, the phases of peak values for each transcript are largely different between the results reported from the two research groups[10,11]. Moreover, it should be pointed out that the transcriptional and translational reactions are totally endergonic and so they have to be driven by an exergonic metabolic pathway like EMO suggesting that EMO primarily controls the network of gene transcription.

The organization of EMO as a closed circular process fits the dissipative structure according to the theory established by Prigogine and coinvestigators[12,13]. The dissipative structures are spontaneously-occurring and sustained oscillators which operate autonomously dissipating energy (that is, creating free energy and entropy by decomposing high-ordered macromolecules), thereby they self-organize vivid structures (or systems) forming patterns and rhythms like turbulent flows, Benard cell, Belousov-Zhabotinsky (BZ) reaction and living organisms[12,13]. Principally, dissipative structures consist of a feedback loop which shows a periodic action of feed-forward activation and feedback inhibition. The glycolytic pathway has been theoretically proven to be a dissipative structure which oscillates under the primary control of phosphofructokinase 1 (Pfk1p) activated auto-catalytically by its own product ADP leading to a non-linear accumulation of NADH in combination with glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[12,13]. NADH acts as the feed-forward activator of a glycolytic pathway facilitating the production of ethanol. Then, ATP produced in the lower part of the glycolytic pathway acts as a feedback inhibitor by inhibiting the kinase reaction of the enzymes hexokinase and Pfk1p. Experimentally, the ATP level oscillated with an inversed phase relative to the NADH level, supporting the theory[3]. EMO in yeast is also a dissipative structure as it is a sustained oscillator with the dissipation of energy but the feedback mechanism is much complicated due to implication of mitochondrial respiration as described above[7].

Apparently, all living organisms are considered to be dissipative structures because they show either circadian or ultradian rhythms suggesting the presence of sustained oscillators and require nutrients for the dissipation of energy. The fact raises the possibility that all living organism besides yeast contain EMO as a central component of the sustained oscillator. To address the possibility, I reviewed findings in biochemical and behavioral studies of circadian rhythms of mammals and cyanobacteria whose energy metabolism and circadian rhythms have been extensively studied.

Some lines of evidence for the presence of EMO in mammals

It is now believed that, in mammals, there are

multiple circadian pacemakers (populations of cells containing circadian clocks) in the brain that work as master pacemakers to control slave clocks in peripheral tissues depending on environmental entrainment-cues. First, it was found that circadian rhythms entrained in a light/dark cycle are driven by a master oscillator in the SCN[14-16] because total lesion of the SCN causes arrhythmicity in circadian rhythms in light-dark cycles in animals[17]. Later, the presence of a food-entrainable oscillator (FEO) independent of SCN was indicated; when food availability is restricted to a single period in the subjective daytime for nocturnal rodents (restricted feeding, RF), animals adapt to this condition within a few days and biological rhythms persist even when SCN function is physically or genetically disrupted[18,19]. Further, Honma *et al.*[20] found that chronic administration of the stimulant methamphetamine (MAP) induced a robust rhythmicity in locomotor activity in SCN-lesioned rats suggesting the presence of a MAP-inducible oscillator. Although the central oscillators are thought to control the slave oscillators in peripheral tissues, it has become evident that the peripheral oscillators are as robust as the central oscillators[21,22], raising the possibility that the former can function independent of the latter under certain conditions. The peripheral pacemakers are entrained by RF independently of the SCN[22,23] and also by various hormonal stimuli related to the metabolism of food like cAMP and glucocorticoids[24], suggesting that the circadian oscillators in peripheral tissues are robust oscillators involved in the metabolism of food, that is, energy metabolism. In fact, there have been many reports suggesting the circadian oscillation of energy metabolism (i.e., EMO) in mammals (Fig. 2). For example, the respiratory quotient in rats increased in the nighttime compared with that in the daytime[25,26] (Fig. 2A) indicating that carbohydrates are a main energy source in the active (dark) phase and more lipids were used as an energy source during the resting (light) phase than in the active phase. In agreement with this finding, in the active phase in rat liver, the glucose level in plasma is high with a high insulin level and the synthesis of fatty acids and glycogen was activated[27,28] (Fig. 2B and C). Similarly, in humans, glycogen accumulates most in the late daytime when the concentration of glucose in blood is high and the utilization of glucose (for synthesis of glycogen and fatty acids) was facilitated by insulin[29,30]. On the other hand, the glucagon level is high activating the breakdown of glycogen in the early resting phase in rat liver[27], showing that the metabolism is catabolic in this phase. In the nighttime, glycogen is degraded by glucagon (Fig. 2C) and fatty acids are degraded by ACTH and adrenaline via a process mediated by cAMP as an intracellular messenger[31,32]. Furthermore, in both the mouse and rat, liver mitosis peaks in the middle of the resting phase[33,34]. Thus, assuming that the resting and active phases in mammals correspond to the respiratory and respiro-fermentative phases of EMO in yeast, respectively, based on the main carbon-source (glucose and fatty acid (in mammals) or acetate (in yeast) which is oxidized during respiration via acetyl-CoA), the energy-metabolism in the peripheral tissues of mammals is circadian-oscillated with principally a similar, if not identical, mechanism to that of EMO in yeast.

