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RESEARCH ARTICLE

Significant dissociation of expression patterns of the basic helix–loop–helix transcription factors *Dec1* and *Dec2* in rat kidney

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SUMMARY

***Dec1* and *Dec2* are regulators of the mammalian molecular clock that show robust circadian rhythms in the suprachiasmatic nucleus and various peripheral tissues. Although the expression of *Dec1* and *Dec2* is altered by multiple stimuli in different organs, their transcriptional regulatory mechanisms have not been fully elucidated for the kidney. In the present study, we describe for the first time significant dissociation of expression patterns with arrhythmic expression of *Dec1* and rhythmic expression of *Dec2* in rat kidney under a normal light–dark (LD) cycle. Daytime restricted feeding (RF) significantly altered the expression patterns of these two clock genes, and even induced circadian expression of *Dec1* with an amplitude of 2.2 on day 3 and 4.2 on day 7. However, when a reversed feeding schedule was coupled with a reversed LD cycle, the expression of *Dec1* but not *Dec2* reverted to being arrhythmic. Moreover, exogenous injection of the glucocorticoid analogue dexamethasone (Dex) at certain times of the day resulted in rhythmic expression of *Dec1*, which was similar to that seen following RF for 7 days. In contrast, endogenous disruption of glucocorticoids by adrenalectomy abolished RF-induced rhythmic expression of *Dec1* in the kidney. These observations suggest the existence of a glucocorticoid gating mechanism in the circadian expression of *Dec1* in rat kidney.**

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Key words: daytime restricted feeding, light–dark cycle, glucocorticoids, adrenalectomy.

INTRODUCTION

Dec1 and *Dec2*, basic helix–loop–helix (bHLH) transcription factors, are emerging as very important regulators in a vast array of biological processes including development, cell differentiation, cell growth, oncogenesis and immune systems (Ivanova et al., 2001; Rossner et al., 1997; Shen et al., 2002; Sun et al., 2001). Moreover, expression of *Dec1* and *Dec2* shows circadian rhythm in most organs, including the suprachiasmatic nucleus (SCN) (Honma et al., 2002; Kawamoto et al., 2006; Kawamoto et al., 2004; Noshiro et al., 2004). In the mammalian clock system, CLOCK and BMAL1 form heterodimers that bind to E-boxes in the promoter region of various target genes, including negative regulators (e.g. *Per1*, *Per2*, *Cry1*, *Cry2*, *Dec1* and *Dec2*) and positive regulators (e.g. *Clock* and *Bmal1*), resulting in transcriptional activation (Lowrey and Takahashi, 2004; Reppert and Weaver, 2002). DEC1 and DEC2 serve as transcriptional repressors for CLOCK/BMAL1-enhanced promoter activity, through binding to E-boxes or interaction with BMAL1 (Hamaguchi et al., 2004; Honma et al., 2002; Kawamoto et al., 2004; Sato et al., 2004). In contrast, PER and CRY form a complex and interact with the CLOCK/BMAL1 heterodimer but cannot bind directly to E-boxes (Griffin et al., 1999; Shearman et al., 2000). Thus, multiple regulatory feedback loops are formed, which may be interlocked to stabilize the mammalian circadian system.

Circadian rhythms in behaviour and physiology are mainly driven by the master clock located in the SCN, which entrains to the environmental light cycle. Many peripheral clocks existing in the liver, heart, kidney and other organs primarily entrain to the environmental feeding schedule (Green et al., 2008; Kowalska and Brown, 2007; Le Minh et al., 2001). For example, the circadian phases of clock genes in the peripheral tissues of nocturnal animals can be uncoupled from those of the master clock by daytime restricted feeding (Damiola et al., 2000; Stokkan et al., 2001; Schibler and Sassone-Corsi, 2002). Moreover, some humoral signals (i.e. glucocorticoids) can influence the food-induced phase shifting of peripheral clock genes (Le Minh et al., 2001). The change of rhythmicity in clock genes will further result in the circadian alteration of other genes, called clock-controlled genes (CCGs) (Reppert and Weaver, 2002). CCGs then deliver a rhythmic output to control physiology and behaviour.

Diurnal fluctuations in blood pressure and urinary volume excretion have been known for decades. It has been reported that CLOCK–BMAL1 heterodimers directly regulate the renal circadian expression of the NHE3 Na^+/H^+ exchanger (Saifur Rohman et al., 2005). A deficiency in core clock genes such as *Per1* or *Clock* results in the alteration of urine electrolyte excretion (Gumz et al., 2009; Zuber et al., 2009). Thus, the molecular mechanism of the renal circadian clock is important for the physiological function of the kidney. Although it has been shown that *Dec1* and *Dec2* are regulated in a circadian fashion in many peripheral tissues (Boivin et al., 2003; Grechez-Cassiau et al., 2004; Noshiro et al., 2004), they exhibit distinct expression levels in the liver (Noshiro et al.,

2004) and in colon carcinomas (Li et al., 2003; Li et al., 2002), and display area-dependent expression patterns in the brain (Sun et al., 2001). Moreover, *Dec1* and *Dec2* gene expression was differently altered in the pineal gland of rats by photoperiod (Engel et al., 2005) and in some peripheral tissues of mice by fasting and re-feeding (Dong et al., 2010; Kawamoto et al., 2006). Therefore, in the present study, we investigated whether *Dec1* and *Dec2* are actually expressed in a similar manner in rat kidney and then examined the effects of multiple external and internal cues on the expression of these two genes.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar rats (body mass, 90–100 g) were purchased from the China National Laboratory Animal Resource Centre (Shanghai, China). Animals were housed under a light–dark (LD) cycle of 12 h L:12 h D at constant temperature and given food only during the dark period and water *ad libitum* for 2 weeks before experimentation. The onset of light was defined as Zeitgeber time 0 (ZT0); the onset of darkness was ZT12.

