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# System-level identification of transcriptional circuits underlying mammalian circadian clocks

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**Mammalian circadian clocks consist of complexly integrated regulatory loops[1–5], making it difficult to elucidate them without both the accurate measurement of system dynamics and the comprehensive identification of network circuits[6]. Toward a system-level understanding of this transcriptional circuitry, we identified clock-controlled elements on 16 clock and clock-controlled genes in a comprehensive surveillance of evolutionarily conserved *cis* elements and measurement of their transcriptional dynamics. Here we report the roles of E/E′ boxes, DBP/E4BP4 binding elements[7] and RevErbA/ROR binding elements[8] in nine, seven and six genes, respectively. Our results indicate that circadian transcriptional circuits are governed by two design principles: regulation of E/E′ boxes and RevErbA/ROR binding elements follows a repressor-precedes-activator pattern, resulting in delayed transcriptional activity, whereas regulation of DBP/E4BP4 binding elements follows a repressor-antiphasic-to-activator mechanism, which generates high-amplitude transcriptional activity. Our analysis further suggests that regulation of E/E′ boxes is a topological vulnerability in mammalian circadian clocks, a concept that has been functionally verified using *in vitro* phenotype assay systems.**

Circadian rhythms are endogenous self-sustained oscillations with a period of ~24 h that are manifested in diverse physiological and metabolic processes[9,10]. Genetic analyses have identified numerous mammalian clock genes, including two basic helix-loop-helix–PAS transcription factors[11,12] (*Clock* and *Arntl*, also called *Bmal1* or *Mop3*), two period genes[13,14] (*Per1* and *Per2*), two cryptochrome genes[15] (*Cry1* and *Cry2*), casein kinase I epsilon[16] (*Csnk1e*) and two orphan nuclear hormone receptors[4,5] (*Nr1d1* and *Rora*, also called *RevErbAα* and *Rorα*, respectively). A number of other transcription factors also thought to function in the circadian regulation of gene expression, including two bZip-family genes[17,18] (*Dbp* and *Nfil3*, also called *E4bp4*), two basic helix-loop-helix transcription factors[19] (*Bhlhb2* and *Bhlhb3*, also called *Dec1* or *Stra13* and *Dec2*, respectively), one period-related gene[20] (*Per3*), one clock-related gene[21] (*Npas2*) and three genes[4,22] related to *Nr1d1* and *Rora* (*Nr1d2*, *Rorb* and *Rorc*, also called *RevErbAβ*, *Rorβ* and *Rorγ*, respectively), have been cloned as well.

Gene-expression analyses have shown that the mRNA levels of 16 of these 18 clock and clock-related genes have high-amplitude circadian oscillations in the central (suprachiasmatic nucleus; SCN) or peripheral (liver, etc.) clock tissues[2,4,17–27] (**Supplementary Fig. 1** online). The organization of the circadian regulation of the transcription of clock and clock-related genes is suggestive of an integrated network of regulatory loops of great complexity, as regulators of circadian clocks are themselves regulated by circadian clocks.

To address this complexity, we sought to identify functional clock-controlled elements in these 16 transcription factors by using an *in vitro* cell culture system[22] that allows us to monitor circadian transcriptional dynamics using a destabilized luciferase reporter (dLuc) driven by clock-controlled promoters (**Figs. 1–4** and **Supplementary Note** online). Of the genes analyzed, nine participate in the circadian regulation of gene expression through E boxes[11–15,19–21], two through DBP/E4BP4 binding elements (D boxes)[17,18] and five through RevErbA/ROR binding elements (RREs)[4,5,22]. An analysis of the effects of clock-controlled elements in these 16 transcription factors identified functional E boxes (CACGTG) or E′ boxes (CACGTT) in nine of the genes (**Figs. 1** and **4**), functional D boxes (TTA[T/C]GTAA) in seven genes (**Fig. 2**) and functional RREs ([A/T]A[A/T]NT[A/G]GGTCA)[4,22] in six genes (**Figs. 3** and **4**). The fact that the E′ box in *Clock* does not direct circadian transcriptional activity (H.R.U. *et al.*, unpublished data) despite being perfectly conserved in human and mouse demonstrates the importance of functional analysis.

We classified the 16 transcription factors described above into three groups: E/E′-box regulators, D-box regulators and RRE regulators (**Fig. 5a**). We next categorized these regulators according to their upstream regulation into five subgroups: regulated by E/E′ boxes, regulated by both E/E′ boxes and D boxes, regulated by D boxes,