Although mammals usually show circadian rhythms of activity/rest cycles, they occasionally exhibit ultradian rhythms under various conditions in which the persistence of the circadian rhythms is disturbed[20,35-38]. For example, rats exposed to prolonged continuous light and those with a lesioned SCN exhibited regular ultradian rhythms of locomotion activity[20,35].

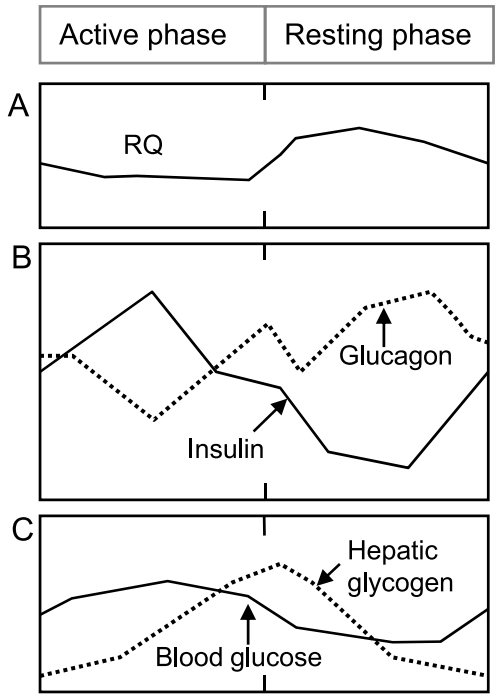

Fig. 2. A schematic presentation of the changes in various parameters during the circadian oscillation between the resting (sleep) and the active (wake) phases in rodent as a representative of mammals. (A) A time course of respiratory quotient (RQ)[22]. (B) Time courses of the changes in the levels of insulin (solid line) and glucagon in plasma (broken line)[23]. (C) Time courses of the changes in the levels of hepatic glycogen (solid line)[136] and plasma glucose (broken line)[23].

Recently, van der Veen *et al.*[39] have shown that, in the common vole *Microtus arvalis* which normally shows an ultradian feeding rhythm without fluctuations of clock-gene mRNA levels in liver, a high-amplitude circadian oscillation of the expression of the clock-gene mRNA was elicited by subjecting the animals to a circadian feeding regimen and that a low-amplitude oscillation was elicited by giving them access to a running wheel. These results suggested that mammals contain endogenous ultradian oscillators that occasionally emerge when they are physically and genetically separated from the circadian clock pacemakers or when animals encounter perturbations of the environment like the loss of photic cues in light/dark cycles and restriction of locomotor activity in the absence of a wheel running.

