Molecular Analysis of Growth Factor and Clock Gene Expression in the Livers of Rats with Streptozotocin-Induced Diabetes

Joo-Heon Kim[1,#], Cheol-Soo Shim[1,#], Jinyoung Won[2,#], Youngji Park[2], Sookyoung Park[2], Jae Seon Kang[3] and Yonggeun Hong[2,†]

[1]*Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea*
[2]*Department of Physical Therapy, College of Biomedical Science & Engineering, Inje University, Gimhae 621-749, Korea*
[3]*Department of Pharmacy, Kyungsung University, Busan 608-736, Korea*

ABSTRACT

Many biological systems are regulated by an intricate set of feedback loops that oscillate with a circadian rhythm of roughly 24 h. This circadian clock mediates an increase in body temperature, heart rate, blood pressure, and cortisol secretion early in the day. Recent studies have shown changes in the amplitude of the circadian clock in the hearts and livers of streptozotocin (STZ)-treated rats. It is therefore important to examine the relationships between circadian clock genes and growth factors and their effects on diabetic phenomena in animal models as well as in human patients. In this study, we sought to determine whether diurnal variation in organ development and the regulation of metabolism, including growth and development during the juvenile period in rats, exists as a mechanism for anticipating and responding to the environment. Also, we examined the relationship between changes in growth factor expression in the liver and clock-controlled protein synthesis and turnover, which are important in cellular growth. Specifically, we assessed the expression patterns of several clock genes, including *Per1, Per2, Clock, Bmal1, Cry1* and *Cry2* and growth factors such as *insulin-like growth factor (IGF)-1* and *-2* and *transforming growth factor (TGF)-β1* in rats with STZ-induced diabetes. Growth factor and clock gene expression in the liver at 1 week post-induction was clearly increased compared to the level in control rats. In contrast, the expression patterns of the genes were similar to those observed after 5 weeks in the STZ-treated rats. The increase in gene expression is likely a compensatory change in response to the obstruction of insulin function during the initial phase of induction. However, as the period of induction was extended, the expression of the compensatory genes decreased to the control level. This is likely the result of decreased insulin secretion due to the destruction of beta cells in the pancreas by STZ.

(Key words : Diabetes, Growth factor, Clock gene, Circadian rhythm, Streptozotocin)

INTRODUCTION

Living organisms exhibit an ongoing circadian rhythm with a period of about 24 h. The circadian clock is located in the suprachiasmatic nucleus (SCN) in mammals. The circadian rhythm is associated with two organs: the pineal gland and SCN. In some lower vertebrates, exposure to light sets off a chain reaction involving enzymes, hormones, and neuroreceptors that may regulate the circadian rhythm (Moore *et al.*, 1967). Upon the detection of light, the retina directly signals and entrains the SCN (Axelrod, 1970). The expression of the circadian rhythm in mammals is influenced by the hypothalamic SCN. Ablation of the SCN abolishes or dampens circadian rhythms in locomotor activity, sleep-wake cycles, body temperature, heart rate, and feeding; however, circadian patterns in feeding may depend on a separate food-entrainable oscillator (Hoogerwerf *et al.*, 2007).

The decreased expression of clock genes leading to changes in the circadian rhythm has been demonstrated in previous studies (Katsutaka *et al.*, 2004; Oishi *et al.*, 2004; Ando *et al.*, 2006); however, few studies have addressed the association of diabetes with growth factor and clock gene expression. Thus, in this study we examined the roles played by clock gene and growth factor expression in diabetes.

* This work was supported by a grant (Code #20070401-034-006-009-02-00 to Y. Hong) from BioGreen 21 Program, Rural Development Administration, KRF (Code No. E00344 to Y. Hong), Republic of Korea.

