

## Circadian clock and vascular disease

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## Abstract

Cardiovascular functions, including blood pressure and vascular functions, exhibit diurnal oscillation. Circadian variations have been clearly shown in the occurrence of cardiovascular events such as acute myocardial infarction. Circadian rhythm strongly influences human biology and pathology. The identification and characterization of mammalian clock genes revealed that they are expressed almost everywhere throughout the body in a circadian fashion. In contrast to the central clock in the suprachiasmatic nucleus (SCN), the clock in each tissue or cell is designated as a peripheral clock. It is now accepted that peripheral clocks have their own roles specific to each peripheral organ by regulating the expression of clock-controlled genes, although the oscillation mechanisms of the peripheral clock are similar to that of the SCN. However, little was known about how the peripheral clock in the vasculature contributes to the process of cardiovascular disorders. The biological clock allows each organ or cell to anticipate and prepare for changes in external stimuli. Recent evidence obtained using genetically engineered mice with disrupted circadian rhythm showed a novel function of the internal clock in the

pathogenesis of endothelial dysfunction, hypertension and hemostasis. Loss of synchronization between the central and peripheral clock also contributes to the pathogenesis of cardiovascular diseases since restoration of clock homeostasis could prevent disease progression. Identification of clock-controlled genes in each organ, as well as discovery of tools to manipulate the phase of each biological clock, will be of great help in establishing a novel chronotherapeutic approach to the prevention and treatment of cardiovascular disorders.

## Introduction

It is well known that some cardiovascular physiological functions such as heart rate (HR) and blood pressure (BP) exhibit apparent circadian variation. In addition, many cardiovascular disorders occur in a circadian fashion. For example, acute myocardial infarction (AMI) and cerebral infarction most often occur in the early morning whereas subarachnoid hemorrhage and a subtype of atrial fibrillation are usually seen in the afternoon. The diurnal variation in cardiovascular events is believed to be the consequence of both external and internal biological clock rhythms. Most of these disorders, once they happen, can be fatal or induce severe damage; therefore, it is important to elucidate the precise mechanism of the onset of such diseases to establish a preventive strategy. In this article, we would like to review the role of the molecular clock in the pathogenesis of vascular diseases.

## Molecular clock in mammalian cells

Accumulating evidence has elucidated the molecular mechanisms of the circadian clock<sup>1-4</sup>.

Several positive and negative feedback loops exist in the biological clock at transcriptional and post-translational levels. Among them, the core negative feedback loop comprises positive limb (CLOCK, NPAS2, BMAL1 and CLIF/BMAL2) proteins and negative limb (three period, PER1, PER2, PER3 and two cryptochrome, CRY1, CRY2) proteins. Most are basic helix-loop-helix (bHLH)/*per-arnt-sim* (PAS) domains containing transcription factors. CLOCK or NPAS2 forms a heterodimer with BMAL1 or CLIF/BMAL2 and binds to the E-box element with CACGTG sequences upstream of the *per* or *cry* gene <sup>2</sup>. They enhance the transcription of *per* and *cry*, and the PER protein forms a complex with the CRY protein and inhibits CLOCK/BMAL-mediated transcription of the *per* or *cry* gene itself, therefore resulting in a negative feedback loop (Figure 1). PER proteins are also phosphorylated with serine-threonine kinase casein kinase 1 epsilon (CK1ε) and degraded by the proteasomal pathway <sup>5</sup>. Thus, post-translational mechanisms, including phosphorylation and ubiquitination, also control the timing of the circadian clock <sup>6</sup>. In contrast to the ubiquitous expression of BMAL1, CLIF/BMAL2 is mainly expressed in vascular endothelial cells <sup>7</sup>. However little is known about the redundancy or the dynamic function of BMALs in the

vasculature, although CLIF/BMAL2 was shown to have a higher affinity to PER2 than BMAL1<sup>8</sup>.