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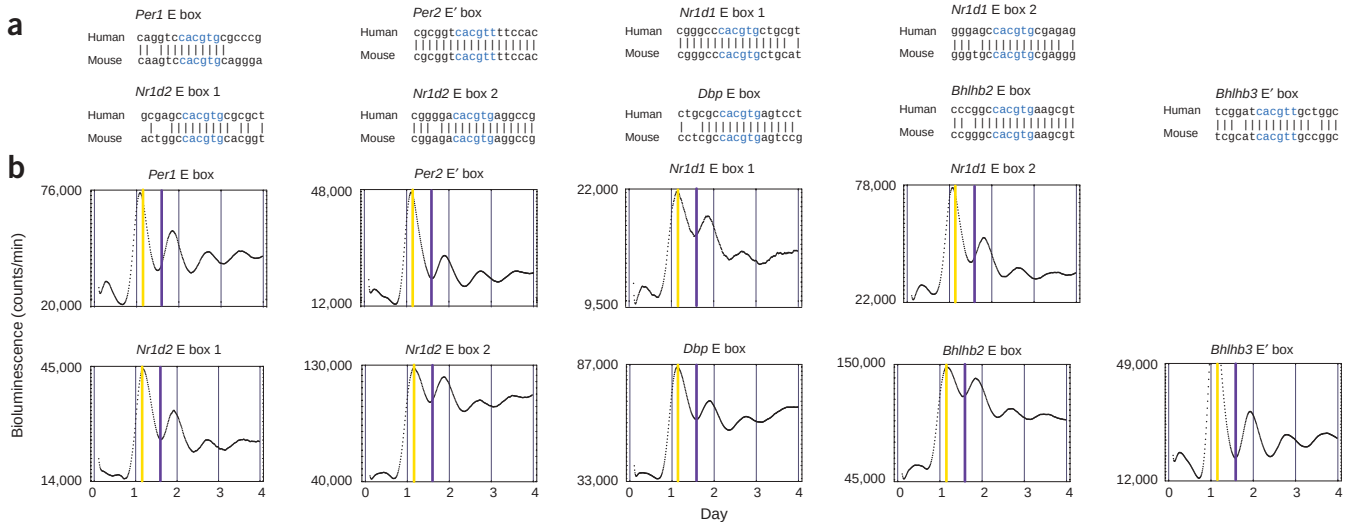


**Figure 1** E/E′ boxes sufficient for circadian oscillation in phase with *Per2* and antiphase to *Arntl* oscillation. (**a**) Evolutionary conservation of E/E′ boxes in noncoding regions of clock and clock-controlled genes. (**b**) Representative circadian rhythms of bioluminescence from wild-type E/E′ boxes fused to the SV40 basic promoter driving a dLuc reporter. The phases measured as the second peak from stimulation were 26.5 ± 0.4 h (*Per1* E box, $n = 3$, mean ± s.e.m.), 26.8 ± 0.18 h (*Per2* E′ box, $n = 3$), 26.7 ± 0.4 h (*Dbp* E box, $n = 3$), 27.6 ± 0.6 h (*Nr1d1* E box 1, $n = 3$), 26.1 ± 0.2 h (*Nr1d1* E box 2, $n = 3$), 27.6 ± 0.2 h (*Nr1d2* E box 1, $n = 3$), 28.3 ± 0.3 h (*Nr1d2* E box 2, $n = 3$), 27.9 ± 0.1 h (*Bhlhb2* E box, $n = 3$) and 26.4 ± 0.23 h (*Bhlhb3* E′ box, $n = 3$), which were in phase with levels of circadian luminescence from the *Per2* promoter (27.7 ± 0.1 h, $n = 3$, yellow lines; see also **Supplementary Fig. 3** online) and antiphase to those from the *Arntl* promoter (38.3 ± 0.3 h, $n = 3$, purple lines; see also **Supplementary Fig. 3** online). See also **Supplementary Figure 4** online for confirmation of E/E′ boxes by transfection assays.

regulated by both E/E′ boxes and RREs, and regulated by RREs. Expression patterns of repressors and activators of E/E′-box, RRE and D-box regulation were temporally distinct (**Fig. 5a** and **Supplementary Fig. 1** online). Gene expression of repressors preceded that of activators in E/E′-box and RRE regulation, whereas the expression patterns of repressors were nearly antiphasic to those of activators in D-box regulation.

As an example, gene expression in the mouse SCN of the strongest E/E′-box repressor *Cry1* preceded that of the E/E′-box activator *Arntl* (**Supplementary Fig. 1** online). Similarly, expression of the RRE

