PER2 Mediates CREB-Dependent Light Induction of Per1

Andrea Brenna  
University of Fribourg

Jürgen A. Ripperger  
University of Fribourg

Gabriella Saro  
University of Fribourg

Dominique Glauser  
University of Fribourg

Zhihong Yang  
University of Fribourg

Urs Albrecht (✉ urs.albrecht@unifr.ch)  
University of Fribourg

---

Research Article

Keywords: Clock resetting, PER2, CRTC1, CBP, acetylation, FRET, SCN, WC-1

DOI: https://doi.org/10.21203/rs.3.rs-644410/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Light affects many physiological processes in mammals such as entrainment of the circadian clock, regulation of mood, and relaxation of blood vessels. At the molecular level, a stimulus such as light initiates a cascade of kinases that phosphorylate CREB at various sites, including serine 133 (S133). This modification leads CREB to recruit the co-factor CRCT1 and the histone acetyltransferase CBP to stimulate the transcription of genes containing a CRE element in their promoters, such as Period 1 (Per1). However, the details of this pathway are poorly understood. Here we provide evidence that PER2 acts as a co-factor of CREB to facilitate the formation of a transactivation complex on the CRE element of the Per1 gene regulatory region in response to light. Using in vitro and in vivo approaches, we show that PER2 modulates the interaction between CREB and its co-regulator CRTC1 to support complex formation only after a light or forskolin stimulus. Furthermore, the absence of PER2 abolished the interaction between the histone acetyltransferase CBP and CREB. This process was accompanied by a reduction of histone H3 acetylation and decreased recruitment of RNA Pol II to the Per1 gene. Collectively, our data show that PER2 supports the stimulus-dependent induction of the Per1 gene via modulation of the CREB/CRTC1/CBP complex. Remarkably, our results indicate that the molecular mechanism that transduces the light signal to the clock is similar to the one in the filamentous fungus Neurospora crassa to induce frequency (Frg). This suggests an evolutionarily conserved mechanism of this process despite the divergent sequences of the individual components.

Introduction

Light perception is one of the most important mechanisms that provoke biological responses in organisms, from resetting the circadian clock to cell division, metabolism, and redox state regulation 1–5. In mammals, light can entrain the circadian clock, regulate mood behaviors, and blood vessel relaxation 6–8. Light is perceived by the retina by intrinsically photosensitive retinal ganglion cells (ipRGCs) and transduced via the retinohypothalamic tract (RHT) to many different brain regions. For instance, in the suprachiasmatic nuclei (SCN) located above the optic chiasm in the ventral part of the hypothalamus, the signal is responsible for the release of neurotransmitters such as glutamate and PACAP 9. Light-evoked signals provoke Ca\(^{2+}\) influx in SCN neurons and activate a phosphorylation cascade, including protein kinase A (PKA), protein kinase G (PKG), Ca\(^{2+}\)/calmodulin-dependent protein kinase (CaMK), and mitogen-activated protein kinases (MAPK) also known as extracellular-signal-regulated kinases (ERK). This cascade finally promotes phosphorylation of cAMP response element-binding protein (CREB) at serine 133 and 142 10–12. CREB dimers recognize a specific motif of an 8-base pair palindromic sequence (TGACGTCA) called cAMP response element (CRE) present on regulatory regions of target genes such as Per1 13,14. In neurons, cAMP-regulated transcriptional co-activator 1 (CRTC1) is required for efficient induction of CREB target genes during neuronal activity 15,16. Upon stimulation of L-type voltage-gated calcium channels, CRTC1 is dephosphorylated by calcineurin. Subsequently, it translocates into the nucleus, where it interacts via its N-terminal CREB-binding domain (CBD) with CREB 17. Finally, the histone acetyltransferase CREB binding protein (CBP) binds to phospho-Ser133 CREB (pS133-CREB) 18.
The subsequent stabilization of the CREB: CBP complex stimulates the expression of target genes by acetylating nucleosomal histone H3 at lysine 27 (AcH3K27), followed by recruitment of RNA polymerase II \(^{19,20}\). Within the wide range of light-responsive genes, the clock gene \(\text{Per1}\) and the immediate-early gene \(\text{cFos}\) are targets of pS133-CREB dependent gene activation \(^{21-24}\).

