JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
EDITORIAL

Molecular clock as a regulator of β-cell function

INTRODUCTION

Type 2 diabetes mellitus is characterized by the loss of β-cell function and mass, resulting from interactions between genetic predisposition and various environmental factors[1]. One environmental condition identified as a risk factor for type 2 diabetes mellitus is circadian rhythm disruption, which is induced by shift work or sleep disturbance. However, the mechanism whereby circadian disruption leads to impaired glucose metabolism is not well understood.

A circadian rhythm is an approximately 24-h cycle in the physiological processes of living beings, including cyanobacteria. The circadian clock system is based on transcriptional–translational regulation of 'core clock genes' in mammals. Clock genes are expressed throughout mammalian organ systems, and they play an important role by generating both behavioral and physiological rhythms, and synchronizing metabolism in anticipation of the sleep/wake and fasting/feeding cycle. The circadian clock is encoded by the heterodimeric basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) transcription factors CLOCK, and brain and muscle Arnt-like 1 (BMAL1), which trigger the expressions of period (PER)/cryptochrome (CRY) repressors that inhibit CLOCK/BMAL1 in a cycle that repeats itself every 24 h. According to genetic studies, *Clock* mutant mice show altered expressions of genes known to regulate islet growth, survival, maturation and proliferation[2]. *Bmal1* deletion in β-cells also results in failed metabolic adaptation to a high-fat diet, characterized by hyperglycemia, glucose intolerance and loss of glucose-stimulated insulin secretion[3]. These results suggest that circadian disruption-induced metabolic disorder is, at least in part, attributable to β-cell failure, though the mechanisms remain largely unknown.

The $Wfs1^{-/-}A^{y/a}$ mouse is an animal model of Wolfram syndrome with mild obesity[4]. These mice develop diabetes with severe insulin deficiency as a result of endoplasmic reticulum (ER) stress-induced β-cell failure. The precise mechanism whereby Wolfram syndrome 1 (*WFS1*) mutation induces ER stress, followed by β-cell failure, is not fully understood. However, various studies have shown that the WFS1 protein localizes to the ER membrane, and exerts a protective effect against ER stress[5]. In gene expression studies, we showed alteration of clock-related gene expressions in $Wfs1^{-/-}A^{y/a}$ mice islets, as compared with those in $A^{y/a}$ mice; D site of albumin promoter (*Dbp*) messenger ribonucleic acid (RNA) levels of $Wfs1^{-/-}A^{y/a}$ mice islets were decreased by 50%, whereas their E4 promoter-binding protein 4 (*E4bp4*) messenger RNA levels were increased by 50% at Zeitgeber Time 12. Notably, similar alterations were observed with chemically-induced ER stress in *in vitro* experiments using MIN6 cells.

ROLE OF E4BP4/DBP

E4BP4 and DBP are basic leucine zipper transcription factors containing a DNA-binding domain that interacts with the same D-box deoxyribonucleic acid (DNA) element. These factors are regulated by core essential components of the circadian clock feedback loop, and they show clear reciprocal circadian rhythms. DBP activates, while E4BP4 suppresses, target gene transcriptions. The DBP and E4BP4 expression patterns show opposite directions depending on the time of day. It is thought that E4BP4 and DBP are paired components of a reciprocating mechanism[6].

Simple questions arose from the aforementioned results. Is the alteration of E4BP4/DBP involved in the development of β-cell failure, or does hyperglycemia itself cause E4BP4/DBP changes? To answer this question, we generated transgenic mice (MIP-E4BP4-TG mice) expressing E4BP4 under the control of the mouse insulin I gene promoter. Herein, constitutively expressed E4BP4 was expected to compete with DBP for D-box in β-cells. Interestingly, these mice showed remarkable glucose intolerance with severely impaired insulin secretion[7]. These data suggest that the transcriptional activities of DBP and E4BP4 have a pivotal role in the regulation of insulin secretion, in connection with circadian regulation, and in ER stress responses. Pancreatic-specific *Bmal1* knockout (PdxCre $Bmal1^{fx/fx}$) mice also reportedly showed severe hypoinsulinemic diabetes. However, the mechanisms of impaired insulin secretion might differ between MIP-E4BP4-TG mice and *Bmal1* knockout mice.

Perelis *et al.*[8] showed cell-autonomous expressions of clock genes in pancreatic islets by synchronizing the intrinsic oscillation of islets *ex vivo* using forskolin. Whole transcriptome analysis (RNA sequencing) showed that approximately 27% of the β-cell transcriptome exhibited circadian oscillation. Gene ontology analysis revealed that many of these genes were involved in vesicle trafficking and membrane fusion. In RNA sequencing studies from β-cell-specific *Bmal1* knockout mice, many RNAs with altered expressions were identified as the cycling RNAs and related to exocytosis networks similar to those observed in synchronized wide-type islets. Furthermore, chromatin immunoprecipitation analysis showed that approximately 30% of CLCOK/BMAL1 binding sites were localized to rhythmically

†These authors contributed equally.

expressed genes. These data suggest that BMAL and CLOCK directly regulate insulin secretion through alteration of genome-wide gene expressions.

