Supporting Information

Maywood et al. 10.1073/pnas.1220894110

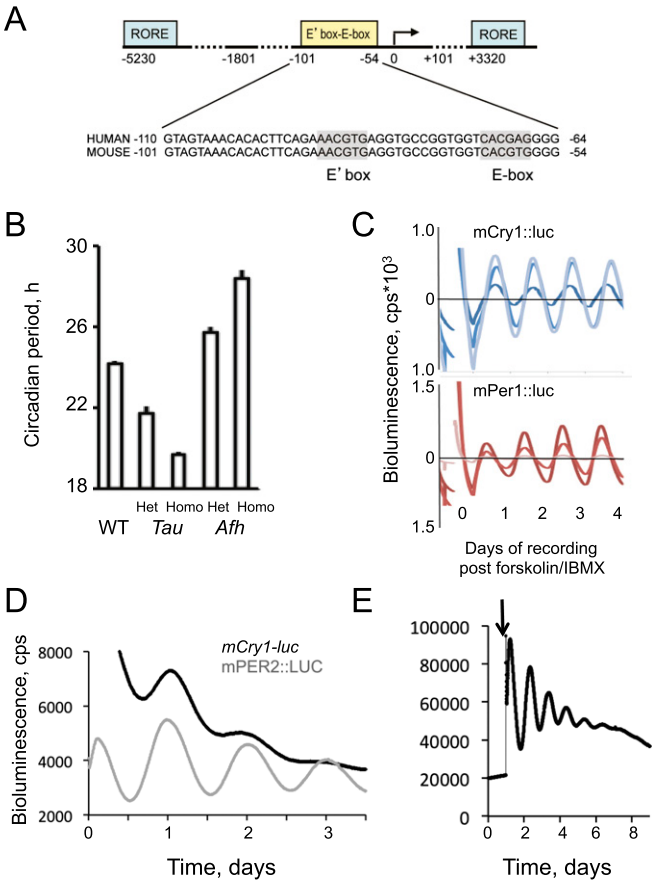


Fig. S1. Circadian *mCry1* reporter expressed in suprachiasmatic nucleus (SCN) and peripheral tissues. (*A*) Schematic of genomic *mCry1* region (−1,504 to +107) used to create circadian reporter, indicating location of included E/E′ boxes and RORE elements (not included). (*B*) Circadian period of SCN *mCry1-luc* bioluminescence rhythms from WT ($n = 28$), heterozygous ($n = 10$) and homozygous ($n = 6$) $CK1\varepsilon^{Tau}$, and heterozygous ($n = 3$) and homozygous ($n = 5$) $Fbxl3^{Afh}$ mice (group data; mean ± SEM). (*C*) Circadian bioluminescence rhythms of SCN from *mCry1-luc* and *mPer1-luc* mice ($n = 3$ for each) previously treated with forskolin/isobutyl-1-methylxanthine (IBMX) and then released to free run by transfer to fresh medium. Note synchronous oscillations of slices within a genotype and phase delay of *mCry1* slices relative to *mPer1* slices. (*D*) Representative bioluminescence recordings from kidney explants of *mCry1-luc* and mPER2::LUC mice. Note weaker definition in the former. (*E*) Representative bioluminescence recording from *mCry1-luc* MEFs. Culture was given a medium change (arrow) after the original rhythm had previously damped out after 8 d of recording.

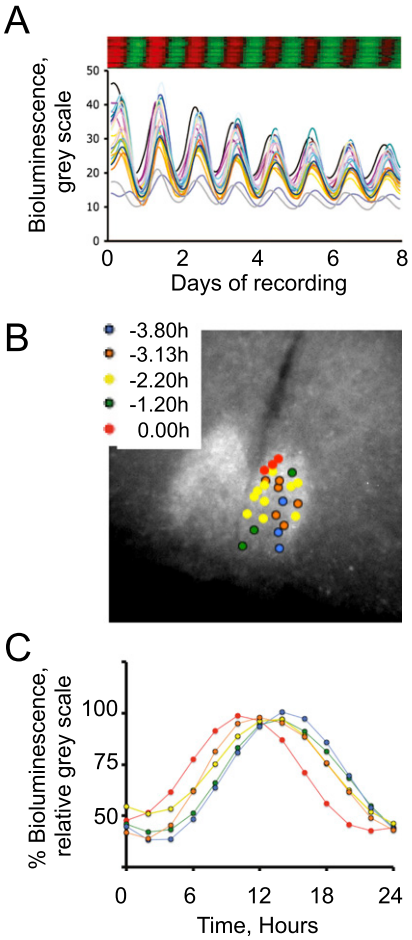


Fig. S2. Temporal and spatial patterns of cellular bioluminescence rhythms in *mCry1-luc* SCN. (*A*) Cellular rhythms recorded by CCD from representative SCN plotted graphically and as a raster plot (*Upper*) reveal tight synchrony and phase dispersion. (*B*) Location within SCN of representative groups of cells color-coded by phase of bioluminescence rhythm. Legend indicates mean phase of oscillation of each group in relation to the phase-leading cells at the dorsomedial lip of the SCN (red). (*C*) Twenty-four-hour profile of cellular bioluminescence (normalized to peak of 100% to facilitate comparison) of representative cells from color-coded phase clusters identified in *B*. Note dorsomedial-to-ventrolateral phase gradient.