Within the eukaryote domain, the filamentous fungus \(\text{Neurospora crassa}\) is the most used model system for studying light-inducible pathways, including entrainment and biosynthesis of photoprotective pigments, to name a few \(^{25-28}\). The NGF1: White Collar complex (WCC) regulates the light signaling system in this organism. The WCC consists of a heterodimer formed by the product of the \(\text{white collar-1 (wc-1)}\) and \(\text{white collar-2 (wc-2)}\) genes \(^{29-31}\). The blue light photoreceptor WC-1 interacts in the dark with the histone acetyltransferase NGF1 \(^{32,33}\) via an LXXLL consensus sequence, a known motif responsible for the interaction between nuclear receptors and their co-activators \(^{34}\). After a light pulse, WC-1 changes conformation and directs NGF1 to the chromatin to acetylate histones. At the same time, it stabilizes the transcriptional factor WC-2 on the promoter of target genes, and target gene expression is promoted \(^{35}\).

Structural similarities between the WC-1 and PER2 protein suggest that PER2 may be a functional homolog of WC-1 in mammals. Both proteins are similar in size and contain 3 PAS domains. They are both essential regulators of the circadian clock and can act as co-activators via LXXLL domains \(^{33,36}\). PER2 accumulates in the SCN between zeitgeber time (ZT) 14 and 16, which corresponds to the time window of light-responsiveness that leads to induction of genes such as \(\text{Per1}\) \(^{37}\). Since WC-1 is involved in light-dependent responses, and due to the resemblance of PER2 with the fungal protein, we wondered whether the mammalian clock factor would modulate the light-mediated CREB signaling pathway to induce genes such as \(\text{Per1}\) or \(\text{cFos}\) in a similar fashion. This resembles the function of WC-2, which is the transcription factor that modulates light responses in \(\text{N. crassa}\).

Here, we describe a novel function of PER2 as a mediator of the light-dependent CREB signaling in the early night. Using an \(\text{in vitro}\) (forskolin-induced cell lines) and \(\text{an in vivo}\) system (light-stimulated mice), we show that PER2 modulates the light/forskolin mediated assembly of the pSer133-CREB/CRTC1/CBP complex. In particular, we show that PER2 gates the CREB: CRCT1 complex to the early night. Förster resonance energy transfer (FRET) analysis revealed that the complex formation between CREB and CBP depends on the presence of PER2. Consequently, PER2 is important for the histone H3 acetylation at lysine 27 by CBP, and hence induction of \(\text{Per1}\) gene expression. We conclude that PER2 is a scaffold facilitating the formation of the CREB: CBP complex after a light pulse or forskolin stimulus. Our data also suggest functional homology between PER2 and WC-1 in the regulation of light dependent gene expression.

**Results**

\(\text{Per2}\) \textit{modulates light/forskolin-induced Per1 and cFos gene expression in an opposite manner}
A light pulse (LP) applied to mammals in the dark phase of a 12 h light:12 h dark cycle, where zeitgeber time (ZT) 0 is lights on and ZT12 is light off, elicits phase-shifts in locomotor activity\textsuperscript{10,38} paralleled by induction of \textit{Per1} gene expression\textsuperscript{6,39}. Here we measured the expression of pre-mRNA of \textit{Per1} and \textit{Per2} genes in the SCN of wild type (wt) mice in the SCN before and after an LP of 15 minutes duration at ZT14 (Fig. 1A). The pre-mRNA of \textit{Per1} was strongly induced after the LP, whereas the pre-mRNA of \textit{Per2} was significantly less responsive (Fig. 1A). To corroborate this observation \textit{in vitro}, we stimulated NIH3T3 cells with forskolin, which mimics the effect of phase-shifting \textit{via} activation of the Protein kinase A (PKA) signaling pathway\textsuperscript{40}. We observed that \textit{Per1}, but not \textit{Per2}, pre-mRNA was induced after 25 and 40 minutes of forskolin treatment (Fig. 1B). These results suggested that the \textit{Per2} gene is less inducible by light or forskolin than \textit{Per1}. Because \textit{Per2} KO mice do not phase delay after a light pulse at ZT14\textsuperscript{41}, our general question was whether \textit{PER2} was an upstream factor rather than a downstream target of stimulus-mediated clock resetting.