To test the effect of external time cues on the circadian expression of *Dec1* and *Dec2*, we divided the rats into three groups. In the control group, rats were sampled (see below) at 4 h intervals of the daily cycle, after adaptation to the normal lighting and feeding conditions. In the daytime restricted feeding (RF) group, rats were starved for 1 day and then subjected to the reversed feeding schedule for 7 days without altering the L:D cycle. Then, the animals were sampled at 4 h intervals on day 3 and day 7. In the dual reversal group, rats were starved for 1 day and then subjected to the reverse conditions of the feeding schedule and LD cycle for 7 days by extending the light period for an additional 12 h. Rats in the dual reversal group were sampled at 4 h intervals on day 0 (DL), day 3 (LD) and day 7 (LD).

To examine the effect of the internal cue (i.e. glucocorticoids), rats were divided into two groups: exogenous and endogenous. Animals used in the following experiments had the same photoperiod and same food availability conditions as in the previous experiments (12 h L:12 h D with food only at night). In the exogenous group, phosphate-buffered saline (PBS) or 1 mg kg^{-1} body mass of the glucocorticoid analogue dexamethasone (Dex) was intraperitoneally injected into the rats at ZT6 everyday for 7 days. The animals were sampled at 4 h intervals on day 7. In the endogenous group, rats were anaesthetized by i.p. injection of 45 mg kg^{-1} sodium pentobarbital and bilateral surgical adrenalectomy was performed using the dorsal approach. The adrenalectomized rats were provided with 0.9% NaCl as their water source. After the operation, animals were allowed to recover from surgery for 1 week. Alert and healthy rats were selected for subsequent experiments. The adrenalectomized rats were divided into two subgroups. In the control subgroup, animals were continually housed under normal lighting and feeding conditions. In the RF subgroup, the supply of food to adrenalectomized rats was changed from the dark to the light phase for 7 days without altering the LD cycle. Rats in each subgroup were sampled at the same time starting at ZT0 in 4 h intervals on day 7.

To further investigate the sensitivity of the kidney to glucocorticoid stimulation, we injected 1 mg kg^{-1} body mass of Dex into adrenalectomized rats at either ZT0 or ZT12 everyday for 7 days. Then animals in both the ZT0 and ZT12 groups were sampled at 4 h intervals on day 7.

All of the rats were killed under anaesthesia by i.p. injection of 45 mg kg^{-1} sodium pentobarbital. The kidneys and heart were removed quickly, frozen immediately in liquid nitrogen, and kept at –80°C until the RNA was extracted. During the dark phase, the dissection was carried out under dim red light. All experiments were performed according to international ethical standards, and the study was approved by the Research Committee of Zhejiang University of Technology.

RNA isolation and reverse transcription

Total RNA from the kidneys and heart was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions as described previously (Wu et al., 2008b).

Real-time PCR

Real-time PCR was carried out using the SYBR® ExScript™ PCR Kit (Takara Biochemicals, Dalian, China) in a total volume of 10 µl. The primer sequences were designed using the software Primer Premier 5.0 as follows: 5′-CCA GGA AAC CAT TGG ACT CAG-3′ and 5′-AGA GGT CGG ATA CCA GCA TTT-3′ for *Dec1*; 5′-GAA GGA ATC CCT CAT TTGC-3′ and 5′-TGC TCC GTT AAG GCT GTTA-3′ for *Dec2*. PCR amplification and quantification were carried out using an Eppendorf MasterCyclers ep RealPlex4 (Wesseling-Berzdorf, Germany) as described in our previous report (Wu et al., 2008a). The profiles of the mRNA levels under the control and experimental conditions were determined within the same RT-PCR run. The data were normalized to the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA.

Corticosterone concentration

Serum corticosterone concentration was measured with an enzyme immunoassay kit (Assay-Designs Inc., Ann Arbor, MI, USA) according to the manufacturer's instructions (serum samples were diluted 1:50). The corticosterone ELISA had a sensitivity of 27.0 pg ml^{-1}. All samples, standards and replicates were assayed in duplicate.

Cosine wave analysis

We estimated the peak phase of each cycling gene from the peak time of the most highly correlated cosine wave. The mRNA levels were fitted using Matlab 7.0.1 by a non-linear least-squares regression with the following cosine wave equation (cosinor): $y=\{A+B\times\cos[2\pi\times(t–C)/24]\}$ where y represents the level of mRNA, A is the mean level of mRNA, B is the amplitude of the mRNA oscillation, C is the acrophase of the mRNA oscillation and t is time (h). The peak time was considered when the mRNA level at a given time point was within 95% confidence bounds of the expected acrophase. The cosine fit was considered to be statistically significant when F was greater than $F_{0.05}$ ($F>F_{0.05}$).