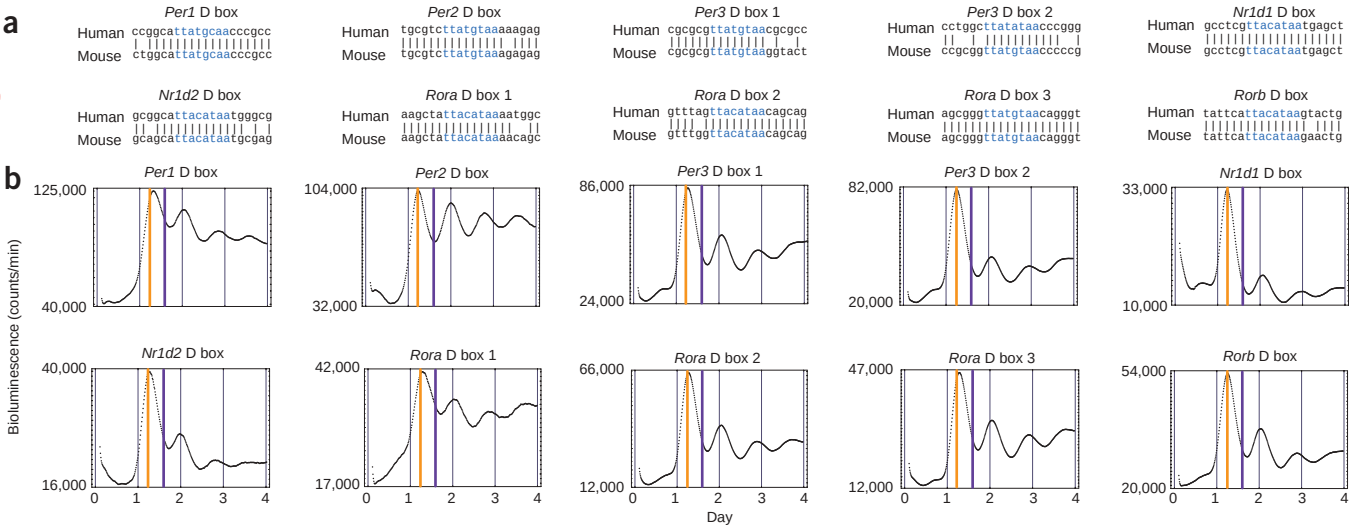


**Figure 2** D boxes sufficient for circadian oscillation in phase with *Per3* and antiphase to *Arntl* oscillation. (**a**) Evolutionary conservation of D boxes in noncoding regions of clock and clock-controlled genes. (**b**) Representative circadian rhythms of bioluminescence from wild-type D boxes fused to the SV40 basic promoter driving a dLuc reporter. The phases measured as the second peak from stimulation were 31.3 ± 0.5 h (*Per1* D box, $n = 3$, mean ± s.e.m.), 29.1 ± 0.1 h (*Per2* D box, $n = 3$), 29.9 ± 0.2 h (*Per3* D box 1, $n = 3$), 30.9 ± 0.9 h (*Per3* D box 2, $n = 3$), 29.9 ± 0.5 h (*Nr1d1* D box, $n = 3$), 30.6 ± 0.3 h (*Nr1d2* D box, $n = 3$), 30.4 ± 0.1 h (*Rora* D box 1, $n = 3$), 30.9 ± 0.1 h (*Rora* D box 2, $n = 3$), 31.4 ± 0.2 h (*Rora* D box 3, $n = 3$) and 30.3 ± 0.3 h (*Rorb* D box, $n = 3$), which were in phase with levels of circadian luminescence from the *Per3* promoter (29.7 ± 1.2 h, $n = 3$, orange lines; see also **Supplementary Fig. 3** online) and antiphase to those from the *Arntl* promoter (38.3 0.3 h, $n = 3$, purple lines; see also **Supplementary Fig. 3** online). See also **Supplementary Figure 5** online for confirmation of D boxes by transfection assays and EMSAs.

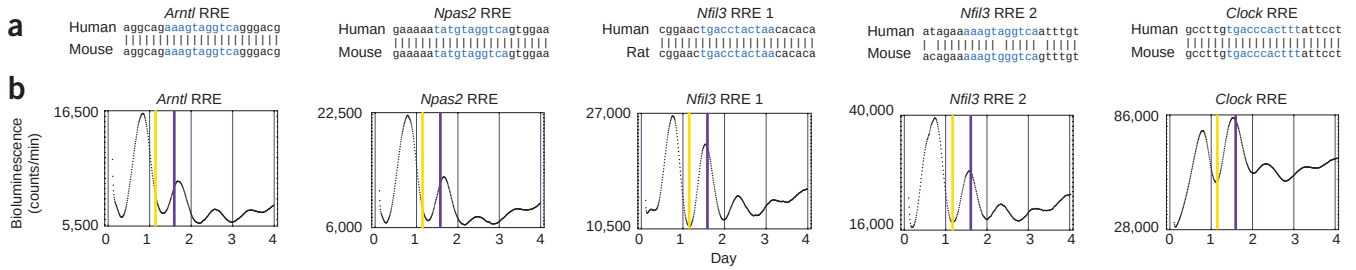


**Figure 3** RREs sufficient for circadian oscillation in phase with *Per2* and antiphase to *Arntl* oscillation. (**a**) Evolutionary conservation of RREs in noncoding regions of clock and clock-controlled genes. (**b**) Representative circadian rhythms of bioluminescence from wild-type RREs fused to the SV40 basic promoter driving a dLuc reporter. The phases measured as the second peak from stimulation were 38.3 ± 0.9 h (*Arntl* RRE, $n = 3$, mean ± s.e.m.), 38.5 ± 0.7 h (*Npas2* RRE, $n = 3$), 37.4 ± 0.2 h (*Nfil3* RRE 1, $n = 3$), 37.6 ± 0.1 h (*Nfil3* RRE 2, $n = 3$) and 38.8 ± 1.3 h (*Clock* RRE, $n = 3$), which were in phase with levels of circadian luminescence from the *Arntl* promoter (38.3 ± 0.3 h, $n = 3$, purple lines; see also **Supplementary Fig. 3** online) and antiphase to those from the *Per2* promoter (27.7 ± 0.1 h, $n = 3$, yellow lines; see also **Supplementary Fig. 3** online). See also **Supplementary Figure 6** online for confirmation of RREs by transfection assays and EMSAs.