To challenge this hypothesis, we analyzed \textit{Per1} pre-mRNA expression in SCN samples obtained from wt and \textit{Per2} knock-out (KO) mice\textsuperscript{42} collected either in the dark or 15 min after LP at ZT14. We observed that, in \textit{Per2} KO mice, the light inducibility of \textit{Per1} is dampened compared to wt (Fig. 1C, S1A upper panels). Although \textit{Per2} pre-mRNA was less light-inducible compared to \textit{Per1}, it was not inducible in the \textit{Per2} KO mice (Fig. 1C, S1A lower panels). Subsequently, we analyzed \textit{Per1} and \textit{Per2} pre-mRNA expression in fibroblast cells derived from wt or \textit{Per2} KO animals. After forskolin treatment of the cells we observed induction of \textit{Per1} but not \textit{Per2} after 25 min. in wild-type cells (Fig. 1D, S1B, upper panels). In contrast, \textit{Per1} was not induced in \textit{Per2} KO cells, but \textit{Per2} was slightly increased after 40 min. (Fig. 1D, S1B, lower panels). These results are in line with the observation that in the SCN \textit{Per1} induction is affected by the loss of \textit{Per2}. Taken together, our observations suggest that \textit{Per2} is important for the regulation of the light- and forskolin-dependent induction of \textit{Per1} expression. Of note, other clock genes did not respond in the same way as \textit{Per1} after both light and forskolin stimuli, \textit{in vivo} as well as \textit{in vitro} (Fig. S1 C-D).

Because \textit{cFos} is an immediate-early gene (IEG) and can be induced by light in the SCN\textsuperscript{23,24}, we wanted to test whether \textit{cFos} induction may also be affected by the lack of \textit{Per2}. In the SCN, we observed an induction of \textit{c-Fos} after an LP in wt animals and surprisingly an even stronger induction in \textit{Per2} KO mice (Fig. 1E, S1E). Similarly, forskolin treatment caused stronger \textit{cFos} induction in fibroblasts after forskolin \textit{Per2} KO fibroblasts than in wt cells (Fig. 1F, S1F). These results suggest that \textit{Per2} plays an important role in regulating light/forskolin responsive genes, which is different from its role as a circadian regulator. Furthermore, \textit{Per2} can modulate light/forskolin responses in a positive (e.g., \textit{Per1}) or negative (e.g., \textit{cFos}) fashion.

**PER2 physically interacts with CREB and modulates its binding to a CRE element in the \textit{Per1} regulatory region**

Light and forskolin activate signaling pathways that lead to CREB phosphorylation at serine 133 (Ser-133) and subsequently evoke target gene expression such as \textit{cFos} and \textit{Per1}\textsuperscript{10–12}. The \textit{Per1} gene contains a CREB response element (CRE)\textsuperscript{14} (Fig. S2A) and is regulated by CREB\textsuperscript{14,43}. Since induction of
Per1 gene expression by light or forskolin is affected by the presence or absence of the Per2 gene (Fig. 1C, D), we set out to test whether the PER2 protein affects CREB binding to the Per1 promoter. For this purpose, we studied the CRE-element present in the Per1 regulatory region, including intron 1 (Fig. S2A) that was shown to be functional. We applied forskolin to wt and Per2 KO fibroblast cell lines and collected samples at various time points. Then, we performed chromatin immunoprecipitation (ChIP) using an antibody against CREB, followed by RT-qPCR to measure CREB occupancy at the CRE-element of Per1 and an unrelated element in the Per1 promoter as control (Fig. S2A). We found that forskolin modulated CREB occupancy at the intronic CRE-element of the Per1 gene in a time-dependent fashion with a peak at 25 min after the stimulus. On the other hand, this time-dependent change was absent in the control region (Fig. 2A, black columns). This transient CREB occupancy increase mirrored the Per1 pre-mRNA profile observed in Fig. 1B. Hence, it appeared that CREB was not recruited in a constant fashion to the Per1 promoter but bound the chromatin upon specific stimulation. Surprisingly, lack of Per2 led to an increase of CREB binding to the Per1 promoter already after 10 min (Fig. 2A, grey columns), but did not further increase after 25 min. This indicated that the two profiles of the occupancy of the regulatory region was different between the two genotypes (two-way ANOVA, p < 0.01). Taken together, these results indicate that PER2 modulates CREB recruitment onto the chromatin of Per1 in a stimulus-dependent manner.