Data analysis

All data are presented as the mean ± s.e.m. of N=4 animals. Quantitative representations of multiple results are expressed as values relative to the minimum value of the control group. The daily oscillation of each circadian gene was analysed using one-way ANOVA. Differences between groups were analysed by two-way ANOVA. Significant differences at each time point were analysed by the Student–Newman–Keuls test.

RESULTS

Diurnal expression patterns of *Dec1* and *Dec2* in rat kidney and heart

As shown in Fig. 1, the expression of *Dec1 and Dec2* was detected in the kidney and heart of rats over the course of a 24 h LD cycle.

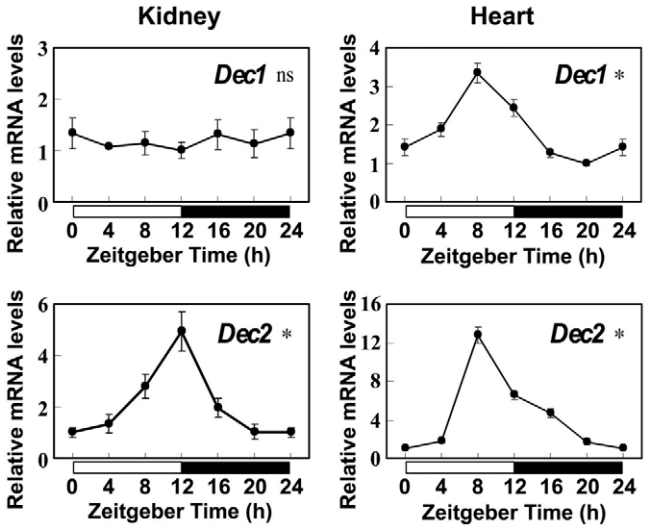

Fig. 1. Daily expression patterns of *Dec1* and *Dec2* in the kidney and heart of rats under a normal light–dark (LD) cycle. The rats were adapted to the 12 h L:12 h D cycle for 2 weeks. Then, animals were sampled at 4 h intervals of the daily cycle starting at Zeitgeber time (ZT)0. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h L:12 h D cycle. Quantitative representations of multiple results are expressed as values relative to the minimum value. The ZT24 value represents a replotting of the ZT0 value. Each value represents the mean ± s.e.m. derived from four animals. The *P*-value calculated by one-way ANOVA for each clock gene is indicated in each panel (**P*<0.05; ns, not significant).

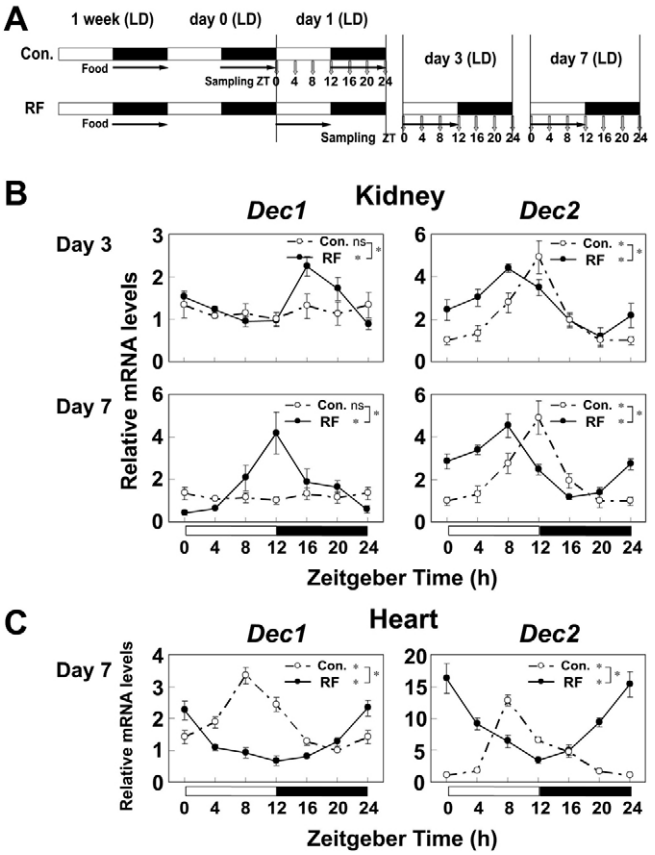

Fig. 2. The expression profiles of *Dec1* and *Dec2* in rat kidney and heart after the daytime restricted feeding (RF) treatment. (A) Experimental design. After adaptation to the 12 h L:12 h D cycle, rats were starved for 1 day and then subjected to the reversed feeding schedule for 7 days without altering the LD cycle. The animals were then sampled at 4 h intervals on day 3 and day 7. Con., control. (B) Expression profiles of *Dec1* and *Dec2* in the kidney of the RF and control group. (C) Expression profiles of *Dec1* and *Dec2* in the heart of the RF and control group. The ZT24 value represents a replotting of the ZT0 value in the control group. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h L:12 h D cycle. Quantitative representations of multiple results are expressed as values relative to the minimum value of the control group. Each value represents the mean ± s.e.m. derived from four animals. The *P*-values calculated by one-way ANOVA for each group and two-way ANOVA for RF *vs* control are indicated in each panel (**P*<0.05; ns, not significant).