It is now believed that circadian clocks whose components are largely common between master and slave pacemakers control circadian rhythms. Among the clock proteins, Clock was first shown to be essential in the circadian clocks, as homozygous Clock mutant mice became arrhythmic when kept in constant darkness in both LEO-[40] and FEO-driven circadian locomotor rhythms[41]. Here, it should be pointed out that, in these reports, the homozygous Clock mutant mice kept in light/dark cycles were rhythmic in locomotor activity suggesting that Clock is dispensable in normal light/dark cycles. Moreover, homozygous Clock-mutant[42] and Clock-null[43] mice were found to sustain circadian activity/rest rhythms even in constant darkness indicating the total dispensability of Clock in the regulation of circadian rhythms. Although it remains possible that some homologous protein(s) substitute for Clock in these animals, no candidate proteins have not emerged[44]. Thus, it is likely that circadian clock in SCN is dispensable for circadian rhythms in light/dark cycles although SCN as a whole is essential[17]. Furthermore, Lakin-Thomas[45] postulated that the standard transcription/post-translation mechanism of the circadian oscillators is no longer an adequate model for the circadian oscillator because rhythmicity is observed in various model organisms including mammals in the situation where either the transcription of various clock genes is held constant or clock gene function is eliminated in knock-out mutants. In addition, there are reports suggesting a strong link between the circadian rhythm and metabolism although the precise

mechanism behind such a link is not clear[46-49].

Presentation of a model of circadian oscillators featuring EMO in mammals

To study the potential role of energy metabolism in the circadian rhythms in mammals, I further reviewed literatures published to date and would like to present a model of circadian EMO in mammals using a small animals like laboratory rodents as representative (Fig. 3). The model is principally identical to that of yeast in that the oscillation is a periodic change between the anabolic and catabolic states (compare Figs. 1 and 3A). Roughly, Energy metabolism in the active phase is substantially anabolic under the control of insulin activating the synthesis of glycogen and lipid, while that in the resting phase is regulated mainly by glucagon in combination with orexin and glucocorticoids[50,51]. However, metabolic states in the transition between the active and resting phases in which the locomotor activity increases (thus, belong to the active phase) are catabolic accompanying gluconeogenesis stimulated mainly by glucocorticoids[52,53]. These reactions caused by glucagon and corticosterones are also found to occur during exercise apart from the circadian rhythms[50,54] suggesting that the "anticipatory locomoter activity" in the circadian rhythm is the action that facilitates the catabolism of glycogen and proteins in muscles leading to hepatic gluconeogenesis. During the transition from the active to resting phase, the metabolism may shift relatively smoothly from the anabolic to catabolic state because the secession of food intake gradually causes hypoglycemia which induces the secretion of catabolic hormones[55] and energy sources, especially hepatic glycogen, are stored in abundance. However, if not enough hepatic glycogen has accumulated by the end of the active phase, animals synthesize glycogen via gluconeogenesis by activating exercise. Especially, in diurnal rodents with a crepuscular pattern (associated with dusk and dawn) of locomotor activity, the secretion of catabolic hormones increases in this phase[56,57]. On the other hand, during the transition from the resting to active phase, animals (regardless of nocturnal, diurnal and crepuscular) are required to produce glucose via gluconeogenesis by promoting locomotor activity[58-62] as the hepatic glycogen level has decreased.

The regulatory mechanism of metabolic oscillation in mammals is, of course, very different from that in the unicellular organism yeast: it is operated by a network of peripheral tissues like the liver, muscle, and adipose tissue, in a coordinated fashion under the control of hormonal and neural signals originating from the central pacemakers in the brain (Fig. 3B). At the end of the anabolic phase, insulin transported in the brain suppresses food intake inhibiting the expression of neuropeptide Y (NPY), a hypothalamic neuropeptide which activates food intake[63-65]. It should be added that some animals like diurnal rodents transiently increase the levels of orexin and glucocorticoids to produce glycogen by activating the locomotor activity (Fig. 3B). The decrease in blood glucose due to cessation of food intake stimulates orexin neurons in the hypothalamus[66,67] and orexins induce catabolic hormones like glucagon[68] and glucocorticoids[50,51]. The catabolic metabolism is facilitated mainly by glucagon in the early resting phase inducing the degradation of hepatic glycogen and fatty acids, and then, in the beginning of the active phase, animals are required to produce glucose via gluconeogenesis by promoting locomotor activity and so the levels of orexins and glucocorticoids are usually highest in this phase of the circadian rhythm[58-62]. The increase in glucocorticoids in the brain, likely in