Next, we asked whether PER2 could affect CREB recruitment to the Per1 gene regulatory region via modulation of CREB phosphorylation at serine 133 (Ser-133), which is associated with light or forskolin-induced phase shifts. Therefore, we performed a ChIP assay using the pSer133-CREB specific antibody as a bait. We observed that this phosphorylated form of CREB was recruited to chromatin in a similar manner as we observed when using the available CREB antibody for both wt (Fig. 2B, black columns) and Per2 KO (Fig. 2B, grey columns) derived chromatin. This result suggests that lack of Per2 did not alter the CREB binding profile to chromatin via changes in phosphorylation of Ser-133.

Above, we described that lack of the PER2 protein affected the recruitment dynamics of CREB to the CRE-element of the Per1 gene regulatory region. This effect could be of direct or indirect nature. In order to test this, we performed a ChIP assay on the same genomic region using a PER2 specific antibody. Our experiments revealed that the PER2 profile of recruitment to the chromatin (Fig. 2C) was similar to the one for CREB (Fig. 2A) with a maximum at 25 min after forskolin. Interestingly, the circadian clock component BMAL1 was not recruited to the Per1 CRE element in response to forskolin (Fig. S2B). This observation indicated that the recruitment of PER2 was not due to a general effect on the circadian clock.

Since CREB and PER2 show the same chromatin binding profile upon forskolin stimulation, we wondered whether they might physically interact. We stimulated fibroblast cells with forskolin and collected samples at various time points. Subsequently, we performed a Western blot (WB) and adjusted the protein loading to CREB accumulation. A proper forskolin induction was confirmed by monitoring CREB phosphorylation at serine 133, which was detected with a peak about 25 min after the forskolin stimulus (Fig. 2D left panel). An immunoprecipitation assay (IP) followed by WB revealed that PER2 binds CREB independently from the forskolin induction and that the interaction is specific (Fig. 2D, right panel, Fig.
Next, we investigated whether we could observe this interaction in vivo in SCN tissue of mice. We immunoprecipitated CREB from SCN protein extracts collected in the dark or 15 min after LP, followed by immunodetection. We observed that PER2 specifically binds CREB (Fig. S2D) and that this was independent of the LP and of CREB phosphorylation (Fig. 2E), as evidenced by the PER2 signal in the absence of the pSer133 signal in the dark. Our in vitro and in vivo data suggest that PER2 physically interacts with CREB and it does so independently from the stimulus (forskolin or light). Therefore, we hypothesize that this interaction influences CREB occupancy at the CRE element in the Per1 regulatory region.

**PER2 modulates CREB: CRTC1 interaction upon light or forskolin stimulation**

CREB occupancy of CRE elements in various promoters has been described to involve a family of factors called CRTCs. It has been demonstrated that in particular CRTC1 localized to the nucleus and dimerized with CREB after exposure to cAMP or calcium. Interestingly, CRTC1 is the main CREB co-activator regulating light responses in the SCN. Upon an LP, CRTC1 was observed to translocate into the nucleus to support CREB activity. These observations prompted us to investigate whether the light or forskolin mediated effect on the induction of the Per1 gene involved CREB: CRTC1 dimerization and whether PER2 modulated this complex.