# These authors contributed equally.

† Corresponding author : Phone: +82-55-320-3681, E-mail: yonghong@inje.ac.kr

Several circadian genes have been identified, including *Period (Per)1, Per2, Per3, Clock, NPAS2, GSK3b, Timeless, Mtnr1A, brain and muscle Arnt-like protein 1 (Bmal1)b, Dbp, Cryptochrome (Cry)1,* and *Cry2*. We examined the expression of several clock genes (*Per1, Per2, Clock, Bmal1b, Cry1,* and *Cry2*) and growth factors (*insulin-like growth factor* [*IGF*]-1 and -2 and *transforming growth factor* [*TGF*]-β1) in the livers of rats with streptozotocin (STZ)-induced diabetes. STZ, which may be used to induce diabetes, is particularly toxic to the insulin-producing beta cells of the pancreas in mammals. Its toxicity is due to the damage of cellular DNA, although other mechanisms may also play a role. STZ is similar enough to glucose to be transported into the cell by the glucose transporter GLUT2 (Schnedl *et al.*, 1994; Wang and Gleichmann, 1998). We expected to see a decrease in clock gene and growth factor expression; however, no significant difference in expression was noted for any of the genes except *Clock*.

Increased levels of *TGF-β1* mRNA in response to diabetes or a high-glucose environment *in vitro* have been shown to prevent diabetic renal hypertrophy in experimental models of diabetes (Ando *et al.*, 2006). TGF-β1, a multifunctional growth factor that plays a key role in the development of tissue fibrosis, may be involved in the pathophysiology of diabetic nephropathy (Patel *et al.*, 2005).

IGFs regulate cellular proliferation, differentiation, and anabolism. For example, IGF-1 is an important growth and metabolic regulatory hormone that not only induces cellular replication and differentiation, but also suppresses myocardial apoptosis. Thus, it improves myocardial function (Froesch *et al.*, 1990; Frayling *et al.*, 2002; Janssen and Lamberts, 2002). The parenchymal cells of the adult liver synthesize and secrete IGF-1 and -2. However, hepatocytes are the main contributors in the liver to IGF-1 synthesis and secretion (Scott *et al.*, 1985). Hepatocytes as well as resident macrophages (Kupffer cells), endothelial cells, and hepatic stellate cells synthesize IGF-2 (Scharf *et al.*, 1998). IGF-1 and -2 are ubiquitous peptides that share structural homology with insulin (Vambergue *et al.*, 2007). The level of IGF-1 in patients with type 2 diabetes is significantly lower than that in non-diabetic controls (Akturk *et al.*, 2007), whereas the level of IGF-2 in patients with type 2 diabetes is increased compared to the level in non-diabetic controls (Shen *et al.*, 1986; Devedjian *et al.*, 2000). The present study investigated differential expression of growth factors and clock related genes in liver between female and male rats following STZ-induced diabetes.

MATERIALS AND METHODS

Animals and Induction of Diabetes

Sprague-Dawley rats (Samtaco, Korea) were housed at the Animal Care Center of Inje University until they reached 4 weeks of age. The rats (175~250 g initial weight) were maintained under specific pathogen-free conditions at 24±1℃, 48% humidity, and a 12-h light/12-h dark cycle. The rats received standard laboratory food and water *ad libitum*.

Experimental Design

In total, twenty-four rats were studied; twelve were treated with STZ while the other twelve were not (control group). Diabetes was induced by injection of the beta-cell toxin STZ (Sigma Chemicals, St. Louis, MO, USA) into the abdominal cavity (60 mg/kg). The blood glucose level was measured using a glucometer (Super Glucocard, ARKRAY, Kyoto, Japan) as per the manufacturer's instructions. The blood glucose level in the twelve rats that developed diabetes (six males and six females) was >200 mg/dl, whereas that in another twelve control rats (six males and six females) used in our experiment was <200 mg/dl. The rats were sacrificed every 3 h from 13:00 to 16:00 during 1-wk and 5-wks. To ensure the reproducibility of the gene expression cycles, the animals were sacrificed on the same day, 4-wks apart. The liver was isolated from each animal for use in analyzing growth factor and clock gene expression.

RNA Extraction and RT-PCR

The tissues were homogenized in 1 ml of Tri-Reagent (Sigma-Aldrich, Dorset, UK), and total RNA was extracted according to the manufacturer's instructions. The RNA was treated with RNase-free distilled water and then subjected to RT-PCR in the presence of reverse transcriptase and Oligo-dT^{TM} in a thermal cycler (Thermo Electron Corp., Waltham, MA, USA). Primer sequences, the size of amplified products, and GenBank accession numbers are in Table 1. The primers used were designed for each growth factor (*Igf-1, Igf-2,* and *Tgf-β1*) and clock gene (*Per1, Per2, Clock, Bmal1b, Cry1,* and *Cry2*) using Primer Express 2.0 software from TaqMan® and synthesized by Genotech (Daejeon, Korea). PCR conditions were as follows: For all subjected genes, denaturing was performed at 94℃ for 1 min, annealing at 62℃ for 1 min, and extension at 72℃ for 2 min. Following 35 PCR cycles, all samples were allowed to extend at 72℃ for 10 min. Twenty μl of each of the PCR products were electrophoresed in 1% agarose (Invitrogen) gels. The gels were then stained with ethidium bromide, placed on a UV transilluminator, and photographed.