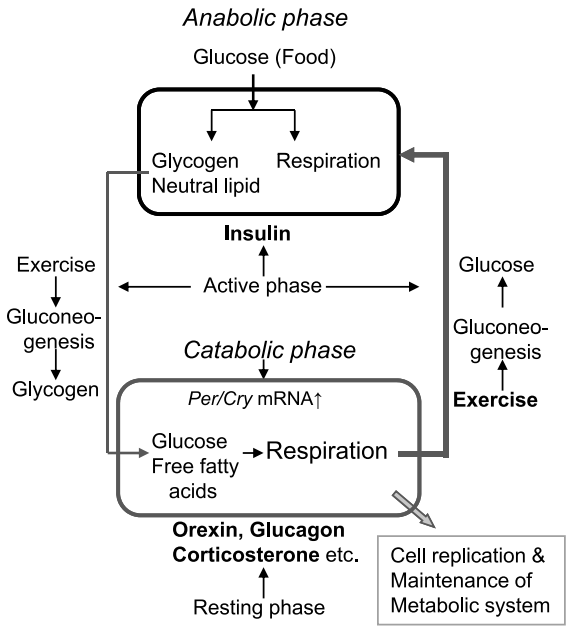

Fig. 3 A

Fig. 3. A model of the mechanism of circadian oscillation of energy-metabolism in a rodent as representative of mammals. (A) A gross presentation of energy-metabolism oscillations in animals showing a periodic change of catabolic and anabolic phases. In the active (wake) phase, the energy metabolism is in an anabolic state under the control of insulin synthesizing glycogen and lipid and in the resting (sleep) phase animals are in a catabolic state under the control of catabolic hormones like glucagon, corticosterones and orexins degrading glycogen, protein and lipid. During the activity-to-rest phase transition, animals synthesize glycogen via gluconeogenesis through exercise if the glycogen level in the liver is not high enough. On the other hand, during the rest-to-activity transition, animals are required to produce glucose via gluconeogenesis from lactate and amino acids in the liver as the glycogen level in the liver is reduced. (B) A schematic presentation of the network of hormonal and neural signals engaged in the mechanisms of regulation and entrainment of the circadian rhythm. At the end of the anabolic phase, insulin transported in the brain suppresses food intake inhibiting the expression of NPY, while some animals like diurnal rodents transiently increase the levels of orexin and glucocorticoids to produce glycogen by activating the locomotor activity (in the box surrounded by a broken line). The decrease in blood glucose due to cessation of food intake stimulates hypothalamic orexin neurons and orexins induce secretion of glucagon and glucocorticoids. The catabolic metabolism is facilitated early by glucagon degrading hepatic glycogen and fatty acids, and then glucocorticoids gradually increase reaching a peak value prior to the beginning of the active phase when animals are required to produce glucose via gluconeogenesis by promoting locomotor activity. The increase in glucocorticoids in the brain facilitates the intake of food mediated by NPY. The increase in blood glucose induces secretion of insulin which facilitates the anabolism of glucose and other nutrients. The central pacemakers like LEO, FEO, and the MAP-responsive oscillator seem to target the catabolic phase for synchronization of the circadian rhythms. LHA, lateral hypothalamic area; SCN, suprachiasmatic nucleus; DMH, dorsomedial hypothalamic nucleus; CPu, caudate-putamen; MAP, methamphetamine.

combination with transient declines of blood glucose by insulin[69-71], facilitates the intake of food mediated by NPY[65] (Fig. 3B). The increase in blood glucose induces secretion of insulin[72,73] which facilitates the anabolism of glucose and other nutrients and thus a feed-back-loop of the energy metabolism is completed. Exercise in the early active phase may be evolutionally conserved in small animals, as it is advantageous for the food-seeking movement. On the other hand, in large mammals like humans, the anticipatory locomotor-activity prior to the active phase is unusual suggesting that the metabolic changes may be regulated by hormonal and neural regulations without exercise. Therefore, the metabolic changes in the whole body in mammals are regulated principally by the fluctuation of the blood levels of glucose and related hormones, while those in the peripheral tissue cells are regulated by fluctuations in the intracellular levels of metabolites like NADH/NAD^+, ATP/ADP/AMP, and acetyl-CoA, as shown during the metabolism in FAA in rodents[74].