In addition to this core feedback loop, the nuclear receptor REV-ERB $\alpha$  is also transactivated by the CLOCK/BMAL1 heterodimer. The REV-ERB $\alpha$  protein represses *bmal1* transcription, which is essential for circadian *bmal1* expression. Another feedback loop includes the bHLH domain containing transcription factors, *deleted in esophageal cancer* (*dec1*, *dec2*). The heterodimer of CLOCK and BMAL1 binds to the E-box upstream of *dec1* and *dec2*, and activates their transcription. DEC proteins in turn repress the transcriptional activity of CLOCK/BMAL1, thus forming another negative feedback loop. The CLOCK/BMAL1 heterodimer binds to the E-box upstream of not only the *per* or *cry* gene, but also to other target genes designated as clock-controlled genes (CCGs). The CCGs include arginine vasopressin (AVP), *wee1* or other target genes, and mediate the rhythmicity of the biological clock and account for the circadian variation in humoral or metabolic functions. The CCGs also comprise three proline- and acid-rich (PAR) basic leucine zipper (bZip) transcription factors, D-element binding proteins (*dbp*), hepatic

leukemia factor (*hlf*), and thyrotrophic embryonic factor (*tef*)<sup>4</sup>. In addition to CLOCK/BMAL1, PAR transcription factors also induce the circadian expression of CCGs and therefore act as mediators or amplifiers of CLOCK/BMAL1-induced CCG expression. The induction of CCG expression is antagonized by another bHLH transcription factor, E4BP4<sup>9</sup>, which is induced by REV-ERB $\alpha$ . The phase of the three PAR transcription factors are antiphase to that of E4BP4, resulting in the circadian expression of CCGs.

The center of the biological clock, that is, the central clock, exists in the suprachiasmatic nucleus (SCN) in the hypothalamus<sup>3</sup>. The central clock regulates physiological functions through the autonomic nervous system, humoral mediators or unknown factors. The phases of the internal clock can be entrained by external stimuli. Zeitgebers (timekeepers) are factors that could reset the rhythm. Clock genes express in a circadian fashion in SCN, and light is the main zeitgeber for the central clock and can reset the phase of its rhythm. In addition to the central clock, circadian expression of clock genes can be detected in each peripheral organ or cell, suggesting that each organ has its own internal clock. This clock system is called the peripheral clock in contrast to the central

clock in the SCN (Figure 2). The molecular mechanism of the peripheral clock is considered to be similar to that of the central clock<sup>10,11</sup>. The central clock synchronizes each of the peripheral clocks within the body<sup>1</sup>; however, little is known about how peripheral clocks are regulated by the central clock. In contrast to the central clock, the phase of the peripheral clock cannot be entrained with light; thus, the phases of each peripheral clock seem to be synchronized by neuronal or other unknown humoral factors derived from the SCN. Finding the appropriate zeitgeber for each organ will help not only in understanding the clock system, but also in establishing a novel type of therapeutic approach, named chronotherapy.

## Molecular/peripheral clock in vasculature

The existence of a peripheral clock system in each organ or cell was demonstrated using *in vitro* cultured fibroblasts<sup>10</sup>. Balsalobre et al. stimulated fibroblasts with 50% serum for a short time and observed the circadian oscillation of clock gene expression. A single cell in culture has its own oscillation rhythm, whereas cell populations in *in vitro* culture are

usually arrhythmic because of the asynchronous circadian rhythm among cells <sup>12</sup>. However, once a phase-aligning stimulus such as 50% serum is applied, they start to show uniform circadian rhythm <sup>13</sup>.

Diurnal variation in clock genes was also reported in cardiac organs including the heart, aorta and kidney <sup>14,15</sup>. A study based on microarray analysis revealed that about 8-10% of genes show circadian expression in the heart and liver; however, most of these genes are organ specific <sup>16</sup>. Therefore, in addition to the central clock in the SCN, a peripheral clock in each organ seems to regulate tissue-specific physiological functions, and identification of peripheral CCG will greatly help in understanding the role of the biological clock in cardiovascular organs <sup>17</sup>.