However, the oscillation patterns differed greatly between these two members of the bHLH family in the kidney. For *Dec2*, the most abundant mRNA level was found at ZT12 and the least at ZT0 (ZT24), and the ratio of peak to trough (amplitude) of the diurnal rhythm was approximately 5.0 (one-way ANOVA, $P<0.05$). In contrast, the expression of *Dec1* did not exhibit rhythmicity (cosine wave analysis, $F<F_{0.05}$). However, both *Dec1 and Dec2* displayed rhythmic expression in rat heart (cosine wave analysis, $F>F_{0.05}$), which peaked at ZT8 and reached the lowest levels between ZT20 and ZT24.

Effect of reversing the feeding schedule on expression profiles of *Dec1* and *Dec2* in rat kidney and heart

The effect of RF on the expression of *Dec1* and *Dec2* in rat kidney and heart can be seen in Fig. 2B,C (filled circles and solid lines). In the kidney, significant differences in the 24 h expression profiles were found on both day 3 and day 7 after RF treatment compared with profiles of the control condition for each gene (two-way ANOVA, $P<0.05$). The peak phase of *Dec2* was consistently shifted by 4 h on the examination days (cosine wave analysis), while the peak expression level of this gene hardly changed ($P>0.05$). RF treatment for 3 days was sufficient to induce the rhythmic expression of *Dec1* (cosine wave analysis, $F>F_{0.05}$). Moreover, the daily fluctuating amplitude of *Dec1* increased with the duration of RF treatment from 2.2 on day 3 to 4.2 on day 7. In the heart, both *Dec1* and *Dec2* showed a robust daily rhythm (cosine wave analysis, $F>F_{0.05}$), and their peak phases were shifted by 8 h after RF treatment for 7 days (cosine wave analysis).

Effect of the combined reversal of the feeding schedule and LD cycle on the expression profiles of *Dec1* and *Dec2* in rat kidney and heart

To investigate the effect of feeding reversal coupled with LD reversal, we subjected rats to the reversed feeding schedule and LD cycle by extending the light period for 24 h (Fig. 3A). The expression profiles of *Dec1* and *Dec2* in the kidney on day 0 (DL, open circles/dashed lines indicated as control), day 3 (LD, filled circles and solid lines) and day 7 (LD, filled circles and solid lines) are illustrated in Fig. 3B. Concomitantly changing the feeding schedule and the LD cycle differently affected the expression profiles of these clock genes in the kidney. The circadian pattern of *Dec2* was significantly altered on both day 3 and day 7 compared with day 0 (two-way ANOVA, $P<0.05$). The peak phase of *Dec2* was shifted by 8 h and 12 h on day 3 and day 7, respectively (cosine wave