Interestingly, in MIP-E4BP4-TG mice, expression levels of core clock genes including *Bmal1* did not change. However, chromatin immunoprecipitation analysis showed that E4BP4 binds to the rhythmically expressed genes, including *Ins1*, *Ins2*, *Slc2a2* and *Rab37*. These findings suggest that DBP and E4BP4 directly regulate, at least to some extent, genes related to insulin synthesis and secretion. Whether BMAL/CLOCK's regulations of the cycling insulin secretion-related genes are through DBP/E4BP4 remains to be elucidated. Other distinct findings in islets from MIP-E4BP4-TG mice were that the adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio completely lost responsiveness to glucose, in association with markedly reduced glucose-stimulated insulin secretion. Basal ATP/ADP ratios in MIP-E4BP4-TG islets were elevated without the circadian oscillations observed in wild-type islets. Neither the ATP/ADP ratio nor the intracellular Ca^{2+} concentration was elevated after glucose stimulation. Our results suggest that disrupted DBP/E4BP4 circadian regulation altered mitochondrial respiration, and glucose-stimulated insulin release could, at least in part, be inhibited by high basal ATP/ADP ratios in MIP-E4BP4-TG. Similar findings were obtained in *Wfs1*$^{-/-}$*A*$^{y/a}$ islets. In contrast, Perelis *et al.*[8] reported that elevation of ATP/ADP, which reflects glycolysis and oxidative phosphorylation in β-cells, did not differ between wild-type and *Bmal1* knockout islets after glucose stimulation. There might be distinct mechanisms whereby BMAL1 and DBP/E4BP4 regulate insulin secretion through activation and inhibition of different types of target genes by binding to their E-box and D-box portions, respectively (Figure 1).

There is a major difference between diurnal and nocturnal insulin secretion in normal mice and also in humans[9]. With regard to the physiological role of DBP/E4BP4 transcriptional activity in β-cells, in the circadian cycle, DBP transactivity is expected to be the strongest around the beginning of the respective active times of day (mice: night-time, humans: daytime), whereas transactivity is expected to be the weakest around passive times of day. In general, an hour or so before we awaken, body temperature increases, the gastrointestinal tract starts moving again and cellular metabolism accelerates to provide energy to support the day's activities. Digestion/absorption systems also prepare for the ingestion of breakfast based on the local time. At the same time, pancreatic β-cells probably prepare to secrete insulin. Our findings suggest that DBP transcriptional activity is an essential component of preparing β-cells to release insulin (Figure 2).

Circadian rhythms of the peripheral clock genes are profoundly affected by fasting/feeding cycles. Therefore, feeding

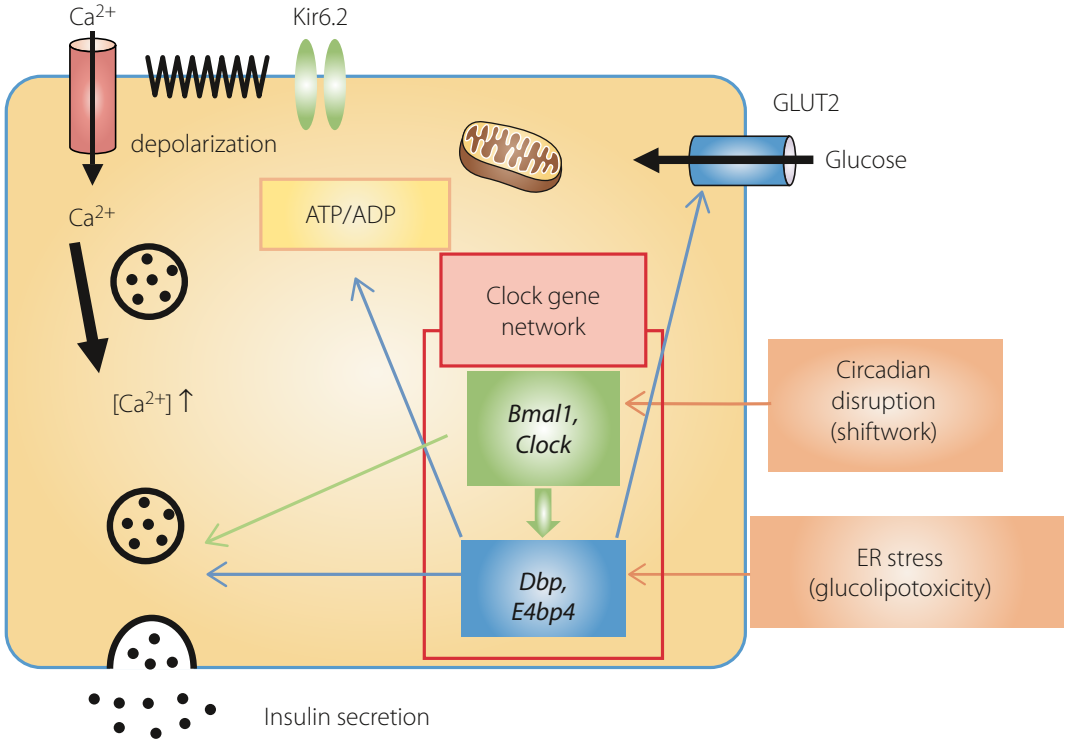

Figure 1 | Schematic representation illustrating potential mechanisms by which circadian disruption and endoplasmic reticulum (ER) stress increase susceptibility to β-cell failure through the molecular clock. ADP, adenosine diphosphate; ATP, adenosine triphosphate; DBP, D site of albumin promoter; E4BP4, E4 promoter-binding protein 4; GLUT2, glucose transporter 2.