In order to prove the existence of an intrinsic clock system in cardiovascular tissues, we studied the clock gene expression of *in vitro* cultured vascular endothelial cells and confirmed the circadian clock gene expression <sup>18</sup>. We also searched for CCGs in vascular endothelial cells and identified circadian expression of thrombomodulin, a membrane protein with anti-coagulant activity. Vascular smooth muscle cells also possess

an intrinsic biological clock. In addition to serum shock, angiotensin II (AngII) or retinoic acid also induced circadian clock gene expression, suggesting that AngII or retinoic acid can function as a zeitgeber<sup>15,19</sup>. Chalmers et al. identified the tissue inhibitor of metalloproteinase 1 and 3, collagen 3a1, transgelin1 (sm22 $\alpha$ ) and calponin1 exhibit circadian expression in smooth muscle cell line (Movas-1)<sup>20</sup>. Several vascular functions have been shown to exhibit circadian rhythm, including endothelium-dependent vasodilatory function<sup>21,22</sup>. In human subjects, endothelial function measured by flow-mediated dilation was shown to have circadian oscillation with lower function in the morning<sup>23</sup>.

Recent evidence has illuminated the roles of the molecular clock in endothelial functions. Mice with the *Per2* mutation produced lesser amounts of nitric oxide and vasodilatory prostaglandins and more cyclooxygenase-1 derived vasoconstrictors than the wild type, resulting in impaired endothelium-dependent relaxation in response to acetylcholine<sup>24</sup>. Endothelial dysfunction was also seen in mice with *Bmal1* Knockout (KO) and *CLOCK*<sup>mut</sup><sup>25</sup>. Akt signaling and nitric oxide production were reduced in *Bmal1* KO

arteries, and those arteries became more susceptible to thrombosis formation. The release of hematopoietic stem cells or endothelial progenitor cells (EPCs) from bone marrow is regulated by circadian rhythm <sup>26</sup>. In a diabetic state, a disrupted peripheral clock caused by bone marrow neuropathy impaired circadian release of EPCs from bone marrow and exacerbated diabetic retinopathy <sup>27</sup>. Per2 mutant mice also had impaired EPC mobilization function <sup>28</sup>. EPC mobilization in response to ischemia or VEGF stimulation was reduced in Per2 mutant mice compared with wild-type mice. EPCs from Per2 mutant mice exhibit greater senescence together with Akt activation and impaired angiogenesis in a hind-limb ischemia model. Transplantation of wild-type bone marrow into Per2 mutant mice prevented autoamputation in Per2 mutant mice. Both Bmal1 KO and Per2 mutant mice had endothelial dysfunction; however, the opposite effect was observed with respect to Akt activation. This may be related to the different roles of these clock genes in the core loop formation; that is, Bmal-1 is a positive limb protein whereas Per2 works as a negative limb protein. Senescence also affects the biological clock function. Kunieda et al. revealed that circadian expression of clock genes are attenuated in senescent human smooth muscle cells

(HSMCs)<sup>29</sup>. Telomere shortening and impaired cAMP response element-binding protein (CREB) activation accounted for the loss of circadian rhythmicity in senescent cells, since the introduction of telomerase or restoration of CREB effected a complete recovery of the circadian rhythm.

## Blood pressure/hypertension and the molecular clock

In the normal subjects, BP declines during nighttime, begins to rise in early hours of the morning and reaches near peak or peak values at mid-morning<sup>30,31</sup>. A number of factors can influence the diurnal variation in BP, including the autonomic nervous system<sup>32</sup>, vasoactive intestinal peptide<sup>33</sup>, plasma renin activity<sup>34</sup>, aldosterone<sup>35</sup> and plasma atrial natriuretic peptide (ANP)<sup>36</sup>. It is well known that sympathetic activity as well as renin-angiotensin-aldosterone activity peaks in the morning<sup>32,37</sup>. BP is also affected by external factors such as physical activity, emotional state, eating and the sleep/wake cycle. Results of a study in humans indicated that disharmony in the circadian rhythm can cause hypertension<sup>38</sup>. Human subjects kept under a protocol of circadian misalignment with

behavioral cycle 28 hours instead of 24 hours, demonstrated mild but significant hypertension.