Statistical Analysis

Data were collected from repeated experiments and are presented as mean±S.E.M. One-way ANOVA was

Table 1. Oligonucleotide primers used for RT-PCR

Gene	Primer sequence(5'-3')	Tm(℃)	Product length(bp)	GenBank accession No.
Igf1	F: CAGTTCGTGTGGACCAAG R: TCAGCGGAGCACAGTACATC	59.3 59.3	151	NM001082479
Igf2	F: GTCGATGTTGGTGCTTCTCA R: AAGCAGCACTCTTCCACGAT	57.3 57.3	195	NM031511
Tgf1-β1	F: ATACGCCTGAGTGGCTGTCT R: TGGGACTGATCCCATTGATT	59.3 55.3	153	NM021578
Per1	F : TTTGGAGAGCTGCAACATTCC R : CTGCCCTCTGCTTGTCATCA	57.9 59.4	100	NM011065
Per2	F : GGCTGTGTCCCTGGTTTCTG R : CCACAAACTTGGCATCACTGA	61.4 57.9	100	NM011066
Clock	F : AAATATTCATCGGCAGCAAGAAG R : CAGGGTTTGATTGCTGCAAA	57.1 55.3	100	NM007715
Bmal1	F : GTCGAATGATTGCCGAGGAA R : GGGAGGGGTACTTGTGATGTTC	64.4 66.1	100	AB012601
Cry1	F: CTTCCAACGTGGGCATCAAC R: CCGAATCACAAACAGACGAGAA	59.4 58.4	100	NM007771
Cry2	F: ACACAGGCCCCAGAGCACTA R: TCTAGCCCGCTTGGTCAGTT	61.4 59.4	100	NM009963
Gapdh	F: GTATGACTCCACTCACGGCAAA R: GGTCTCGCTCCTGGAAGATG	60.3 61.4	100	BC094037

used for statistical analysis and Ducan's multiple range-test was used to compare the significant difference of gene expression in each groups. A P value less than 0.05 denoted significant difference. All analyses were performed using the Statistical package for the SPSS ver.16 software.

RESULTS

Blood Glucose Levels

Diabetes was induced by the intraperitoneal injection of the beta-cell toxin STZ. The induction of diabetes was confirmed by measuring the blood glucose level (Fig. 1). The blood glucose levels of the control were lower than 200 mg/dl throughout the 5-week experimental period. In contrast, the blood glucose levels of STZ-induced rats were higher than 200 mg/dl. Thus, insulin-dependent diabetes mellitus was successfully induced by the injection of STZ. Interestingly, as shown at Fig. 1, male rats were more sensitive at STZ treatment compared to female rats in the early stage and slightly recovered the blood glucose levels at 5 week after STZ treatment.