repressor *Nr1d1* preceded that of the RRE activator *Rora* in the mouse SCN (**Supplementary Fig. 1** online). In contrast, gene expression of the D-box repressor *Nfil3* was nearly antiphasic to that of the D-box activator *Dbp* in the mouse SCN (**Supplementary Fig. 1** online). We observed similar temporal organization of E/E′-box, RRE and D-box regulators in the mouse liver (**Supplementary Fig. 1** online). These results suggest that simple principles govern temporal relationships in the expression of these transcriptional factors. In E/E′-box and RRE regulation, repressors precede activators, whereas in D-box regulation, repressors and activators are nearly antiphasic. These design principles are encoded and realized, at least in part, by distinct clock-controlled elements (**Fig. 5a**). The temporal relationships observed in the expression of genes encoding E/E′-box, RRE and D-box regulators have been observed in protein abundances as well[4,17,24].

The predicted durations of E/E′-box and RRE activity follow the previously reported abundances of E/E′-box and RRE activator proteins, indicating delays in transcriptional activity in E/E′-box and RRE regulation. E/E′-box activity in the mouse liver is expected to reach a maximum at circadian time (CT) of ∼7.5–11.5 (**Supplementary Fig. 1** online), which follows the peak in abundance of mBMAL1 that occurs at ∼CT0–CT3 (ref. 24). Similarly, RRE activity in the mouse liver is expected to reach a maximum at ∼CT21.0–CT23.0 (**Supplementary Fig. 1** online), which may follow the peak in abundance of mRORγ bound to RRE at zeitgeber time of ∼14–18 (ref. 4). These findings suggest that the repressor-precedes-activator mechanism in E/E′-box and RRE regulation might delay transcriptional activity.

The predicted period of D-box activity in the mouse liver matches previously reported protein abundances of D-box activators, in contrast to the correspondence observed in E/E′-box and RRE activity. For example, D-box activity is expected to reach a maximum at ∼CT11.0 (**Supplementary Fig. 1** online), which almost matches the peak in abundance of mDBP at ∼CT12 (ref. 17). On the basis of these findings and an idea previously suggested by others[17], we hypothesized that a repressor-antiphasic-to-activator mechanism in D-box regulation might generate high-amplitude transcriptional activity.

To test these hypothetical design principles, we implemented repressor-precedes-activator and repressor-antiphasic-to-activator models *in silico*. For the sake of comparison, we also implemented an activator-precedes-repressor model. Results of these *in silico* analyses showed that the repressor-precedes-activator mechanism could generate delayed transcriptional activity in clock-controlled transcriptional regulation (**Fig. 5b**), whereas the activator-precedes-repressor model generated advanced transcriptional activity (**Fig. 5c**). In the repressor-precedes-activator model, the advance in gene