A decade ago, Janssen et al. studied the role of the internal clock in the rhythm of BP. Lesioning of the rat SCN abolished the circadian rhythm of BP and HR without affecting the 24-hour cycle of locomotor activities<sup>39</sup>. Recent evidence has provided much deeper insights into the role of the molecular clock in BP regulation. Global deletion of *Bmal1* completely abolished the diurnal variation in BP<sup>40</sup>. *Bmal1* mutant mice also exhibit hypotension together with reduced production of catecholamines. Global *Per2* mutant mice also exhibit lower BP<sup>24</sup>. In contrast, the endothelial-specific deletion of *Bmal1* did not affect the variation in BP, suggesting that the peripheral clock in endothelial cells does not solely induce diurnal BP rhythm<sup>41</sup>. A genetic association study demonstrated that a single nucleotide polymorphism within the *bmal1* promoter is associated with hypertension and type 2 diabetes<sup>42</sup>, providing support that the molecular clock is involved in the pathogenesis of metabolic disorders. Recent evidence supported the contribution of PPAR $\gamma$  in the clock system. PPAR $\gamma$  binds to the promoter upstream of *bmal1* and induces its

transcription<sup>43</sup>. The expression of PPAR $\gamma$  also exhibited circadian oscillation in the aorta, and an endothelial- or vascular smooth muscle cell-specific deletion of PPAR $\gamma$  attenuated the BP variation together with reduced catecholamine production.

Plasma aldosterone concentration has a diurnal variation with the peak during night hours<sup>35</sup>. One of the adrenal enzymes involved in aldosterone production, type VI 3 $\beta$ -hydroxyl-steroid dehydrogenase (Hsd3b6), exhibits circadian expression in normal subjects. However, *Cry1/2*-null mice had a constitutive high expression of Hsd3b6 together with overproduction of aldosterone from adrenal glands, which resulted in salt-sensitive hypertension in *Cry1/2*-null mice<sup>44</sup>. Aldosterone regulates the expression of the alpha-subunit of the epithelial sodium channel ( $\alpha$ ENaC) mRNA through the Per1-mediated pathway<sup>45</sup>.  $\alpha$ ENaC is known to affect systemic BP; therefore these findings suggest a novel function of the molecular clock during the pathogenesis of hypertension.

## Acute myocardial infarction and the circadian clock

Beginning a few decades ago, it became well known that AMI or thrombotic events such as

pulmonary embolism frequently occur in the early morning<sup>46,47</sup>. Since these disorders can be fatal, elucidating the mechanisms of circadian onset of cardiovascular disorders will help not only for a better understanding of their pathogenesis but also for establishing preventive strategies<sup>48</sup>. In this section, we would like to discuss how the biological clock contributes to the onset of thromboembolic events.

Diurnal activation of the autonomic nervous system seems to contribute to the circadian onset of cardiovascular events. A morning increase in ischemic events was not observed in patients with autonomic nervous dysfunction induced by diabetes<sup>49</sup>. In addition, patients receiving  $\beta$ -blockers did not demonstrate morning increase of ischemic heart attacks<sup>50</sup>. Several cardiovascular and hematologic functions are related to the circadian onset of cardiovascular events, including BP, HR, coronary blood flow, platelet function, blood coagulability and fibrinolytic activity<sup>48</sup>. In the early morning, BP and HR increase and enhance the demand for oxygen by the heart<sup>51</sup>. In contrast, the vascular tone of coronary arteries increases and, therefore, coronary blood flow decreases in the morning<sup>52</sup>, resulting in a mismatch of oxygen demand and supply during this period. Coronary

segments with endothelial dysfunction exhibit circadian vasomotor activity, whereas segments with normal endothelial function did not demonstrate circadian variations, suggesting a potential protective role of endothelial function in coronary events <sup>53</sup>. Moreover, both platelet aggregation and blood coagulability increase <sup>54</sup> whereas fibrinolytic activity decreases in the morning. These hypercoagulability and hypofibrinolytic activities also elicit the morning onset of thromboembolic events.