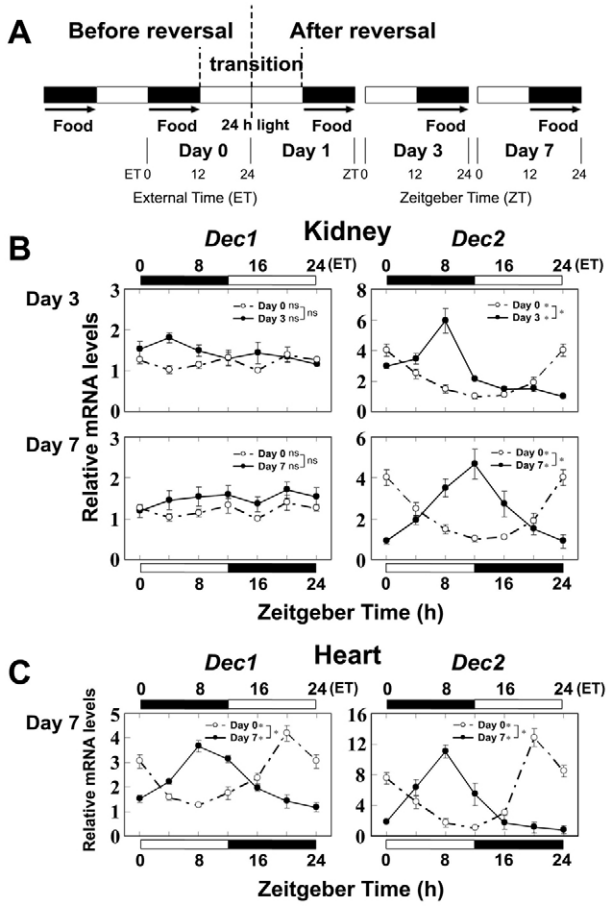

Fig. 3. The expression profiles of *Dec1* and *Dec2* in rat kidney after reversal of the feeding schedule coupled with reversal of the LD cycle. (A) Experimental design. After adaptation to the 12 h L:12 h D cycle, rats were starved for 1 day and then subjected to the reverse conditions for both feeding schedule and LD cycle for 7 days by extending the light period for 24 h. Rats in the dual reversal group were sampled at 4 h intervals on day 0 (DL), day 3 (LD) and day 7 (LD). (B) Expression profiles of *Dec1* and *Dec2* in the kidney of the dual reversal group. (C) Expression profiles of *Dec1* and *Dec2* in the heart of the dual reversal group. The ZT24 value represents a replotting of the ZT0 value from day 0. The black bar at the top of the panel represents the duration of the dark phase in the 12 h L:12 h D cycle for day 0, whereas the black bar at the bottom of the panel represents the duration of the dark phase of the 12 h L:12 h D cycle for day 3 or day 7. Quantitative representations of multiple results are expressed as values relative to the minimum value at day 0. Each value represents the mean ± s.e.m. derived from four animals. The *P*-values calculated by one-way ANOVA for each day and two-way ANOVA for day 3 or day 7 *vs* day 0 are indicated in each panel. Differences between groups were analysed by two-way ANOVA (**P*<0.05; ns, not significant).

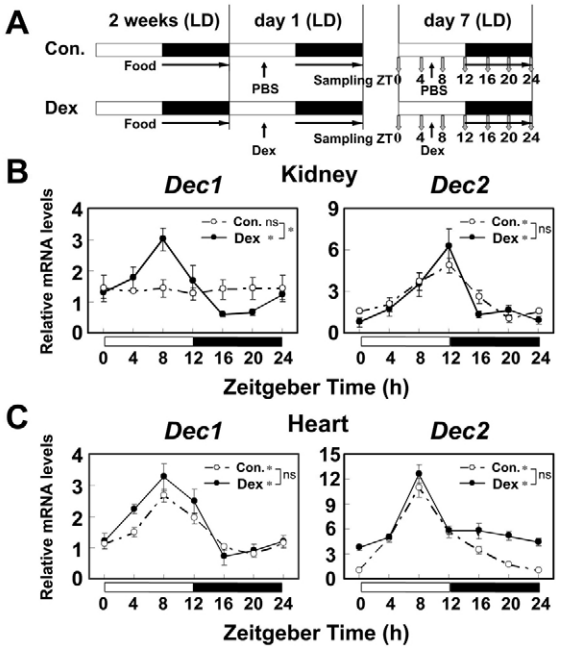

Fig. 4. The expression profiles of *Dec1* and *Dec2* in rat kidney after exogenous injection of dexamethasone. (A) Experimental design. After adaptation to the 12 h L:12 h D cycle, rats were intraperitoneally injected with phosphate-buffered saline (PBS) or 1 mg kg^{-1} body mass of the glucocorticoid analogue dexamethasone (Dex) at ZT6 everyday for 7 days. The animals were sampled at 4 h intervals on day 7. (B) Expression profiles for *Dec1* and *Dec2* in the kidney of the control and Dex groups. (C) Expression profiles for *Dec1* and *Dec2* in the heart of the control and Dex groups. The ZT24 value represents a replotting of the ZT0 value in the control group. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h L:12 h D cycle. Quantitative representations of multiple results are expressed as values relative to the minimum value. Each value represents the mean ± s.e.m. derived from four animals. The *P*-value calculated by one-way ANOVA is indicated in each panel. Differences between groups were analysed by two-way ANOVA (**P*<0.05; ns, not significant).

analysis). As for the peak mRNA level, it had increased by 1.5-fold on day 3 ($P<0.05$) but was not significantly altered on day 7 ($P>0.05$). In contrast, the expression profile of *Dec1* was unaltered on both day 3 and day 7 (two-way ANOVA, $P>0.05$). With regard to the expression pattern of *Dec1* and *Dec2* in the heart, they displayed rhythmic expression before and after the reversed feeding schedule and LD cycle (cosine wave analysis, $F>F_{0.05}$) (Fig. 3C). On day 7, the peak phases of both genes in the heart were shifted by 12 h, similar to the change in *Dec2* expression in the kidney on the same day (cosine wave analysis).

Effect of exogenous glucocorticoids on the circadian expression of *Dec1* and *Dec2* in rat kidney and heart

We next investigated the effect of a representative internal cue, glucocorticoids, on the circadian expression of *Dec1* and *Dec2* in the kidney and heart of rats, to compare this with the effects of the external time cues. We examined the effect of exogenous injection of 1 mg kg^{-1} body mass of the glucocorticoid analogue Dex at ZT6 everyday for 7 days (Fig. 4A). On day 7 (Fig. 4B), *Dec1* displayed rhythmic expression in the kidney of the Dex group (cosine wave analysis, $F>F_{0.05}$), which showed a peak phase at ZT8 with an amplitude of 5.2 ($P<0.05$). Exogenous injection of Dex did not alter the expression pattern of *Dec2* compared with that of the control group in the kidney (two-way ANOVA, $P>0.05$), which showed a peak phase at ZT12 with an amplitude of 8.2 ($P<0.05$). In the heart (Fig. 4C), both *Dec1* and *Dec2* displayed rhythmic expression profiles (cosine wave analysis, $F>F_{0.05}$), which were similar for the Dex and control groups (two-way ANOVA, $P>0.05$).