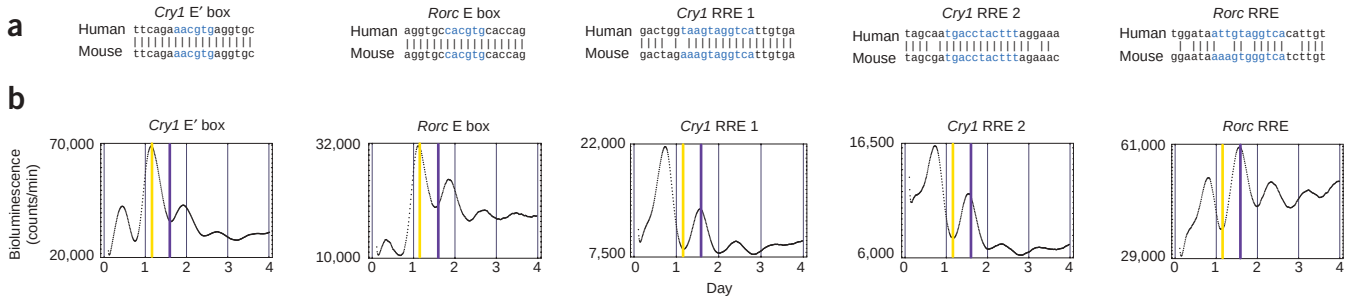


**Figure 4** Distinct roles of E/E′ boxes and RREs in noncoding regions of *Cry1* and *Rorc*. (**a**) Evolutionary conservation of E/E′ boxes and RREs in noncoding regions of *Cry1* and *Rorc*. (**b**) Representative circadian rhythms of bioluminescence from wild-type E/E′ boxes and RREs fused to the SV40 basic promoter driving a dLuc reporter. The phases of circadian oscillations from E/E′ boxes were 28.0 ± 0.2 h (*Cry1* E′ box, $n = 3$, mean ± s.e.m.) and 26.3 ± 0.2 h (*Rorc* E box, $n = 3$), which were in phase with levels of circadian luminescence from the *Per2* promoter (27.7 ± 0.1 h, $n = 3$, yellow lines; see also **Supplementary Fig. 3** online). On the other hand, the phases of circadian oscillations from RREs were 37.6 ± 0.4 h (*Cry1* RRE 1, $n = 3$), 37.3 ± 0.2 h (*Cry1* RRE 2, $n = 3$) and 37.9 ± 0.2 h (*Rorc* RRE, $n = 3$), which were in phase with levels of circadian luminescence from the *Arntl* promoter (38.3 ± 0.3 h, $n = 3$, purple lines; see also **Supplementary Fig. 3** online). See also **Supplementary Figure 7** online for confirmation of E/E′ boxes and RREs by transfection assays and EMSAs.

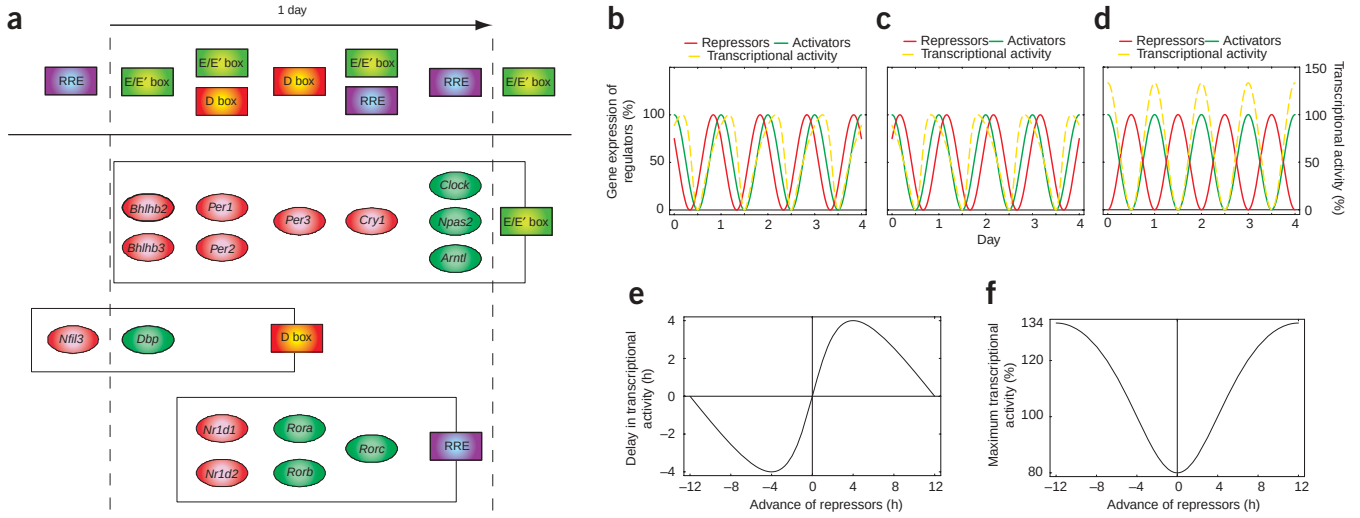


**Figure 5** Simple design principles encoded in the complex transcription circuits underlying mammalian circadian clocks. (**a**) Temporal organization of 16 transcriptional regulators realized, at least in part, by distinct clock-controlled elements. We classified 16 transcriptional regulators into three groups: E/E′-box regulators (*Bhlhb2*, *Bhlhb3*, *Per1*, *Per2*, *Per3*, *Cry1*, *Clock*, *Npas2* and *Arntl*), D-box regulators (*Nfil3* and *Dbp*) and RRE regulators (*Nr1d1*, *Nr1d2*, *Rora*, *Rorb* and *Rorc*). Transcriptional activators (green circles) and repressors (red circles) are indicated. We next categorized them into five groups according to their upstream regulation: regulated by E/E′ boxes, regulated by both E/E′ boxes and D boxes, regulated by D boxes, regulated by both E/E′ boxes and RREs and regulated by RREs. (**b**–**d**) *In silico* analysis of repressor-precedes-activator (**b**), activator-precedes-repressor (**c**) and repressor-antiphasic-to-activator (**d**) mechanisms. Gene expression of transcriptional activators (green lines) and repressors (red lines) and transcriptional activity (yellow dashed lines) are indicated. (**e**,**f**) Delay in transcriptional activity (**e**) and maximum transcriptional activity (**f**) plotted against advance in gene expression of a repressor relative to that of an activator. A 4-h advance in gene expression of a repressor generates a 4-h delay in transcriptional activity (**e**). A 12-h advance or delay in gene expression of a repressor generates the highest-amplitude transcriptional activity (**f**).

expression of the repressor closely matched the delay in transcriptional activity (**Fig. 5e**). In contrast, the repressor-antiphasic-to-activator model generated the highest-amplitude transcriptional activity (~133%; **Fig. 5d**,**f**), which was ~1.67 times higher than the lowest amplitude (80%).