We applied forskolin to wt and Per2 KO fibroblast cell lines and collected samples at various time points. Then, we performed chromatin immunoprecipitation (ChIP) using an antibody against CRTC1, followed by RT-qPCR to measure CRTC1 occupancy at the CRE-element of Per1 and an unrelated element in the Per1 promoter as control (Fig. 3A). We observed that in wt cells, CRTC1 recruitment to the Per1 intronic chromatin after forskolin treatment displayed a profile comparable to CREB (Fig. 2A, black columns) with a peak at 25 min after forskolin treatment (Fig. 3A, black columns). Conversely, in Per2 KO cells, the recruitment of CRTC1 to the Per1 promoter after forskolin application was reduced (Fig. 3A, grey column) and comparable to the CREB profile (Fig. 2A, grey columns). Thus, PER2 appeared to influence CREB and CRTC1 recruitment to the Per1 CRE element in a similar way.

Next, we investigated whether CRTC1 abundance was affected by the lack of PER2. The WB with samples obtained from wt and Per2 KO cell lines collected before and 25 min after forskolin stimulation did not show any difference in CRTC1 levels between the two genotypes. The induction mediated by forskolin increased CRTC1 amounts in both genotypes in a similar manner (Fig. 3B). Interestingly, the interaction between CREB and CRTC1 appeared to be increased by the forskolin stimulus, as shown by immunoprecipitation (IP) using an anti-CREB antibody as bait (Fig S3A). We wondered whether PER2 might affect CREB: CRTC1 interaction. IP with an anti-CRTC1 antibody as bait revealed that PER2 interacted with CRTC1 independently from the forskolin stimulus (Fig. 3C). Hence, PER2 binds to both CREB (Fig. 2D, E) and CRTC1 (Fig. 3C), although CREB and CRTC1 interact only after a stimulus (Fig. S3A). To test whether PER2 could modulate the CREB: CRTC1 interaction, we immunoprecipitated CREB in both wt and Per2 KO cell lines, without and 25 min after forskolin treatment. Surprisingly, CREB and CRTC1 interacted after this treatment only in wt cells. In contrast, in the Per2 KO cells (Per2−/−), the
interaction was stimulus-independent (Fig. 3D), as evidenced by the co-IP of both components before forskolin treatment.

Next, we investigated whether our observations in cells were also manifested in the SCN of mice. Similar to what we observed in the fibroblast cell line, CREB interacted with CRTC1 only after the light stimulus in SCN samples (Fig. S3B). Immunoprecipitation experiments showed that PER2 interacted with CRTC1 in a specific manner (Fig. S3C). This interaction was independent of an LP, as revealed when sample loading between the two conditions was adjusted to equal amounts of CRTC1 (Fig. 3E). Furthermore, light-induced CRTC1 accumulation was not affected by the lack of PER2 (Fig. 3F), and CREB was still phosphorylated at Ser-133 after the LP, as revealed by WB (Fig. 3F). Although CREB phosphorylation and CRTC1 accumulation were unaffected by PER2, the CREB: CRTC1 interaction was modulated by the clock factor (Fig. 3G) in a comparable manner as observed in cells (Fig. 3D) when a stimulus (light or forskolin) was applied. Additionally, CREB and CRTC1 interacted before the LP in Per2 KO mice (Fig. 3G) which was also observed in cells before the forskolin stimulus (Fig. 3D). Altogether these results define PER2 as a modulator of CREB: CRTC1 interaction. This modulation depends on specific triggers, such as forskolin for cell cultures or light for the SCN cells in animals.

**PER2 mediates CREB: CBP interaction upon light or forskolin stimulation**

CREB binding protein (CBP) is a histone acetyltransferase that can heterodimerize with CREB when CREB is phosphorylated at Ser-133. This CREB: CBP complex then promotes gene expression. To determine the CBP recruitment profile to the CRE-element of the Per1 regulatory region we performed a ChIP assay using an antibody against CBP, followed by RT-qPCR (Fig. 4A). In wt animals, recruitment of CBP to the CRE-element was very similar (Fig. 4A, left panel, black columns) to the profiles observed for CREB (Fig. 2A), pSer-133 CREB (Fig. 2B), PER2 (Fig. 2C), and CRTC1 (Fig. 3A). Interestingly, CBP appeared to bind to the control region in the Per1 promoter as well (Fig. 4A, right panel, black columns) possibly due to interaction with BMAL1, since this region contained a BMAL1 binding site (Fig. S2B). In contrast, in Per2 KO cells CBP did not bind to the CRE-element of Per1 and to a reduced extent to the control element after forskolin treatment compared to background signal at time point 0 (Fig. 4A, grey columns). These results suggested that PER2 affected CBP binding to both Per1 promoter regions.