Effect of endogenous glucocorticoids on the circadian expression of *Dec1* and *Dec2* in rat kidney and heart

To investigate the effect of endogenous glucocorticoids, bilateral surgical adrenalectomy was performed on the rats. The results can

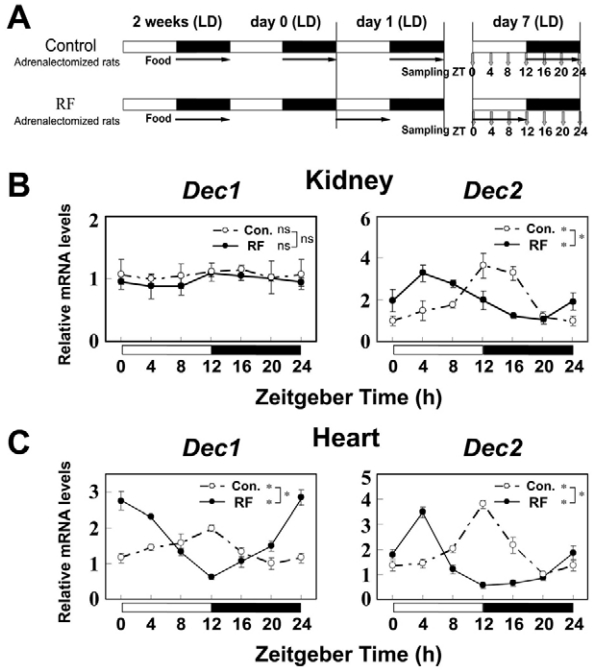

Fig. 5. The expression profiles of *Dec1* and *Dec2* in adrenalectomized rat kidney. (A) Experimental design. After adaptation to the 12 h L:12 h D cycle, the adrenalectomized rats were divided into two groups. In the control group, animals were continually housed under normal lighting and feeding conditions. In the RF group, the food supply of adrenalectomized rats was changed from the dark to the light phase for 7 days without altering the LD cycle. Rats in each group were sampled at the same time starting at ZT0 at 4 h intervals on day 7. (B) Expression profiles of *Dec1* and *Dec2* in the kidney of the control and RF groups of adrenalectomized rats. (C) Expression profiles of *Dec1* and *Dec2* in the heart of the control and RF groups of adrenalectomized rats. The ZT24 value represents a replotting of the ZT0 value in the control group. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h L:12 h D cycle. Quantitative representations of multiple results are expressed as values relative to the minimum value. Each value represents the mean ± s.e.m. derived from four animals. The *P*-value calculated by one-way ANOVA for each group is indicated in each panel (**P*<0.05; ns, not significant).

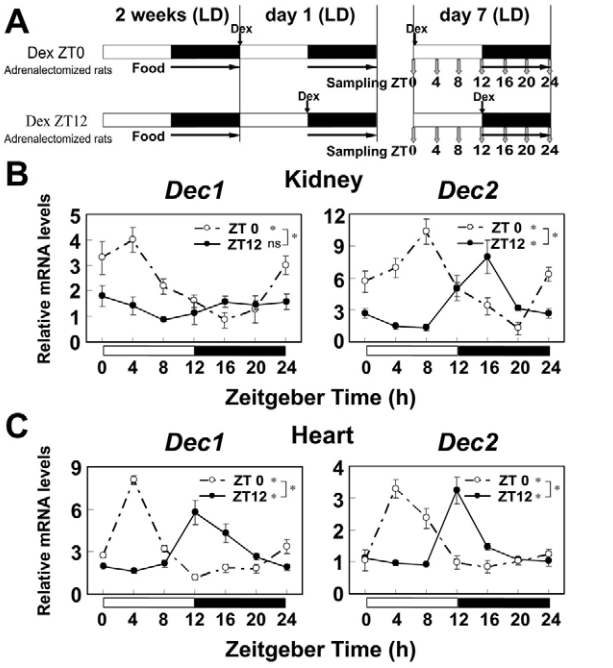

Fig. 6. The expression profiles of *Dec1* and *Dec2* in the kidney and heart of adrenalectomized rats after different times injection of dexamethasone. (A) After adaptation to the 12 h L:12 h D cycle, adrenalectomized rats were intraperitoneally injected with 1 mg kg^{-1} body mass of the glucocorticoid analog dexamethasone (Dex) at either ZT0 or ZT12 every day for 7 days. Rats were sampled at 4-h intervals on day 7. (B) Expression profiles for *Dec1* and *Dec2* in the kidney of the ZT0 and ZT12 groups. (C) Expression profiles for *Dec1* and *Dec2* in the heart of the ZT0 and ZT12 groups. The black bar at the bottom of each panel represents the duration of the dark phase of the 12 h L:12 h D cycle. Quantitative representations of multiple results are expressed as values relative to the minimum value. Data are means ± s.e.m. derived from four animals. The *P*-value calculated by one-way ANOVA for each group is indicated in each panel. Differences between groups were analysed by two-way ANOVA (**P*<0.05; ns, not significant).