Both mechanisms might be important in circadian clock function, as delayed transcription or translation negative-feedback loops have been proposed to generate circadian oscillations[1,2], and high-amplitude transcriptional oscillations have been observed in the circadian expression of key regulators in diverse physiological and metabolic processes[9,10]. We also note that some genes have at least two different types of functional clock-controlled elements, such as *Cry1* and *Rorc*, which have both E/E′-boxes and RREs. Although the biological importance of such combinatorial regulation remains to be investigated in depth, combinatorial regulation of two or more different type of clock-controlled elements might serve to generate new phases of circadian gene expression, as the phases of gene expression of *Cry1* and *Rorc* occur in the middle of the expected phases of E/E′-box and RRE activity (**Fig. 4** and **Supplementary Fig. 1** online).

We next focused on circuit structures in this transcriptional network. Given functional and conserved transcriptional regulation, we could diagram their transcriptional circuits (**Fig. 6a**). This circuit diagram gives an overall picture of the complex integrated transcriptional circuits underlying mammalian circadian clocks. These models indicate the potential for as many as 80 positive-feedback loops and 80 negative-feedback loops composed of up to three genes, and the potential for as many as 89 coherent feedforward loops[28] and 358 incoherent feedforward loops[28] composed of three genes in the circadian transcriptional circuits (**Supplementary Note** and **Supplementary Fig. 2** online).

Topological analysis of these complex transcriptional circuits suggests that E/E′-box regulation may be the Achilles' heel of the entire transcriptional network (**Fig. 6a**), because E/E′-box regulation is the most highly connected node in the circuit diagram; 9 of the 16 genes have E/E′ boxes in their promoter or enhancer regions, and nine transcriptional factors participate in E/E′-box regulation. Highly connected nodes in biological networks are likely to be centrally involved in biological functions, which makes them vulnerable[29,30]. This led us to hypothesize that E/E′-box regulation has a vital role in the regulation of mammalian circadian clocks and that a disturbance of E/E′-box regulation would greatly affect the robustness of circadian rhythm.

To test this hypothesis, we overexpressed *Cry1*, the strongest repressor of E/E′-box regulation, in Rat-1 cultured fibroblasts and measured circadian rhythmicity as evidenced by the real-time activity of the *Per2* and *Arntl* promoters. Consistent with our predictions, we found that circadian rhythmicity of *Per2* and *Arntl* promoter activity was impaired in a dose-dependent manner (**Fig. 6b**, $n = 2$), whereas the activity of the SV40 basic promoter was not affected by overexpression of *Cry1* (**Fig. 6b**, $n = 2$). For the sake of comparison, we also overexpressed repressors of RRE regulation (*Nr1d1*) or D-box regulation (*Nfil3*). We found that overexpression of *Nr1d1* impaired circadian rhythmicity in *Arntl* promoter activity in a dose-dependent manner but did not affect *Per2* promoter activity (**Fig. 6d**, $n = 2$). Overexpression of *Nfil3*, however, did not impair circadian rhythmicity in either *Per2* or *Arntl* promoter activity (**Fig. 6c**, $n = 2$). Such different modes of effect cannot be explained by mere quantitative differences in the strength of these three repressors, indicating that there is some qualitative difference between E/E′-box, D-box and RRE regulation in circadian rhythmicity. The results of these topological and functional studies suggest that E/E′-box regulation