Not only platelet aggregation activity, but also the number of circulating platelets have circadian oscillation <sup>55,56</sup>. Platelets are activated by catecholamines, which are secreted from the autonomic nervous system. However, it is not clear whether the peripheral clock directly affects platelet function, since no surface markers characteristic of platelet activation have been shown thus far to exhibit circadian expression <sup>55</sup>.

High concentration of coagulation factor VII is considered to be a risk factor for coronary artery diseases <sup>57</sup>. Circadian oscillation has been shown not only in the factor VII level in blood, but also in levels of fibrinogen, prothrombin, factor VIII and tissue factor pathway inhibitor, a direct inhibitor of the FXa/TF/FVIIa complex <sup>58,59</sup>. Microparticles from

endothelium induces coagulation through the tissue factor mediated pathway <sup>60</sup>. A recent report by Madden et al. showed that the number of vascular cell adhesion molecule-1 (VCAM-1) positive microparticles in human plasma had a significant diurnal variation with a peak at 9 a.m. <sup>61</sup>. These findings support the presence of hypercoagulability in the morning hours.

Fibrinolytic activity also was shown to have circadian variation with a peak in the afternoon and trough in the early morning, which is an antiphase to that of coagulation activity <sup>62-64</sup>. The level of plasmin-plasmin inhibitor complex, a marker of intravascular plasmin generation, decreases in the morning. Because of the morning decrease in fibrinolytic activity, recovery of patency of occluded coronary vessels by tissue-plasminogen activator (tPA) therapy for AMI treatment is more difficult in the morning hours <sup>65</sup>. The level of tissue plasminogen activator inhibitor-1 (PAI-1), which regulates the activity of t-PA, mainly determines fibrinolytic activity. High concentration of PAI-1 or t-PA can become a risk factor for the occurrence of a first AMI <sup>66</sup>. There is circadian oscillation in the concentration and activity of PAI-1 with a morning peak,

resulting in reduced t-PA activity during that period <sup>67,68</sup>. All these data support the notion that circadian oscillation of PAI-1 activity significantly contributes to the formation of a diurnal variation in fibrinolytic function. The homeostasis of the coagulation cascade is achieved by the balance between coagulation activity and fibrinolytic activity. Activation of coagulation is normally accompanied by an increase in fibrinolytic activity. Therefore, the mismatch of these two cascades also elicits the morning onset of cardiovascular events.

The mechanisms of the diurnal variation in PAI-1 activity have been well studied. We and other groups analyzed the roles of the molecular clock in circadian PAI-1 activation <sup>7,69</sup>. PAI-1 mRNA and protein levels clearly reflect a circadian rhythm in the heart and aorta with a peak expression in the evening. The phase of circadian PAI-1 expression in mice is antiphase to that of the human, since humans are diurnal whereas rodents are nocturnal. Therefore, PAI-1 expression in rodents also accounts for the human circadian oscillation. We have shown that CLIF/BMAL2 forms a heterodimer with CLOCK and binds to the E-boxes upstream of the *pai-1* gene and transactivates its expression <sup>7</sup>. The heterodimer of CLOCK/BMAL1 also activates the PAI-1 promoter <sup>70</sup>. Oishi et al. showed that a ketogenic

diet induces the phase shift of peripheral clock gene expression including PAI-1, suggesting that PAI-1 expression is regulated by the peripheral clock <sup>71</sup>. Westgate et al. studied the susceptibility to thrombotic events using a mouse photochemical injury model and observed a diurnal variation in thrombogenicity in this *in vivo* model <sup>41</sup>. CLOCK<sup>mut</sup> mice have lost this dynamic variation. Surprisingly, the endothelial-specific deletion of the Bmal1 gene (Bmal1<sup>fx/fx</sup>Cre<sup>Tek</sup>) also abolished the circadian oscillation of thrombogenic events; however, diurnal variation in systemic PAI-1 activity was sustained in this mouse model. This finding suggests that the peripheral clock within endothelial cells contributes to prevention of thrombosis through mechanisms other than those affecting systemic PAI-1 activity.