Apparently, the central pacemakers in the brain target the catabolic state for synchronization of the circadian rhythms and increase their activity reaching the maximum level in the early active phase (Fig. 3B). The dorsomedial hypothalamic nucleus (DMH) as the FEO pacemaker targets the catabolic state by activating orexin neurons in the lateral hypothalamic area (LHA) prior to the food-available period in which animals become anabolic[75-78] whereas an alternative pathway is suggested[79]. Although the SCN as the LEO pacemaker have only sparse projections to orexin neurons[80], SCN inputs reach them indirectly via DMH[81]. Further, while

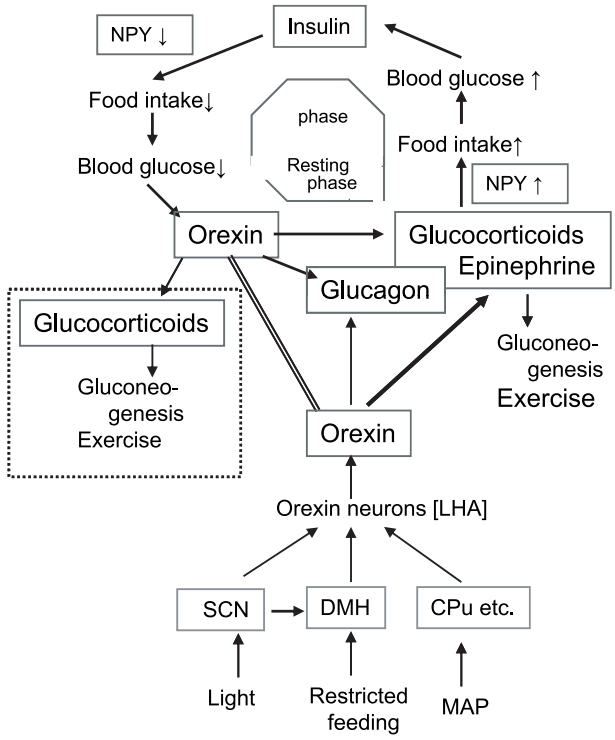

Fig. 3B

acutely-administrated MAP immediately increases locomotor and feeding activities by stimulating the secretion of orexin[82], chronic administration of MAP sensitizes animals causing MAP-inducible oscillator(s) to appear outside the SCN such as in the parietal cortex, caudate-putamen (CPu) and striatum[83-88]. The oscillators once established increase the corticosterone level during the resting phase leading to the elongation of the active phase[83]. Thus, the MAP-sensitive oscillator entrains the circadian rhythm stimulating a catabolic state in the resting phase prior to the activation of the locomotor activity as the other oscillators do.

Potential role of the circadian clock suggested by the target genes of BMAL1/Clock heterodimer

The circadian clocks in both central and peripheral pacemakers contain the BMAL1/Clock heterodimer as a major component which acts as a transcription factor targeting genes that contain E-box elements in their promoters[89]. Recently, there has been increasing evidence that most, if not all, genes with E-box elements also contain cAMP-response element (CRE) for the binding of CRE-binding protein (CREB) which is activated through the cAMP-protein kinase A (PKA) signaling pathway. For example, in addition to the clock genes Per1 and Per2[90], Dec1 (also called Stra13 and Sharp2), a regulator of the mammalian circadian clock belonging to the family of basic helix loop helix (bHLH) transcription factor[91], is transcribed by the BMAL1/Clock heterodimer, depending on E-box elements[92] and is also inducible by cAMP, which probably depends on a putative CRE in its promoter[93,94]. The transcription of the cholesterol 7α-hydroxylase gene CYP7A1 is suppressed by Dec2 (also named Sharp1), mediated by E-box[95], and also inhibited by cAMP by activating phosphorylation[96] or/and recruitment of hepatocyte nuclear factor 4α (HNF4α)[97]. The expression of AC1, which encodes a neurospecific adenylate cyclase and plays an important role in melatonin synthesis in the pineal gland, is mediated by E-box elements[98] and the expression is inhibited by increases in cAMP[99]. The expression of the gene encoding arylalkylamine N-acetyltransferase (AA-NAT)[100], a key enzyme in melatonin synthesis, is mediated by an E-box in the first intron and is also induced by cAMP depending on CRE[101]. Albumin D-element binding protein (DBP) is transcribed, mediated by E-box elements[102], and induced by administration of forskolin (adenylate cyclase activator) in rat-1 cells[103], although it is unknown whether the gene contains CRE in its promoter. These findings, taken together, suggest that genes expressed through mediation of E-box elements are a subset of genes that are activated though the cAMP-PKA-CREB signaling pathway and are involved in the regulation of circadian rhythms targeting the catabolic phase. Thus, it is likely that circadian clocks are a kind of backup system for the cAMP-signaling pathway and they become essential when the environmental light/dark condition is disturbed.