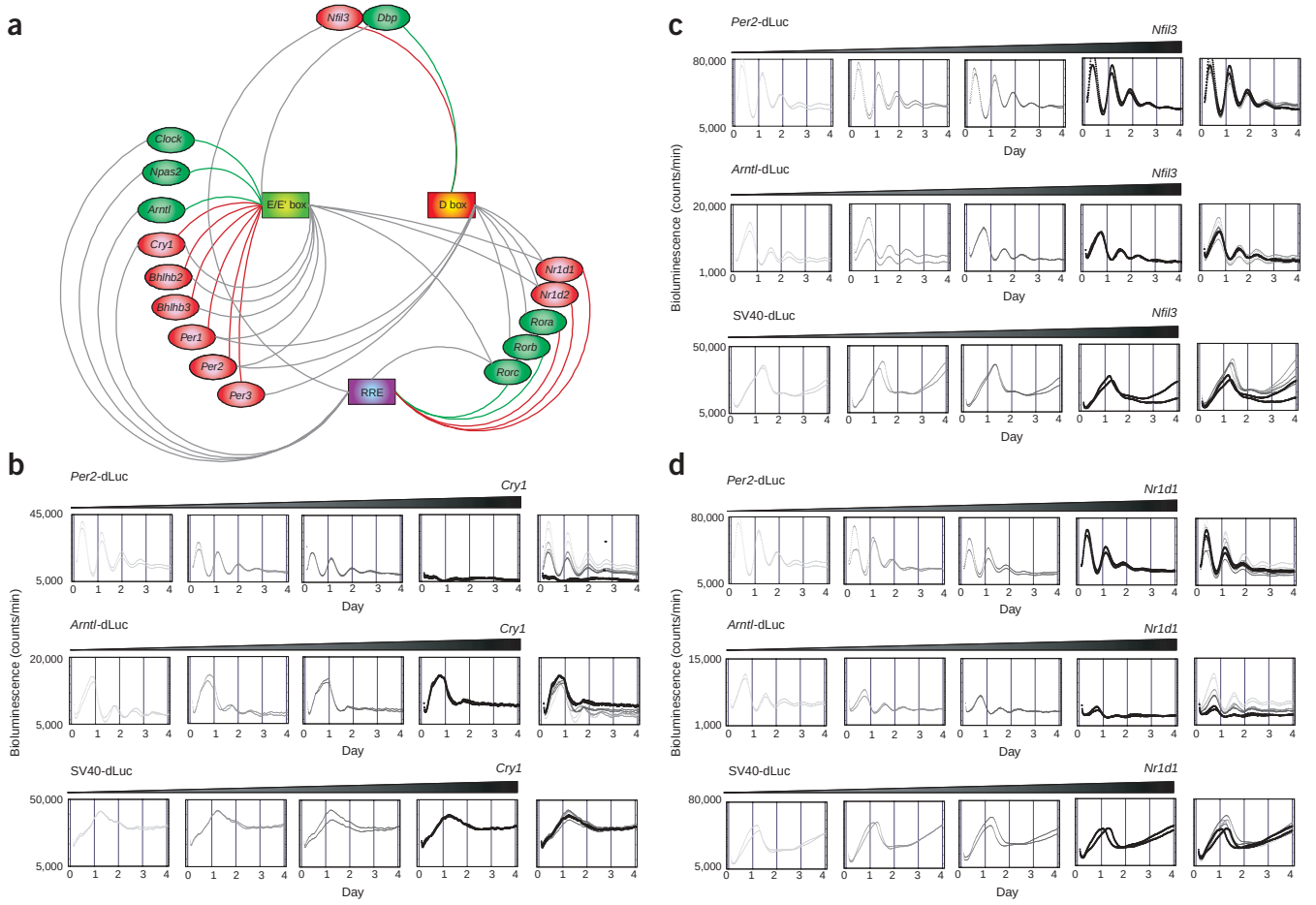


**Figure 6** Topological and functional identification of the Achilles' heel of transcriptional circuits underlying mammalian circadian clocks. (**a**) Topology of transcriptional circuits composed of 16 transcription factors underlying mammalian circadian clocks. Transcriptional activators (green circles) and repressors (red circles) and transcriptional activation and repression (gray lines) are indicated. For example, E/E′ boxes are located in promoters or enhancers of nine genes, D boxes are located in those of seven genes and RREs are located in those of six genes. E/E′ boxes are the most highly connected node in the transcriptional circuit diagram: nine genes have E/E′ boxes in their promoter or enhancer regions, and nine transcriptional factors can participate in E/E′-box regulation. (**b**–**d**) Bioluminescence from *Per2*, *Arntl* and SV40 basic promoter driving a dLuc reporter in the presence of 0, 0.1, 0.3 or 1.0 μg of *Cry1* (**b**), *Nfil3* (**c**) or *Nr1d1* (**d**) expression vectors.

has a crucial role, and is thus inherently vulnerable, in mammalian circadian systems.

This study presents a systems-biological approach to identifying design principles in the transcriptional regulation of mammalian circadian rhythms. Having identified numerous conserved and functional E/E′ boxes, D boxes and RREs in clock and clock-related genes, we formulated a pair of simple design principles: a repressor-precedes-activator mechanism in E/E′-box and RRE regulation and a repressor-antiphasic-to-activator mechanism in D-box regulation, encoded in the transcriptional circuits underlying mammalian circadian clocks. *In silico* models verified that these principles can generate delayed transcriptional activity and high-amplitude transcriptional activity, respectively. We further identified E/E′-box regulation as a structural keystone of mammalian circadian clocks. We have no doubt that there are additional degrees of complexity in these systems, where the regulation of clock function is achieved not only by transcriptional circuits, but also by signaling circuits involving the phosphorylation of clock proteins, chromatin modifications, clock protein stability and intracellular localization[10]. The challenge of identifying and characterizing systems in these signaling circuits lies ahead.