Thrombomodulin has an opposite effect to that of PAI-1 in terms of the coagulation cascade; that is, thrombomodulin inhibits thrombin activation and also activates protein C <sup>72-74</sup>. We revealed that thrombomodulin is expressed with a circadian oscillation in vascular endothelial cells <sup>18</sup>. The phase of circadian thrombomodulin expression is similar to that of PAI-1 with a peak in the morning. Based on these findings,

we can raise the hypothesis that circadian expression of thrombomodulin may be beneficial in protecting endothelium from diurnal thrombogenic activation induced by PAI-1 expression. Further studies are required to fully elucidate the role of circadian thrombomodulin expression in cardiovascular events.

## Roles of the peripheral clock in cardiovascular diseases.

The central questions related to the molecular clock and cardiovascular diseases are whether the biological clock is affected in cardiovascular disorders, and, in turn, whether impairment of the molecular clock induces the progression of those diseases. The impairment of the peripheral clock in pathology already has been demonstrated in several disease models. Young et al. showed that the phase of circadian rhythm of core clock genes, such as *bmal1*, *per2* and *hlf*, was advanced 3 hours in diabetic rats <sup>75</sup>. Also, in rat heart with pressure-overload hypertrophy, the rhythmic expression of PAR transcription factors (*dbp*, *hlf*) and *anp* was markedly reduced <sup>76</sup>. Myocardial ischemia/reperfusion (I/R) was also shown to affect the circadian clock system. Clock gene oscillations were rapidly diminished

in the I/R area of the heart whereas they were not affected in non-ischemic regions. E4BP4 antagonizes the transcriptional activity of PAR family members, such as DBP, HLF and TEF. At the I/R site of the heart, E4BP4 expression was strongly induced, resulting in the suppression of circadian *pdk4* and *ucp3* expression <sup>77</sup>. Moreover, aging and hypertension were also known to affect the internal circadian rhythm <sup>78,79</sup>.

Several studies have addressed the second question, which is whether an impaired circadian clock affects disease progression. Penev et al. repeated phase shifts of the Light/Dark (L/D) cycle in cardiomyopathic hamsters and found that disruption of rhythmicity strikingly enhanced disease progression and resulted in shortened longevity <sup>80,81</sup>. Martino et al. also analyzed the effect of impaired rhythm in cardiac hypertrophy <sup>82</sup>. They performed transverse aortic constriction (TAC) surgery as a murine model of pressure overload cardiac hypertrophy, and kept the mice in a rhythm-disruptive 20-hour (L/D 10:10) or normal 24-hour (L/D 12:12) environment after TAC surgery. Rhythm-disturbed TAC animals exhibited decreased left ventricular systolic function together with increased perivascular and interstitial fibrosis. Decreased left ventricular function was recovered

when the mice were kept under conditions of a normal 24-hour rhythm.

Martino et al. also performed an elegant study using hamsters with a point mutation in the circadian regulatory gene *casein kinase-1ε* as a heterozygote, termed the *+/tau* mutation. The *+/tau* heterozygous animals had a reduced circadian period of 22 hours with disrupted behavior rhythmicity, and they developed cardiomyopathy and extensive cardiac fibrosis, resulting in death at a young age. However, when these mutant animals were maintained under conditions of their own rhythm period (22 hours), the progression of the cardiac disorders was reversed. Ablation of the SCN at a young age also rescued the cardiac phenotype. There exist two clock systems with different periods in *+/tau* heterozygotes, since their peripheral clock is controlled by the intrinsic 22-hour clock as well as the 24-hour cycle from the SCN. Under a 22-hour L/D cycle or an SCN lesioned condition, the discrepancy between the central and peripheral clock disappeared together with rescue of the cardiac pathology. These results raise the hypothesis that it is not disruption of the peripheral clock but disharmony between the external and internal clock or between the central and peripheral clock that elicited cardiovascular disorders. Therefore,

loss of synchronization between the central and peripheral clock could elicit progression of the disease.