be seen in Fig. 5. In the control group of adrenalectomized rats, the oscillation patterns of *Dec1* and *Dec2* in the kidney were similar to those in normal rats under the same conditions as described in Fig. 1. *Dec1* still displayed arrhythmic expression (cosine wave analysis, $F<F_{0.05}$), and the peak phase of *Dec2* was also found at ZT12 with an amplitude of 3.6 (one-way ANOVA, $P<0.05$). It is interesting that the RF-induced circadian expression of *Dec1*, which was observed in the kidney of normal rats, did not appear in the RF group of adrenalectomized rats (cosine wave analysis, $F<F_{0.05}$). *Dec2* continued to display circadian expression (cosine wave analysis, $F>F_{0.05}$), while the peak phase of this gene was advanced by 8 h (cosine wave analysis). In the heart, both *Dec1* and *Dec2* showed circadian expression in the adrenalectomized rats before and after the RF treatment (cosine wave analysis, $F>F_{0.05}$). The peak phase of *Dec1* and *Dec2* was shifted by 12 h and 8 h, respectively (cosine wave analysis). In addition, we confirmed that the expression patterns of clock genes in the sham-operated rats (data not shown) were similar to those in the normal rats (Fig. 2) under both control and RF conditions.

Effect of exogenous glucocorticoids at different times on the circadian expression of *Dec1* and *Dec2* in the kidney and heart of adrenalectomized rats

To confirm the effects of glucocorticoids on the circadian expression of *Dec1* and *Dec2* at different times of the day, we further examined the response of these genes after exogenous injection for 7 days of 1 mg kg^{-1} body mass of Dex at either ZT0 or ZT12 in adrenalectomized rats (Fig. 6A). In the kidney (Fig. 6B), *Dec1* displayed arrhythmic expression in the ZT12 group after 7 days of Dex injection (cosine wave analysis, $F<F_{0.05}$). However, this gene showed circadian expression in the ZT0 group (cosine wave analysis, $F>F_{0.05}$) with a peak phase at ZT4 and an amplitude of 4.7 ($P<0.05$). *Dec2* displayed circadian expression in both the ZT0 and ZT12 groups (cosine wave analysis, $F>F_{0.05}$), with a peak phase at ZT8 and ZT16, respectively. In the heart (Fig. 6C), both *Dec1* and *Dec2* displayed rhythmic expression (cosine wave analysis, $F>F_{0.05}$) with peak phases at ZT4 in the ZT0 group and at ZT12 in the ZT12 group, respectively.

DISCUSSION

In the present study, we describe for the first time significant dissociation of expression patterns for arrhythmic expression of *Dec1*

and rhythmic expression of *Dec2* in the kidney of rats under a normal LD cycle. This differs not only from the circadian expression of *Dec1* and *Dec2* in the heart (present study) and in the SCN (Butler et al., 2004; Honma et al., 2002) and other peripheral tissues (Kawamoto et al., 2006; Kawamoto et al., 2004; Wu et al., 2008a; Wu et al., 2008b) of rats and mice but also from observations in mouse kidney (Hamaguchi et al., 2004; Noshiro et al., 2005), which suggests tissue- and species-specific mechanisms underlying the circadian expression of these two clock genes. This unusual phenomenon may be the cause of overlapping functions of *Dec1* and *Dec2* in maintaining and entraining circadian rhythms. It has been shown that DEC2 can also interact with BMAL1, bind to the E-box, and exhibit suppressive activity similar to that of DEC1 (Honma et al., 2002; Kawamoto et al., 2004). Similar DNA binding domains and flanking sequences with a high degree of identity for DEC1 and DEC2 suggest that they have overlapping target genes and are consequently functionally redundant for some target genes (Li et al., 2003; Sato et al., 2004). In support of this notion, expression profiles of clock genes such as *Bmal1*, *Clock*, *Per2*, *Per3*, *Dbp* and *Rev-erbα* did not show any significant changes in the liver of *Dec1*$^{-/-}$ mice (Grechez-Cassiau et al., 2004). Moreover, *Dec1*-knockout mice were capable of developing to adulthood and showed no discernible phenotypic differences from their wild-type littermates (Sun et al., 2001). Thus, functional disruption induced by arrhythmic expression of *Dec1* might be compensated for by the rhythmic expression of *Dec2* in rat kidney. Previous studies have also shown species-specific expression profiles of *Dec1* and *Dec2* in the liver of rat and mouse (Noshiro et al., 2004; Noshiro et al., 2005). The mechanisms involved and the physiological significance of the discrepancy between rat and mouse need to be studied further.