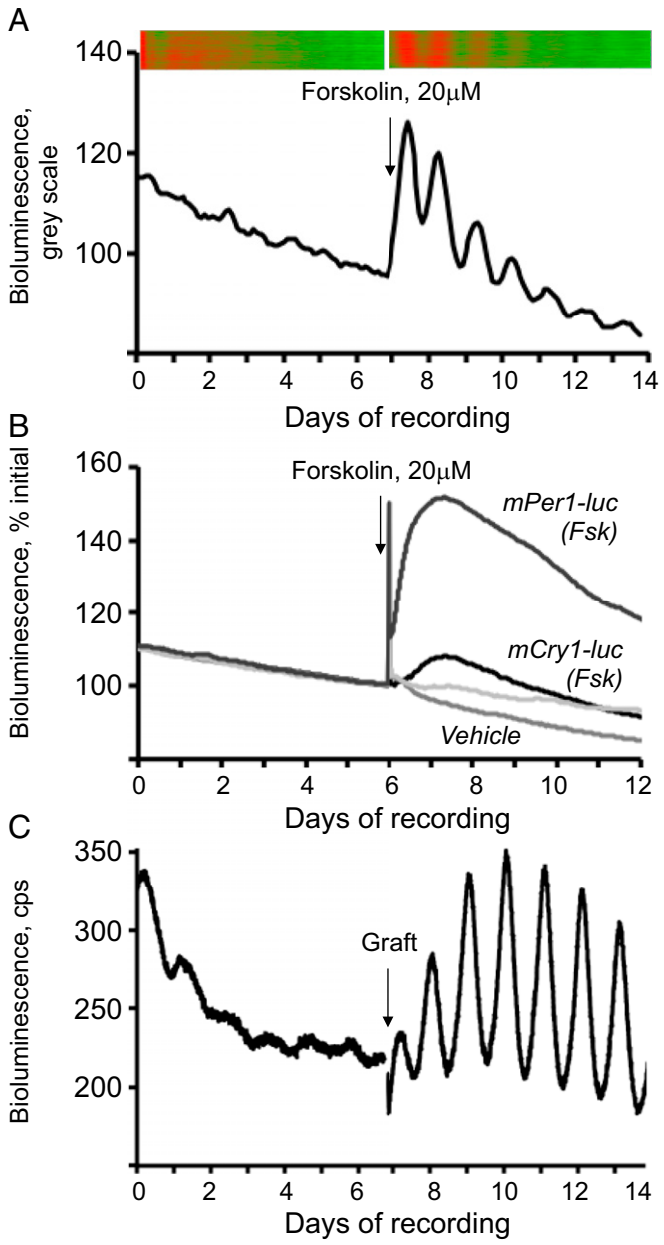


Fig. S3. Regulation of *mCry1* expression in SCN by extracellular cues. (*A*) Bioluminescence emission recorded by CCD of representative VPAC2-deficient *mCry1-luc* SCN treated with forskolin (20μM). (*Upper*) Raster plots of bioluminescence from cells before and after forskolin. (*B*) Bioluminescence emission from PER1/PER2-deficient SCN carrying *mCry1-luc* (black, medium gray) or *mPer1-luc* reporters (dark gray, light gray) and treated with vehicle ($n = 3$ and $n = 5$) or forskolin ($n = 6$ and $n = 5$). Data plotted as mean without SEM for clarity. SEMs were ≤10% of mean for all measures. (*C*) Bioluminescence emission from representative VIP-deficient *mCry1-luc* SCN that received a WT SCN graft at time indicated. Note damped rhythm before grafting and restoration of rhythm by WT SCN graft. (Before graft: amplitude, 26.25 ± 8.68; relative amplitude error (RAE), 0.140 ± 0.032; with graft amplitude, 75.45 ± 17.50; RAE, 0.044 ± 0.004; both $P < 0.05$ by paired *t* test; mean ± SEM; $n = 5$.)