In healthy subjects, the peripheral clock seems to be beneficial for anticipation and preparation for external stimuli such as BP rise in the morning. It may help the organ to respond rapidly and easily to the environmental change at each time of the day. Mice had a diurnal variation in BP with a peak in the evening. In mouse cardiomyocytes, the expression of cardioprotective gene *anp* also results in a diurnal variation with a peak in dark phase, which is consistent with high BP periods <sup>76</sup>.

Discrepancy between the two clock systems could occur among peripheral tissues as well. In the aorta, the phase of circadian *Per2* expression is distinct from the phase in SCN, suggesting that the timing of clock rhythm is determined by each peripheral organ <sup>83</sup>.

Davidson et al. reported that the time phases of circadian rhythm in arteries and veins varies significantly according to the anatomical location <sup>84</sup>. The role of the peripheral clock in arteries may be different from that in veins.

In the acute phase of myocardial infarction, the phase of the circadian clock in the

ischemic heart differs from that in the non-ischemic area <sup>77</sup>. This discrepancy could elicit the incidence of myocardial arrhythmia. These losses of synchronization of circadian rhythms between each organ or tissue may occur more frequently than we have expected.

Resynchronization of the peripheral circadian clock with the environment or within each peripheral organ can become a potential target for establishing a novel preventive strategy or treatment for cardiovascular diseases. Although angiotensin II, endothelin or prostaglandin E2 is known to modify the circadian rhythm <sup>15,85,86</sup>, we must identify the appropriate zeitgebers (timekeepers) to reset or resynchronize the phase of each clock system without directly affecting tissue homeostasis. No direct evidence has been reported that catecholamine or nutrients (glucose or fatty acids) could affect the phase of circadian clock <sup>87</sup>. A recent report revealed that PPAR $\gamma$  induces Bmal1 expression in cardiovascular organs <sup>43</sup>, suggesting that thiazolidinediones, an agonist of PPAR $\gamma$ , may become a potential tool for manipulating the clock system.

## Conclusion

Each cardiovascular organ or cell has its own peripheral clock together with input from the SCN central clock. These peripheral clocks seem to play an important role in the prevention of cardiovascular disorders. Identification of CCGs in each organ will provide significant insights for an understanding of the precise roles of the peripheral clock. Synchronization of clock cycles between the central and peripheral clock, or among peripheral clocks in different organs, is also critical for normal health and homeostasis. Failure to harmonize the central and peripheral clock or internal and external rhythm could result in progression of cardiovascular disorders. Discovery of an appropriate zeitgeber or a small compound that could manipulate the phase of each peripheral clock is required to establish chronotherapeutic approaches.

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## Figure legends

### Figure 1.

The heterodimer of CLOCK and BMAL1 bind to the E-box elements upstream of period (*per*), cryptochrome (*cry*), and nuclear receptor *Rev-erb $\alpha$*  promoters. PER protein accumulates in the cytoplasm and translocates into the nucleus forming a complex with CRY proteins, then inhibits CLOCK-BMAL1-dependent transcription. Rev-erb $\alpha$  protein accumulates quickly and inhibits BMAL1 transcription, resulting in the oscillation of *bmal1* gene expression. CLOCK/BMAL1 heterodimer also binds to the E-box of target genes, designated as clock-controlled genes (CCGs). The heterodimer also transactivates proline-and acid-rich basic leucine zipper transcription factors, *dbp*, *hlf* and *tef*. These transcription factors in turn induce the circadian expression of CCGs.

Figure 2.

The center of the biological clock (central clock) is located in suprachiasmatic nucleus (SCN) in the hypothalamus. Each organ or cell, including the aorta, vascular endothelial cells and vascular smooth muscle cells (VSMC), also has circadian expression of clock genes and is designated as the peripheral clock. Circadian expression of clock-controlled genes (CCGs) are in part regulated directly by the central clock (direct pathway). In addition, peripheral clocks in cardiovascular tissues or cells are also stimulated and synchronized by the central clock and regulate diurnal expression of (CCGs).