Growth Factor Gene Expression

As shown in Fig. 2, gene expression of growth fac-

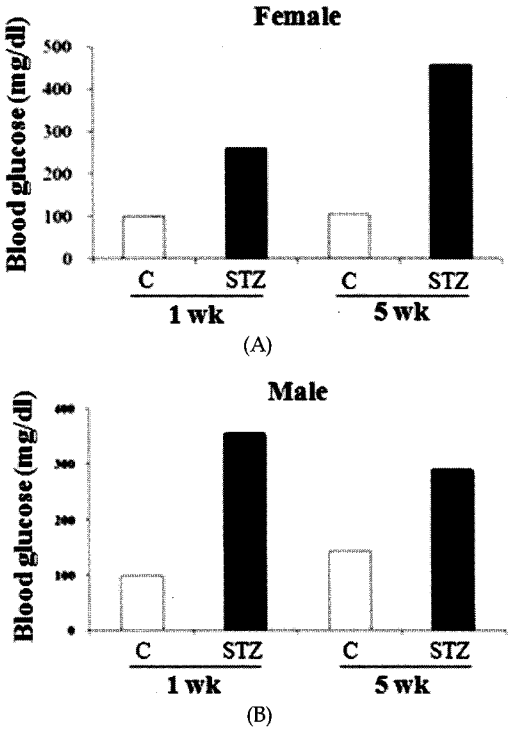

Fig. 1. Blood glucose level in rats with STZ-induced diabetes and control rats. The blood glucose levels in the STZ-induced diabetic rats differed by sex; however, STZ significantly affected the blood glucose level in all rats.

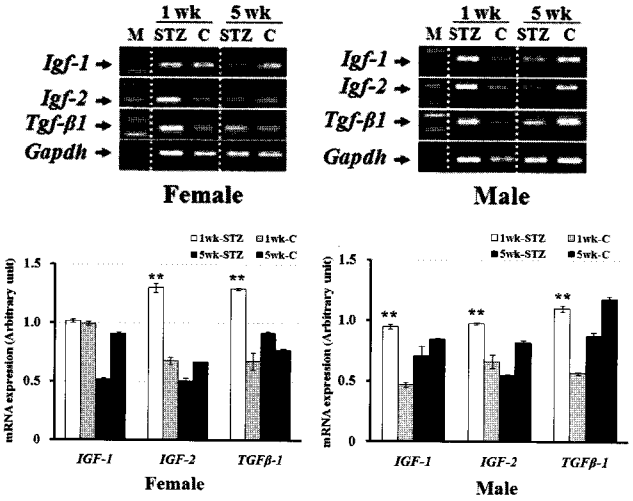

Fig. 2. Effect of STZ on growth factor gene expression. (A) and (B) Experimental and control rats were sacrificed at weeks 1 and 5 and their livers were harvested. The images show the expression of *IGF-1, IGF-2, TGF-β1,* and *Gapdh* (control) as indicated by RT-PCR in the samples obtained from the STZ-injected rats. ** $p<0.05$ compare to control group at 1 week.

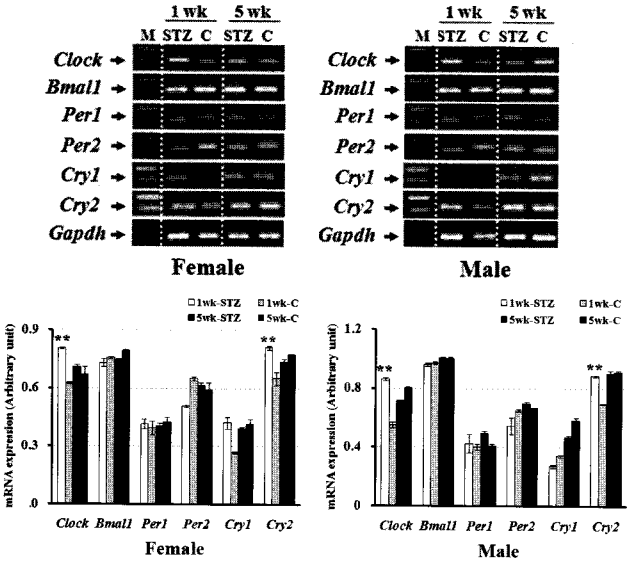

Fig. 3. Effect of STZ on clock gene expression. (A) and (B) Experimental and control rats were sacrificed at weeks 1 and 5 and their livers were harvested. The images show the expression of *Clock, Bmal1b, Per1, Per2, Cry1, Cry2,* and *Gapdh* as indicated by RT-PCR in the samples obtained from the STZ-injected rats. ** $p<0.05$ compare to control group at 1 week.

tors was affected by STZ-induced high blood glucose levles. Gene expression in the liver 1 week after the induction of diabetes mellitus by intraperitoneally injection of STZ was significantly increased compared to that in the control group (Fig. 3). However, clock-related gene expression 5 weeks after the induction of diabetes mellitus was similar to that in the controls.