So far, numerous studies using DNA microarray technology have been conducted to demonstrate the network of gene expression associated with the circadian rhythms[104]. The results obtained so far indicate that the expression of about 10 % of genes oscillates in a circadian fashion at significant amplitude (mostly, 1.3- to 3.0-fold) in mammalian tissues and that, however, the species of circadian-controlled genes are markedly different for every tissue and cell tested, and even for each assay using the same tissues, except the canonical clock genes which prominently oscillate in most tissues[104-106]. Thus, a convincing mechanism for the transcriptional

network has yet to be proposed. In addition, the expression of a large proportion of circadian-controlled genes is affected by the feeding status in the rat liver[107]. Thus, it is likely that the network of gene expression is finely tuned depending on genetic, metabolic and environmental conditions.

Potential role of the circadian clock suggested by the study of circadian rhythms in unicellular cyanobacteria

Unicellular cyanobacteria are prokaryotes that perform oxygen-evolving photosynthesis showing clear circadian rhythms, as evidenced by periodic changes in photosynthesis and nitrogen fixation, and the cyanobacterium *Synechococcus* is the only prokaryote whose circadian clock has been elucidated[108,109]. Kondo and colleagues[110] reported that a central oscillator of the clock is composed of three proteins (KaiA, KaiB and KaiC), and the phosphorylation state of KaiC oscillates with a periodicity of about 24 h *in vivo* in the presence of transcription and translation inhibitors. Furthermore, they reconstituted the circadian oscillation of KaiC phosphorylation *in vitro* by incubating the three proteins in the presence of ATP at molar ratios similar to that measured *in vivo*[111,112]. These results suggested that the levels of the components in the clock are regulated to oscillate with a circadian periodicity *in vivo*. The input signal for the core clock is transferred by the proteins LdpA and CikA. LdpA, an iron-sulphur protein, senses the redox state of the cell and influences the period length of the circadian clock, which affects the abundance and redox sensitivity of CikA[113-115]. CikA, a histidine protein kinase, senses the redox state of the plastoquinone pool in the thylakoid membrane, which varies as a function of the light strength in the environment, and influences the phosphorylation state of KaiC during the resetting of the circadian phase by a dark pulse[115,116]. Thus, phase setting of the circadian clock is regulated by the metabolic state of the cell through a coordinated function of LdpA and CikA. On the other hand, the output signal from the core clock is relayed by two protein kinases, SasA and RpaA, which make up a two-component signal transduction system[117]. SasA is a KaiC-binding histidine kinase and its disruption attenuates or eliminates circadian expression of all tested genes including those of Kai proteins[118]. RpaA, a potential DNA-binding protein, acts as a cognate response regulator of SasA, and its disruption also severely attenuates the circadian expression of all genes tested[117]. Thus, these proteins are thought to mediate temporal information from the core clock to drive the global expression of genes in cyanobacteria. Furthermore, Smith and Williams reported that a chromatin compaction rhythm is found in parallel with the circadian rhythm of global expression of genes in *Synechococcus*, and the rhythm is controlled by the circadian clock depending on Kai proteins[119]. However, because the chromatin compaction rhythm is not affected by the disruption of SasA[118], the precise pathway from the central clock to the global expression of genes remains to be elucidated.