Although the expression of *Dec1* and *Dec2* is altered by multiple stimuli such as light pulses, hypoxia, growth factors and feeding (Yamada and Miyamoto, 2005), their transcriptional regulatory mechanisms are not fully understood. *Dec1* and *Dec2* displayed differential responses to the external time cues. A brief light pulse induced *Dec1* but not *Dec2* expression in the SCN in a phase-dependent manner (Honma et al., 2002). The 24 h pattern of expression of *Dec2* but not *Dec1* was affected by the photoperiod (Engel et al., 2005). More recent studies showed that fasting and re-feeding exhibited a different and even inverse effect on the expression of *Dec1* and *Dec2* in peripheral tissues (e.g. liver and heart) (Dong et al., 2010; Kawamoto et al., 2006). As different tissues respond to different environmental signals for their tissue-specific functions, we examined the previously unknown effect of food and light cues on the expression of *Dec1* and *Dec2* in rat kidney. In the present study, we found that RF (feeding schedule uncoupled from the LD cycle) not only shifted the peak phase of *Dec2* but also induced the rhythmic expression of *Dec1* in rat kidney. When the feeding schedule was reversed together with the LD cycle, the peak phase of *Dec2* shifted more rapidly on day 3 and day 7, which was very similar to the responses of *Per1* and *Per2* in the rat kidney (Wu et al., 2010). The results observed in the heart for both *Dec1* and *Dec2* resembled that of *Dec2* in the kidney in both of the above situations. In contrast, RF-induced circadian expression of *Dec1* did not occur following coupled reversal of the feeding schedule and LD cycle, which still displayed arrhythmic expression as in the control condition. These observations suggest that uncoupling of the feeding schedule and LD cycle induces some disordered internal circadian signal, which may account for the arrhythmic expression of *Dec1* in rat kidney.

To address the possibility that an internal candidate may regulate the expression of *Dec1* in rat kidney, we examined the role of glucocorticoids, which have been implicated in the entrainment of peripheral circadian clocks in mammals (Dickmeis, 2009; Reddy et al., 2009). To investigate induction by glucocorticoids, we injected exogenous Dex at ZT6 for 7 days and found that Dex could also induce the rhythmic expression of *Dec1*, which was similar to the effect of RF over 7 days. The expression profile of *Dec2* in the kidney and of both genes in the heart was almost identical between the Dex and control groups. In contrast, when the RF treatment was carried out in adrenalectomized rats, the RF-induced rhythmic expression of *Dec1* was suppressed by the endogenous disruption of glucocorticoids. *Dec2* expression displayed a larger peak phase shift than in normal rats after 7 days of RF treatment, which resembled *Dec1* expression in the heart. These results suggest that the endogenous disruption of glucocorticoids facilitates an RF-induced phase shift of peripheral clock genes consistent with the observation by Le Minh and colleagues (Le Minh et al., 2001). Thus, glucocorticoids must play key roles in the circadian regulation of *Dec1* in rat kidney. To test the sensitivity of *Dec1* in the kidney to glucocorticoid stimulation at different times of the day, we further examined the response of *Dec1* and *Dec2* after 7 days of exogenous injection of Dex at either ZT0 or ZT12 in adrenalectomized rats. We found that the circadian expression of *Dec1* could only be induced at ZT0 but not ZT12, which suggests that a timed sensitivity of the kidney to glucocorticoid stimulation may gate the circadian expression of *Dec1* in rat kidney.

In addition, we examined the plasma corticosterone values in the control and RF groups (supplementary material Fig. S1). We found that RF can induce a bimodal temporal pattern of corticosterone secretion – the first peak appearing at the beginning of the day, depending on the feeding time, and the second one appearing just before the LD transition controlled by the SCN clock, which was in a good agreement with previous observations in the RF condition (Le Minh et al., 2001). Therefore, the bimodal temporal pattern of corticosterone secretion was probably induced by the uncoupling of the feeding schedule and the LD cycle. To further confirm this hypothesis, we also measured the plasma corticosterone values in the LD reversal group. We found that the circadian pattern of corticosterone secretion on day 7 of LD reversal was similar to that in the control group (supplementary material Fig. S1). Combining the findings of Le Minh and colleagues with our own observations, we propose that RF-induced changes of daily glucocorticoid secretion may account for the 'unusual' circadian expression of *Dec1* in rat kidney.

Furthermore, it has been proposed that tissue-specific conversion of glucocorticoids to receptor inactive metabolites occurs because of the presence of 11β-hydroxysteroid dehydrogenase (11-HSD) (Draper et al., 2005). In rats, the large diurnal variation of plasma corticosterone appeared to be partially buffered in the kidney as the variation was smaller in the renal microdialysate, which might reflect the capacity of renal 11-HSD to maintain a relatively stable level of corticosterone (Usa et al., 2007). Moreover, 11-HSD shows a diurnal rhythm, which may be able to buffer the high level of plasma corticosterone only at a certain time of day. This might explain the presence of the glucocorticoid gating mechanism in the kidney, but not in the heart, of rats.

CONCLUSION

In conclusion, the present study was undertaken to extend existing knowledge of the circadian expression of *Dec1* and *Dec2* in peripheral tissues. For the first time, we have shown significant dissociation of the expression patterns of *Dec1* and *Dec2* in rat kidney. In the normal LD cycle, *Dec1* and *Dec2* display arrhythmic and

rhythmic expression patterns, respectively, which suggests *Dec1* may be functionally redundant in rat kidney. RF treatment significantly altered the expression patterns of these two clock genes and even induced the circadian expression of *Dec1*. However, when reversal of the feeding schedule was coupled with reversal of the LD cycle, the rhythmic expression of *Dec1* was abolished again. Moreover, exogenous injection of glucocorticoids at a certain time of day could induce the rhythmic expression of *Dec1*, which was similar to the effect of RF treatment. The rhythmic expression of *Dec1* under RF treatment was abolished by endogenous disruption of glucocorticoids. These observations suggest the existence of a glucocorticoid gating mechanism for the circadian expression of *Dec1* in rat kidney.

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