Since we were interested in the CREB mediated regulation of the Per1 gene, we wondered how PER2 could affect CBP recruitment to the CRE element. We investigated whether PER2 could favor CBP nuclear localization. We tested the cells for CBP expression before and after forskolin treatment (Fig. 4B). We saw that CBP accumulation and localization in the nuclei were increased after forskolin treatment, but no distinct difference between the two genotypes was observed (Fig. 4B). We performed immunofluorescence on wt and Per2 KO fibroblasts to evaluate the specificity of our PER2 antibody (Fig. S4A). Next, we tested whether forskolin treatment induced Ser133 phosphorylation of CREB in our assay using an anti-pSer133 CREB antibody. The phosphorylation of CREB was induced as expected in both wt and Per2 KO cells 25 min after but not before forskolin treatment (Fig. S4B). Since, CBP can still accumulate in the nuclei after forskolin induction in the absence Per2, we investigated whether PER2
could affect the interaction between CBP and CREB. This interaction is essential for the CBP acetyltransferase activity on chromatin. Therefore, we immunoprecipitated CREB at 0 and 25 min after forskolin treatment of cells and performed IP using an antibody against CREB followed by WB (Fig. 4C). We observed that CBP was co-precipitated with CREB 25 min after forskolin treatment.

In contrast, this co-precipitation was not observed in Per2 KO cells (Fig. 4C). Subsequently, we wanted to see whether a Per2-dependent CREB: CBP interaction could also be observed in the SCN of mice after the LP stimulus. Immunohistochemistry on SCN tissue showed that the amount of CBP was increased after LP in both wt and Per2 KO tissue, indicating that lack of Per2 did not affect the increase of CBP levels in response to LP (Fig. 4D, control Fig. S4C). Next, we immunoprecipitated CREB from SCN extracts collected in the dark and after LP at ZT14 of both genotypes (Fig. 4E). Similar to the observation in cells (Fig. 4C), CBP was co-precipitated with CREB in wt extracts but not in extracts of Per2 KO mice. Taken together, our findings indicate that PER2 is necessary for the formation of the CREB: CBP complex after a stimulus such as forskolin or light pulse.

**FRET analysis indicates that PER2 supports the interaction of CREB and CBP domains**

The results presented above lend support to the notion that PER2 serves as a scaffold for CREB: CBP interaction. To further challenge this idea, we performed Förster resonance energy transfer (FRET) experiments, a widely used method to investigate molecular interactions between proteins such as CREB: CBP in living cells. We used a sensor called ICAP (an indicator of CREB activation due to phosphorylation). The sensor is composed of three different elements: 1) the KID domain of CREB containing the Ser-133, which is phosphorylated upon forskolin induction, 2) the KIX domain of CBP, which is responsible for the dimerization with phospho-CREB and 3) a short linker that separates the KID from the KIX domain. KID is flanked by a cyan fluorescent protein (CFP), while KIX is flanked by a yellow fluorescent protein (YFP). When KID is not phosphorylated at the resting phase, the ICAP conformation allows CFP to transfer energy to YFP, producing FRET resulting in yellow light emission. After a stimulus (forskolin), the serine in KID is phosphorylated and binds to KIX. The dimerization separates CFP from YFP, leading to decreased FRET resulting in blue light emission (Fig. 5A upper panel).