## METHODS

**Real-time monitoring of circadian transcriptional dynamics.** We grew Rat-1 cells in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Sigma). We plated $3.0 \times 10^5$ cells per dish in 35-mm dishes 24 h before transfection. We transfected cells with LipofectAMINE 2000 reagent (GIBCO) in accordance with the manufacturer's instructions. We transfected cells in each dish with 1 μg (total) of expression plasmids. After 68 h, we treated cells in each dish with 0.1 μM dexamethasone (Sigma) and, after 2 h, replaced the media with 2 ml of culture medium (DMEM supplemented with 10% FBS; Sigma) supplemented with 10 mM HEPES buffer (pH 7.2), 0.1 mM luciferin (Promega) and antibiotics (25 U $ml^{-1}$ penicillin and 25 μg $ml^{-1}$ streptomycin). We measured bioluminescence with photomultiplier tube detector assemblies (Hamamatsu).

***In silico* analysis of repressor-preceding-activator and repressor-antiphasic-to-activator mechanisms.** We formulated transcriptional activity at time $t$ regulated by competition between a clock-controlled activator and a repressor as follows:

$$T(t) \equiv \frac{\frac{A(t)}{K_a}}{1+\frac{A(t)}{K_a}+\frac{R(t)}{K_b}}$$

where $1/K_a$ and $1/K_b$ represent the strengths of an activator and a repressor, respectively. $A(t)$ and $R(t)$ represent gene expression of a clock-controlled

activator and repressor, which are defined as follows:

$$A(t) \equiv \alpha\left(1+Cos\left(2\pi\frac{t-a}{24}\right)\right)$$

and

$$B(t) \equiv \beta\left(1+Cos\left(2\pi\frac{t-b}{24}\right)\right),$$

where $\alpha$ and $\beta$ represent half amplitudes of gene expression of an activator and a repressor, and $a(-12 \leq a \leq 12)$ and $b(-12 \leq b \leq 12)$ represent phases of gene expression of an activator and a repressor. Advance $d$ in gene expression of a repressor from that of an activator is defined as follows: $d \equiv a - b$. For simplicity, we used $K_a = K_b = \alpha = \beta = \frac{1}{2}$ and $a = 0$ in the analysis. We used $b = -4$ for the repressor-precedes-activator mechanism (**Fig. 5a**), $b = 4$ for the activator-precedes-repressor mechanism (**Fig. 5c**) and $b = 12$ for the repressor-antiphasic-to-activator mechanism (**Fig. 5d**). For the analysis of transcriptional delay (**Fig. 5e**) and transcriptional activity (**Fig. 5f**), we changed the phase of a repressor within $-12 \leq b \leq 12$ and then measured the peak time (**Fig. 5e**) and peak level (**Fig. 5f**) of transcriptional activity $T(t)$.

***In vitro* circadian phenotype assay.** We grew Rat1 cells in DMEM supplemented with 10% FBS (Sigma). We plated $3.0 \times 10^5$ cells per dish in 35-mm dishes 24 h before transfection. We transfected cells with LipofectAMINE 2000 reagent (GIBCO) in accordance with the manufacturer's instructions. We transfected cells in each dish with 1.0 μg of *Per2*, *Arntl* or SV40 reporter plasmids in the presence of 0, 0.1, 0.3 or 1.0 μg of pCI-*Cry1*, pCMV-*Nfil3* or pCMV-*Nr1d1*. We used the pCI-neo or pCMV-Sport6 plasmids to adjust the amount of DNA (2.0 μg). After 68 h, we treated cells in each dish with 0.1 μM dexamethasone (Sigma) and, after 2 h, replaced the media with 2 ml of culture medium (DMEM supplemented with 10% FBS) supplemented with 10 mM HEPES buffer (pH 7.2), 0.1 mM luciferin (Promega) and antibiotics (25 U $ml^{-1}$ penicillin and 25 μg $ml^{-1}$ streptomycin). We measured bioluminescence with photomultiplier tube detector assemblies (Hamamatsu).

**Other materials and methods.** Detailed information on animals; quantitative PCR; transfection studies; protein synthesis; electrophoretic mobility shift assays (EMSAs); network analysis; construction of pCI-*Arntl*1, pCI-*Clock*, pCI-*Cry1*, pCMV-*Dbp*, pCMV-*Nfil3*, pCMV-*Rora* and pCMV-*Nr1d1* vectors; construction of dLuc, SV40-dLuc, m*Per2*-dLuc, m*Per3*-dLuc and m*Arntl*-dLuc reporter vectors; and construction of SV40-dLuc reporter vectors containing wild-type or mutant *cis* elements are available in **Supplementary Methods** online. Detailed information on possible feedback and feedforward loops, DNA probes used for EMSAs and the insertion sequences used for construction of wild-type or mutant SV40-dLuc reporters is available at the Database for Systems Biology.

**URLs.** The Database for Systems Biology is available at http://www.dbsb.org/.

*Note: Supplementary information is available on the Nature Genetics website.*

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**COMPETING INTERESTS STATEMENT**

The authors declare that they have no competing financial interests.

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