In regard to metabolism, unicellular cyanobacteria photosynthesize glucose from carbon dioxide thereby evolving oxygen, and accumulate polymers of glucose resembling glycogen into dense granules in the daytime[120,121]. In the nighttime, they fix nitrogen into ammonium, which is catalyzed by nitrogenase, leading to the synthesis of amino acids and proteins and other macromolecules[120,121] (Fig. 4). Nitrogen fixation is a process that requires a lot of energy; therefore, stored glycogen is degraded to yield energy via respiration[121]. Thus, the metab-

olism of unicellular cyanobacteria is mainly anabolic in the daytime and catabolic in the nighttime. cAMP plays an important role in the regulation of metabolism in unicellular cyanobacteria as in other living organisms. The level of cAMP increases immediately after a dark-to-light transition and, conversely, decreases after a light-to-dark transition[122]. The major adenylate cyclase Cya1 in unicellular cyanobacteria is activated in response to the irradiation of blue light

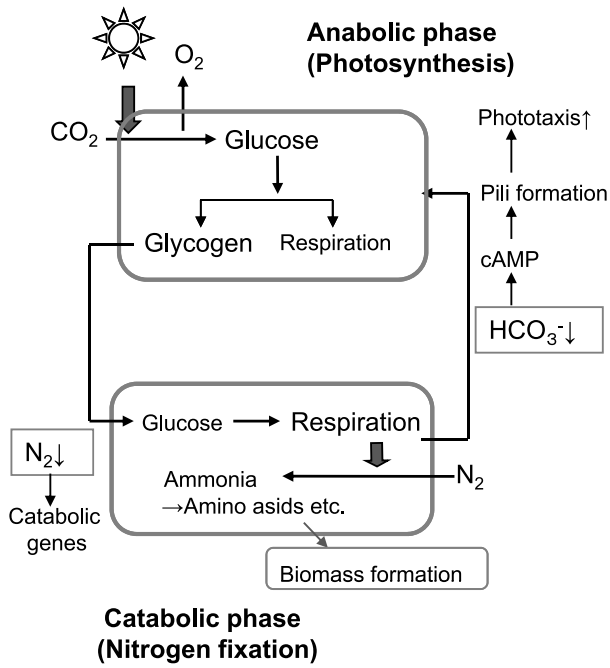

Fig. 4. A schematic presentation of EMO in unicellular cyanobacteria. The cells photosynthesize glucose from carbon dioxide evolving oxygen in the daytime and fix nitrogen into ammonium leading to the synthesis of amino acids and proteins and other macromolecules in the nighttime. In parallel, they accumulate glucose into glycogen in the daytime and degrade the stored glycogen to yield energy for nitrogen fixation via respiration. Thus, the metabolism of unicellular cyanobacteria is mainly anabolic in the daytime and catabolic in the nighttime. After the light-to-dark transition, the decrease in the intracellular level of nitrogen due to nitrogen fixation induces the expression of genes involved in the catabolism of glucose and glycogen. The level of cAMP increases immediately after a dark-to-light transition in response to the blue light via as-yet-unidentified pathway and, in addition, the decrease in the bicarbonate level activates the adenylate cyclase Acy1, which causes the activation of genes involved in the biogenesis of pili, which enhances phototaxis. Thus, in unicellular cyanobacteria, the metabolic oscillation is regulated by their own substrates such as nitrogen and carbon dioxide in combination with the actions of light and cAMP.