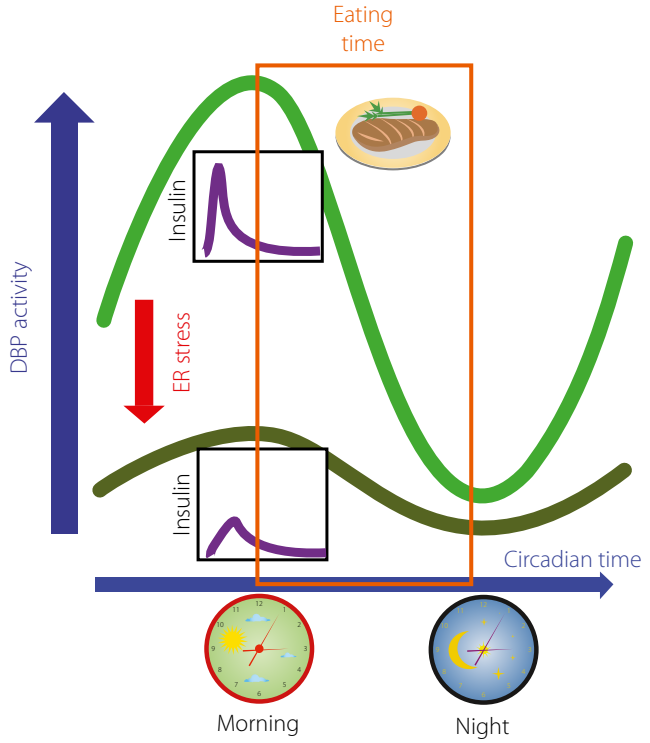

Figure 2 | Schematic diagram of metabolic regulations by D site of albumin promoter (DBP) transcriptional activity throughout the day. ER, endoplasmic reticulum.

behavior, one of the environmental components of diabetes risks, can disrupt the molecular clock in β-cells. In addition, as noted earlier, DBP/E4BP4 expression is susceptible to ER stress. Evidence has accumulated that ER stress is enhanced in the β-cells of many diabetes models including human type 2 diabetes. This is another factor potentially disrupting the β-cell molecular clock and predisposing living beings to diabetes.

FUTURE PERSPECTIVE

Environmental factors affecting the circadian rhythm are inevitable risk factors for type 2 diabetes mellitus in modern society. Recent research approaches to tissue-specific circadian mutant mice have opened windows into how the circadian system is involved in β-cell function. Genome-wide association studies and clinical research have shown a relationship between circadian rhythm regulation and glucose homeostasis in humans[10,11]. Additionally, common variants in *WFS1* are associated with type 2 diabetes mellitus. Recently, our research has shows that DBP/E4BP4 might be a crucial player in connecting the circadian clock and diabetes, including that in Wolfram syndrome and type 2 diabetes mellitus with impaired insulin secretion.

Future studies should focus on translation 'from bench to bedside.' We must determine whether lessons from the pathophysiological features of circadian mutant mice can be applied to human type 2 diabetes mellitus induced by circadian disruptions, such as shift work. One recent study showed clock-modifying drugs to exert positive effects on metabolism[12]. However, currently existing clock-modifying drugs are literally circadian modulators, such that these drugs interfere with the core clock gene network and might thus disrupt circadian rhythms. There is a potential concern as to whether such circadian disruption adversely affects health in the long term. Meanwhile, a modulator of DBP/E4BP4 transcriptional activity might have milder effects on core circadian systems, because DBP/E4BP4 are likely to be components of the circadian output pathway, and modulate clock-controlled genes expressions[13]. In fact, period length in *Dbp*$^{-/-}$ mice was shown to be just 30 min shorter than that in control mice in a free-running experiment under constant dark conditions[13]. Therefore, DBP/E4BP4 might be preferable as drug targets for improving β-cell function with minimal effects on the core circadian system.

ACKNOWLEDGMENTS

This study was supported by grants, from the Japan Society for the Promotion of Science, 26460489 (to YO), and 23390080 and 15H04849 (to YT), as well as a grant from the Takeda Science Foundation.

DISCLOSURE

The authors declare no conflict of interest.

Akihiko Taguchi[1†], Yasuharu Ohta[1,2†], Yukio Tanizawa[1,3*]
[1]*Department of Endocrinology, Metabolism, Hematological Science and Therapeutics, Graduate School of Medicine;*
[2]*Department of Diabetes Research, School of Medicine;*
[3]*The Research Institute for Time Studies (RITS), Yamaguchi University, Yamaguchi, Japan*
**E-mail: tanizawa@yamaguchi-u.ac.jp*

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Doi: 10.1111/jdi.12835