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Figure 1

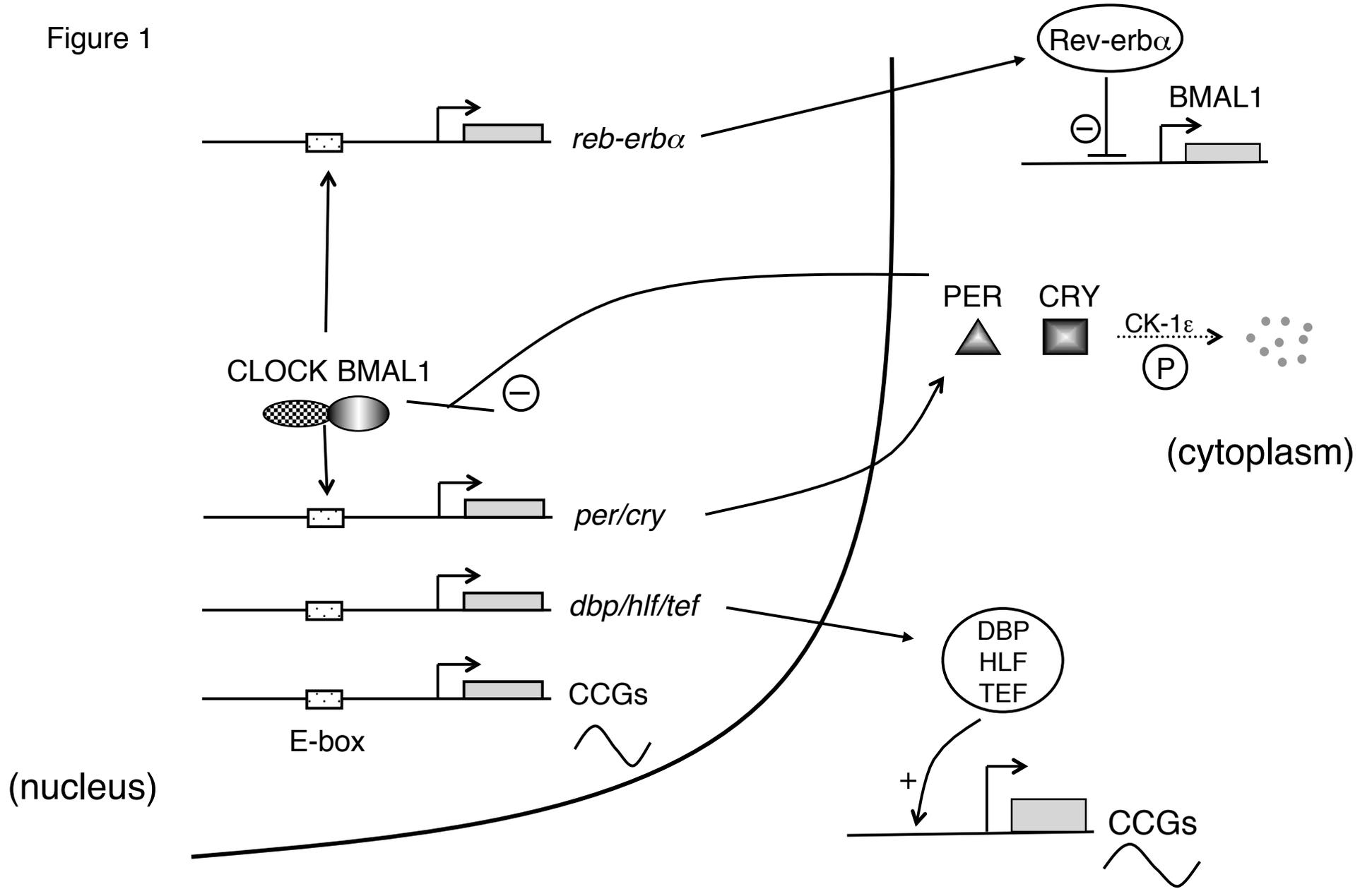


Figure 2