We hypothesized that depletion of the endogenous PER2 could affect the KID: KIX dimerization (Fig. 5A bottom panel). To test this hypothesis, we co-transfected ICAP and either scrambled (scr), or Per2 directed shRNA (shPer2) into NIH3T3 cells. Subsequently, we acquired the FRET signal. 3 min after forskolin treatment, which is the standard latency time for FRET signal detection, we noted that only about 10% of shPer2 transfected cells (Per2 knock-down (KD)) were responsive to the stimulus compared to about 90% of scr transfected cells (Fig. 5B). As evidenced by single-cell traces (Fig. S5A), most shPer2 transfected cells were not responsive, but some with a substantial delay, compared to the scr controls. These results suggested that the knock-down of Per2 affected the KID: KIX interaction. Quantification of the FRET signal acquired over 30 min from 50 cells showed a difference in the profiles of scr and shPer2 transfected cells (Fig. 5C, S5B). 50% of the scr control cells responded to the forskolin treatment within about 3 min, while shPer2 cells needed over 10 min (Fig. 5D). Additionally, the relative KID: KIX
dimerization was significantly higher in scr control cells compared to shPer2 cells up to around 20 min after the stimulus (Fig. 5E). Altogether these results reinforce our notion that PER2 may support the CREB: CBP dimerization via their respective KID: KIX domains.

**PER2 modulates CBP-mediated chromatin acetylation at lysine 27 of histone H3 after forskolin or light stimulation**

CBP binds to chromatin by dimerizing with pSer133-CREB to exert its histone acetyltransferase (HAT) activity. Lysine 27 (K27) of histone H3 is the main target of CBP HAT activity to acetylate (Ac) K27 of histone H3. We wondered whether this epigenetic modification (AcH3K27) could be observed on chromatin containing the Per1 Cre-element in cells after forskolin treatment. A ChIP assay, using an anti-AcH3K27 antibody for IP, followed by RT-qPCR revealed that chromatin remodeling at that specific amino acid of histone H3 is forskolin-dependent (Fig. 6A, black columns). We observed highest acetylation of histone H3 in the Per1 CRE-element containing chromatin between 25 and 40 min after stimulus application (Fig. 6A, black columns). Interestingly, acetylation was dramatically reduced in the Per2 KO cells (Fig. 6A, grey columns). This suggested that CBP needed PER2 as a co-factor for assembling a functional complex allowing acetylation of histone H3 on the Per1 CRE-element. This observation is the first evidence indicating that acetylation at K27H3 is a result of PER2-dependent forskolin stimulation.

Acetylation of histone H3 leads to the assembly of the RNA polymerase II (RNA Pol II) complex to promote gene transcription. Therefore, we tested whether the formation of such an RNA Pol II containing complex on the Per1 CRE-element was promoted by forskolin treatment of cells and whether PER2 may be involved in this process. We performed a ChIP assay using an antibody against RNA Pol II as bait. We noted that RNA Pol II recruitment to chromatin of wt cells (Fig. 6B, black columns) paralleled the acetylation dynamics of histone H3 on K27 (Fig. 6A, black columns). In contrast, the recruitment of RNA Pol II was lower in cells lacking Per2 (Fig. 6B, grey columns), mirroring the absence of acetylation of H3K27 (Fig. 6A, grey columns). These results provide additional evidence for the importance of PER2 as a facilitating component for assembling the stimulus-dependent transcriptional complex on the intronic CRE-element of the Per1 gene.

A previous study that described light-dependent histone phosphorylation in the SCN of mice prompted us to ask whether acetylation of histone H3 at K27 in the SCN was light-dependent. We applied an LP at ZT14 to mice as described before and subsequently collected SCN tissue. We performed an immunofluorescence assay using an anti AcH3K27 antibody. At ZT14 H3 in the SCN of wt mice displayed a basal level of chromatin acetylation, which was strongly increased after application of an LP (Fig. 6C upper row). In contrast the immunofluorescence signal was partially dampened in the SCN of Per2 KO mice (Fig. 6C lower row). To confirm this observation, we performed WB on pooled SCN tissue collected in the dark or 15 min after LP from both genotypes (Fig. S6A). Quantification confirmed that AcH3K27 was increased right after the light stimulus in wt SCN tissue, whereas the induction was blunted in SCN extracts obtained from Per2 KO mice (Fig. 6D). Together, these data suggested that the light signaling cascade did regulate chromatin acetylation and that PER2 modulated this process in the SCN.
Discussion