DISCUSSION

Previously, we showed that the rhythmic expression of clock genes is attenuated in the livers of KK-Ay mice with obese diabetes compared to that in control KK mice (Ando *et al.*, 2006). Given that the molecular clock is believed to regulate the expression of most circadian genes (Reppert and Weaver, 2001; Lowrey and Takahashi, 2004), the rhythmic expression of those genes should be attenuated in obese diabetes. Therefore, the influence of the molecular clock on rhythmic gene expression may be different according to each gene.

The master circadian clock in the SCN is composed of interacting positive and negative transcription-translation feedback loops (Yagita *et al.*, 2001) involving *Per1, Per2, Per3, Clock, Bmal1b, Tim, Cry1,* and *Cry2,* which are expressed in the SCN. Restricted feeding determines the rhythm of *Per* expression in the liver (Damiola *et al.*, 2000) and *Clock* expression in the cerebral cortex and hippocampus, but not the SCN (Wakamatsu *et al.*, 2001). Thus, SCN-independent circadian oscillators must exist in the peripheral organs and those areas of the brain other than the SCN (Iijima et *al.*, 2002). The fundamental unit of the circadian clock is a feedback loop in which clock gene expression is periodically suppressed by the corresponding protein products (Dunlap, 1999).

The serum level of IGF-1 in patients with type 2 diabetes mellitus was found to be significantly lower than that in non-diabetic controls (Akturk *et al.*, 2007). The decrease in serum IGF-1 is greater in type 2 diabetic patients than in healthy controls (Tan and Baxter, 1986); however, the mechanism responsible for this observation is unknown.

Recent studies have revealed that these endogenous rhythms are generated at the cellular level by circadian core oscillators, which are composed of transcriptional/ranslational feedback loops involving a set of clock genes (Reppert and Weaver, 2001; Lowrey and Takahashi, 2004). In mammals, rhythmic transcriptional enhancement by two basic helix-loop-helix Per-Arnt-Sim domain-containing transcription factors, CLOCK and BMAL1, provides the basic drive for the intracellular clock system; the CLOCK-BMAL1 heterodimer activates the transcription of various clock-controlled genes (Gekakis *et al.*, 1998; Bunger *et al.*, 2000). Given that the products of some clock-controlled genes, including albumin D-site binding protein (Dbp), also serve as transcription factors, the expression of numerous genes may be tied to the molecular clock (Reppert and Weaver, 2001; Lowrey and Takahashi, 2004). In parallel, the CLOCK-BMAL1 heterodimer activates the transcription of the *Per* and *Cry* genes (Kume *et al.*, 1999; Okamura *et al.*, 1999; Vitaterna *et al.*, 1999). When the PER and

CRY proteins reach a critical concentration, they attenuate CLOCK-BMAL1 transactivation, thereby generating a circadian oscillation in their own transcription (Gekakis *et al.*, 1998; Kume *et al.*, 1999).

The molecular clock system resides not only in the hypothalamic SCN, which is recognized as the mammalian central clock, but also in various peripheral tissues (Panda *et al.*, 2002; Storch *et al.*, 2002; Yoo *et al.*, 2004). The SCN is not essential for driving peripheral oscillations but acts to synchronize peripheral oscillators (Yoo *et al.*, 2004). Therefore, the local molecular clock may directly control physiological rhythmicity in peripheral tissues.

Recent studies have suggested that dysfunction of the molecular clock system is involved in the development of metabolic syndrome, which is a constellation of metabolic abnormalities including obesity, dyslipidemia, hypertension, and insulin resistance/type 2 diabetes (Staels, 2006). In mice, inactivation of BMAL1 suppresses the diurnal variation in plasma glucose and triglyceride concentrations and can lead to insulin resistance (Rudic *et al.*, 2004). Homozygous *Clock* mutant mice have an attenuated diurnal feeding rhythm, are hyperphagic and obese, and develop hyperglycemia, hyperlipidemia, and hepatic steatosis (Turek *et al.*, 2005). Furthermore, we have shown that the rhythmic expression of clock genes is attenuated in the liver and visceral adipose tissue of KK-Ay mice, a genetic model of severe obesity and overt diabetes (Ando *et al.*, 2006).