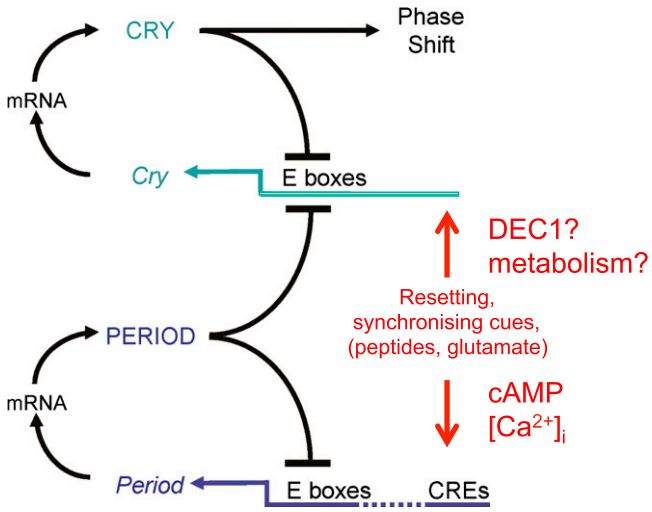


Fig. S4. Schematic model of interactions between extracellular signals and *Per* and *Cry1* expression. E-boxes are central to circadian expression of *Per* and *Cry*. In addition, resetting/synchronizing cues act upon CREs to regulate Per1 and Per2 expression, which in turn negatively regulate the *Cry* E-boxes, leading to complementary resetting of CRY expression. The shift to *Per* may be further stabilized by resynchronized CRY expression acting on *Per* E-boxes. This completes the coordinated phase shift/synchronization of the SCN molecular program and thereby directs shifts in behavioral and metabolic rhythms. Resetting cues can also act via PER1/PER2-independent pathways. These await identification but may involve metabolic signals and/or altered DEC-1 expression.

Table S1. Bioluminescence emissions from explants from mPER2::LUC and *mCry1-luc* reporter mice

Tissue	mPER2::Luc bioluminescence (n = 6)	*mCry1-luc* bioluminescence (n = 12)
Kidney	1,821 ± 416	1,914 ± 461 n.s.
Liver	1,031 ± 270	1,027 ± 255 n.s.
Lung	2,022 ± 559	1,295 ± 305 n.s.

Values presented as mean ± SEM. n.s., no significant difference vs. corresponding mPER2::LUC data by t test.

Table S2. Summary statistics for circadian bioluminescence rhythms recorded from mPER2::LUC and *mCry1-luc* reporter mice

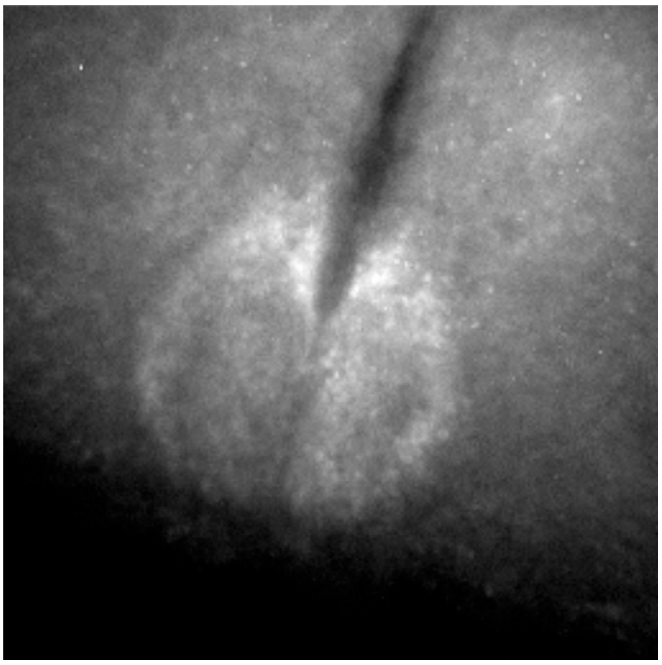
	mPER2::LUC			*mCry1-luc*		
Tissue	Period, h	Amplitude	RAE	Period, h	Amplitude	RAE
Kidney	24.4 ± 0.4 (6)	373 ± 115 (6)	0.10 ± 0.01 (6)	23.5 ± 1.1 n.s. (9)	100 ± 2** (9)	0.17 ± 0.03* (9)
Liver	24.0 ± 0.5 (6)	177 ± 61 (6)	0.13 ± 0.01 (6)	23.3 ± 1.6 n.s. (8)	72 ± 41 n.s.(8)	0.34 ± 0.06* (8)
Lung	25.1 ± 0.7 (6)	425 ± 132 (6)	0.09 ± 0.01 (6)	24.2 ± 0.8 n.s. (10)	65 ± 15** (10)	0.15 ± 0.01** (10)

Values presented as mean ± SEM. Values in parentheses are the numbers of explants. Note that three, four, and two explants of 12 total for each of kidney, liver, and lung from *mCry1-luc* mice failed to display a significant circadian rhythm by FFT analysis. All six mPER2::LUC explains were rhythmic. n.s., no significant difference vs. corresponding mPER2::LUC data by t test.
*P < 0.05, **P < 0.01 vs. corresponding mPER2::LUC data by t test.

Table S3. Summary statistics for circadian bioluminescence rhythms recorded from MEFs derived from mPER2::LUC and *mCry1-luc* reporter mice

Genotype	No.	Period, h	Amplitude	RAE
mCry1-luc	6	23.6 ± 0.1	1,513 ± 244**	0.123 ± 0.005
mPER2::LUC	4	23.6 ± 0.3	445 ± 72	0.128 ± 0.006

Values presented as mean ± SEM.
**P < 0.01 vs. corresponding mPER2::LUC data by t test.



Movie S1. Representative recording of bioluminescence emission from *mCry1-luc* organotypic SCN slice culture.

Movie S1