and increase cellular motility for phototaxis[123] which is caused by the activation of genes involved in the biogenesis of pili via the cAMP-responsive protein SYCRP1[124,125]. Furthermore, bicarbonate decreases the maximal velocity and substrate affinity in Cya1[126], which suggests that the activity of Cya1 is suppressed in the nighttime as the level of carbon dioxide increases in the absence of photosynthesis. In addition, Osanai *et al.*[127] reported that nitrogen depletion induces the expression of genes involved in the catabolism of glucose and glycogen summarizing the results of experiments that used mutants expressing a partially inactivated NtcA, a global nitrogen regulator, or a deficient sigE — a sigma factor specific for sugar catabolic genes. Thus, in the nighttime when the intracellular level of nitrogen decreases due to nitrogen fixation, the expression of catabolic genes is activated in response to the nitrogen depletion rather than cAMP production in unicellular cyanobacteria differing from other prokaryotes wherein the expression is activated by cAMP[128]. This particular action of cAMP in unicellular cyanobacteria may be related to the coexistence of photosynthesis and nitrogen fixation in a cell. This is because, in filamentous cyanobacteria wherein nitrogen fixation is operated exclusively in differentiated cells called heterocysts, cAMP increases during the light-to-dark transition[122,129] and an increased production of cAMP induces the fragmentation of filaments and inhibits growth[130], which suggests that cAMP is engaged in degradative (=catabolic) reactions. Altogether, in unicellular cyanobacteria, the metabolic oscillation is regulated by their own substrates such as nitrogen and carbon dioxide in combination with the actions of light and cAMP, suggesting that the oscillation operates autonomously in light/dark cycles. In consistency with this view, KaiC mutants grow normally as the wild type while they show arrhythmic expression of a reporter gene under constant light conditions[121], and both sasA-null and rpaA-null mutants also grow normally under constant light conditions while the expression of genes is largely arrhythmic[117,118]. These results suggest that the programmed expression of genes by the circadian clock is not essential to maintain metabolic oscillation. Moreover, normal growth under the constant light conditions means that the oscillation of cellular metabolism are normal because cells must die if the oscillation becomes arrythmic as nitrogenase is severely sensitive to evolving oxygen[131]. Furthermore, the arrhythmicity of gene expression in mutants is re-synchronized by application of a light/dark cycle[132-134] suggesting that one of the reasons for the arrhythmic expression of genes in the absence of the clock is the desynchronization of metabolic rhythms among cells in cultures under constant light conditions. Thus, it is likely that the clock is required for synchronization of the circadian oscillation of metabolism in cell populations and for the transfer of temporal information needed to regulate gene expression. Taken together, the study of the circadian rhythm of unicellular cyanobacteria suggested that the circadian clock oscillates with a circadian periodicity by itself in concert with the redox state of the cell and entrain the metabolic oscillator by giving a time signal to a regulatory system for gene expression.

Conclusion: living organisms as autopoietic dissipative structures

Herein I report that the energy metabolism oscillates daily between anabolic and catabolic states and circadian rhythms are redox-regulated by metabolites which fluctuate in their intracellular levels in coupling with the metabolic oscillation. The circadian clocks may oscillate

with a circadian rhythmicity *per se* and mediate temporal information to cells to maintain the synchronization of metabolism in cell populations. The periodic change of the two metabolic states is essential to avoid metabolic equilibrium, that is, death, and so it should be essential for all living organisms. According to the theory of autopoiesis proposed by Maturana[135], the characteristics of living organisms that discriminate them from non-living organisms (dissipative structures) are suggested as follows. First, the metabolism in a living organism is organized in a closed circular process (that is, in a metabolic oscillator) so that the organism can continuously produce itself. Second, the circularity makes a living organism a unity (that is, cell and body) and so makes it an autopoietic (self-productive) system. Third, the circularity makes a living system a self-referring system to maintain its circularity and to define its identity accordingly. Fourth, a living organism undergoes internal structural changes to adapt to the environmental perturbations through a cognitive domain. Considering the theory, I would like to propose here that a living organism uses the genome and gene transcription system as a self-referring system to maintain the metabolic organization assuring identities of individuals and species. Thus, the circadian clocks may be a component of the circular organization of metabolic pathways of EMO and participate in the modulation of metabolism in response to changes in genetic, metabolic, and environmental conditions. In other words, the perturbations met during interaction with the environment having circadian rhythmicity are processed in the circular processing of the metabolism involving the circadian clock mechanism as a component, and the internal changes of metabolism thus induced lead to behavioral changes to adapt to the environment. Thus, the requirement of the circadian clocks may change depending on the metabolic and environmental conditions and it is almost dispensable under light/dark conditions in circadian organisms.

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