The liver and visceral adipose tissues play critical roles in the development of metabolic syndrome/type 2 diabetes (Bugianesi *et al.*, 2005; Schaffler *et al.*, 2005). Approximately 10% of the genes in the liver are expressed according to the circadian rhythm, which may help maintain hepatic physiology (Vitaterna *et al.*, 2001; Okamura, 2004). Therefore, the circadian expression of various genes appears to be dampened in the livers of animals with metabolic syndrome/type 2 diabetes. In general, the *in vivo* effects of metabolic abnormalities on gene expression are studied at only one scheduled time each day. The effects of obese diabetes on the hepatic mRNA expression of several clock genes were observed only at their peak times (Ando *et al.*, 2006). Therefore, differences in timing among experiments may lead to diverse results, especially for genes that are expressed rhythmically. Additional studies are needed to clarify the pathophysiological roles of these effects in obese diabetes.

REFERENCES

1. Ando H, Oshima Y, Yanagihara H, Hayashi Y, Takamura T, Kaneko S, Fujimur A (2006): Profile of rhythmic gene expression in the livers of obese diabetic KK-A^y mice. Biochem Biophys Res Comm 346: 1297-1302.
2. Akturk M, Arslan M, Altinova A, Ozdemir A, Ersoy R, Yetkin I, Ayvali E, Gonen S, Toruner F (2007): Association of serum levels of IGF-I and IGFBP-1 with renal function in patients with type 2 diabetes mellitus. Growth Horm IGF Res 17:186-193.
3. Axelrod J (1970): The pineal gland. Endeavour 29 (108):144-148.
4. Bugianesi E, McCullough AJ, Marchesini G (2005): Insulin resistance: a metabolic pathway to chronic liver disease. Hepatology 42:987-1000.
5. Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA (2000): Mop3 is an essential component of the master circadian pacemaker in mammals. Cell 103:1009-1017.
6. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U (2000): Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes 14:2950-2961.
7. Devedjian JC, Pujol A, Cayla C, George M, Casellas A, Paris H, Bosch F (2000): Transgenic mice overexpression insulin-like growth factor-II in beta cells develop type 2 diabetes. J Clin Invest 105(6):731-740.
8. Dunlap JC (1999): Molecular bases for circadian clocks. Cell 96:271-290.
9. Frayling TM, Hattersley AT, McCarthy A, Holly J, Mitchell SM, Gloyn AL, Owen K, Davies D, Smith GD, Ben-Shlomo Y (2002): A putative functional polymorphism in the IGF-I gene: association studies with type 2 diabetes, adult height, glucose tolerance, and fetal growth in U.K. populations. Diabetes 51(7):2313-2316.
10. Froesch ER, Guler HP, Schmid C, Zapf J (1990): Insulin-like growth factors (IGF I and IGF II) and diabetes. Ther Umsch 47(1):8-14.
11. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ (1998): Role of the CLOCK protein in the mammalian circadian mechanism. Science 280:1564-1569.
12. Hoogerwerf WA, Hellmich HL, Cornélissen G, Halberg F, Shahinian VB, Bostwick J, Savidge TC, Cassone VM (2007): Clock gene expression in the murine gastrointestinal tract: endogenous rhythmicity and effects of a feeding regimen. Gastroenterology 133:1250-1260.
13. Iijima M, Nikaido T, Akiyama M, Moriya T, Shibata S (2002): Methamphetamine-induced, suprachiasmatic nucleus-independent circadian rhythms of activity and mPer gene expression in the striatum of the mouse. Eur J Neurosci 16:921-929.
14. Janssen JA, Lamberts SW (2002): The role of IGF-I

in the development of cardiovascular disease in type 2 diabetes mellitus: is prevention possible? Eur J Endocrinol 146(4):467-477.
15. Katsutaka O, Manami K, Norio I (2004): Gene and tissue specific alterations of circadian clock gene expression in streptozotocin induced diabetic mice under restricted feeding. Biochem Biophys Res Comm 317:330-334.
16. Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, Maywood ES, Hastings MH, Reppert SM (1999): mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98:193-205.
17. Lowrey PL, Takahashi JS (2004): Mammalian circadian biology: elucidating genome-wide levels of temporal organization. Annu Rev Genom Hum Genet 5:407-441.
18. Moore RY, Heller A, Wurtman RJ, Axelrod J (1967): Visual pathway mediating pineal response to environmental light. Science 155(759):220-223.
19. Oishi K, Ohkura N, Kasamatsu M, Fukushima N, Shirai H, Matsuda J, Horie S, Ishida N (2004): Tissue specific augmentation of circadian PAI-1 expression in mice with streptozotocin-induced diabetes. Thromb Res 114:129-135.
20. Okamura H (2004): Clock genes in cell clocks: roles, actions, and mysteries. J Biol Rhythms 19(5):388-399.
21. Okamura H, Miyake S, Sumi Y, Yamaguchi S, Yasui A, Muijtjens M, Hoeijmakers JH, van der Horst GT (1999): Photic induction of mPer1 and mPer2 in cry-deficient mice lacking a biological clock. Science 286:2531-2534.
22. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB (2002): Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 109:307-320.
23. Patel A, Scott WR, Lympany PA, Rippin JD, Gill GV, Barnett AH, Bain SC; Warren 3/UK GoKind Study Group (2005): The TGF-beta 1 gene codon 10 polymorphism contributes to the genetic predisposition to nephropathy in Type 1 diabetes. Diabet Med 22(1):69-73.
24. Reppert SM, Weaver DR (2001): Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 63:647-676.
25. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, Hogenesch JB, Fitzgerald GA (2004): BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol 2(11):1893-1899.
26. Scharf JG, Knittel T, Dombrowski F, Müller L, Saile B, Braulke T, Hartmann H, Ramadori G (1998): Characterization of the IGF axis components in isolated rat hepatic stellate cells. Hepatology 27:1275-1284.
27. Schaffler A, Scholmerich J, Buchler C (2005): Mechanisms of disease: adipocytokines and visceral adipose tissue-emerging role in nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 2:273-280.
28. Schnedl WJ, Ferber S, Johnson JH, Newgard CB (1994): STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes 43 (11):1326-1333.
29. Scott CD, Martin JL, Baxter RC (1985): Production of insulin-like growth factor I and its binding protein by adult rat hepatocytes in primary culture. Endocrinology 116:1094-1101.
30. Shen SJ, Wang CY, Nelson KK, Jansen M, Ilan J (1986): Expression of insulin-like growth factor II in human placentas from normal and diabetic pregnancies. Proc Natl Acad Sci USA 83(23):9179-9182.
31. Staels B (2006): When the clock stops ticking, metabolic syndrome explodes. Nat Med 1:254-255.
32. Storch KF, Lipan O, Leykin L, Viswanathan N, Davis FC, Wong WH, Weitz CJ (2002): Extensive and divergent circadian gene expression in liver and heart. Nature 417:78-83.
33. Tan K, Baxter RC (1986): Serum insulin-like growth factor I levels in adult diabetic patients: the effect of age. J Clin Endocrinol Metab 63:651-655.
34. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J (2005): Obesity and metabolic syndrome in circadian clock mutant mice. Science 308:1043-1045.
35. Vambergue A, Fajardy I, Dufour P, Valat AS, Vandersippe M, Fontaine P, Danze PM, Rousseaux J (2007): No loss of genomic imprinting of IGF-II and H19 in placentas of diabetic pregnancies with fetal macrosomia. Growth Horm IGF Res 17:130-136.
36. Vitaterna MH, Selby CP, Todo T, Niwa H, Thompson C, Fruechte EM, Hitomi K, Thresher RJ, Ishikawa T, Miyazaki J, Takahashi JS, Sancar A (1999): Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. Proc Natl Acad Sci USA 96:12114-12119.
37. Vitaterna MH, Takahashi JS, Turek FW (2001): Overview of circadian rhythms. Alcohol Res Health 25(2):85-93.
38. Wakamatsu H, Yoshinobu Y, Aida R, Moriya T, Akiyama M, Shibata S (2001): Restricted-feeding-induced anticipatory activity rhythm is associated with a phase-shift of the expression of mPer1and mPer2 mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. Eur J Neurosci 13:1190-1196.
39. Wang Z, Gleichmann H (1998): GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. Diabetes 47(1):50-56.
40. Yagita K, Tamanini F, van der Horst GT, Okamura

H (2001): Molecular mechanisms of the biological clock in cultured fibroblasts. Science 292:278-281.
41. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS (2004): Period-2:luciferase real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci USA 101: 5339-5346.

(Received: 29 August 2009 / Accepted: 7 September 2009)