In the present study, we report a role of the clock protein PER2 as a modulator of the light-dependent CREB signaling in the early night. In particular, we observed that PER2 acted as a positive factor in stimulus dependent Per1 gene expression, while it functioned as a negative regulator in cFos gene induction (Fig. 1C-F). This functional dichotomy indicated that the transcriptional complex involving PER2 is not identical on the Per1 and cFos promoters. That PER2 can act as a positive or negative regulator is consistent with previous observations that this protein has a modulatory influence on transcriptional regulation in both directions in mammals $^{52,53}$. Because deletion of cFos in mice attenuated behavioral responses to light only marginally $^{54}$, we focused on the role of PER2 in the stimulus-dependent activation of the Per1 promoter. Since light activates several signaling pathways in mammals culminating in the induction of the Per1 gene $^{39,6}$, we investigated the role of PER2 as co-factor in CREB mediated transcription, a common downstream target of protein kinase cascades $^{18}$.

ChIP analysis on the intronic CRE-element of the Per1 gene (Fig. S2A) revealed that CREB and its known co-factor CRTC1 are recruited in a time-dependent manner after a forskolin stimulus with a peak around 25 min. However, although CREB and CRTC1 bound to the same region in the Per2 KO cell lines, their binding was less pronounced and occurred earlier, with a peak around 10 min (Fig. 2A, 3A). On the other hand, the histone acetyltransferase CBP and the RNA Pol II were recruited to the same element only in wt, but not in Per2 KO cells (4A, 6B). Our observations are in agreement with previous findings that describe CREB as an activator of Per transcription $^{14,55}$. Interestingly, PER2 could be recruited to the CRE-element of the Per1 promoter as well, with the same temporal profile as CREB (Fig. 2A, 2C). These results suggested that PER2 could be part of the CREB-containing transcriptional complex. The hypothesis was further corroborated by the observation of a discrete reduction and temporal profile of CREB and CRTC1 binding to the Per1 promoter in Per2 KO cells.

Immunoprecipitation experiments revealed that PER2 co-precipitated with CREB (Fig. 2D, E) and with CRTC1 (Fig. 3C, E) in a stimulus-independent manner, suggesting that PER2 could interact with both CREB and CRTC1 before the stimulus occurred (Fig. 7, left). Interestingly, CREB bound to CRTC1 independent of a stimulus when PER2 was absent, however in the presence of PER2 this interaction became stimulus-dependent (Fig. 3D, G). In contrast, interaction between CREB and CBP did not occur in absence of PER2, but appeared in presence of it in a stimulus-dependent fashion (Fig. 4C, E). Hence, a stimulus led to rearrangement of the components CREB, CRTC1 and CBP with PER2 most likely acting as a scaffold to facilitate this rearrangement (Fig. 7, right). Thus, we conclude that PER2 is the factor that mediates the stimulus-dependent build-up of the complex facilitating CRE element-dependent transcription. The limitation of our observation is, that we do not know whether this is a general mechanism or whether this mechanism is restricted to a subset of CRE elements that are flanked by specific uncharacterized regulatory sequences that are present in the Per1 gene. However, the conclusion that PER2 appears to be the stimulus-dependent factor that facilitates gene activation in a subset of CREB dependent target genes seems reasonable. A number of previous observations relate Per gene
activation to stimulus-dependent behavioral responses such as entrainment by light\textsuperscript{41}, response to drugs\textsuperscript{56–59}, and adaptation to temperature and humidity\textsuperscript{60}. The role of PER2 in the CREB complex appears not to be essential (Fig. 1C). However, PER2 increases the transcriptional activation potential of the CREB complex. Since PER2 protein is expressed in a circadian fashion the transcriptional potential of the CREB complex is guided to a particular time window and boosted. Hence, PER2 seems to be important to increase environmental signal transduction to particular times of the day.

Dynamic chromatin remodeling in the SCN has been suggested to occur in response to a physiological stimulus such as light\textsuperscript{51}. These findings are in line with our observation that stimulus-dependent activation of \textit{Per1} transcription involved the acetylation of histone H3 at lysine 27 and that this acetylation was depending on the presence of \textit{Per2} (Fig. 6A). The acetylation profile on the \textit{Per1} promoter was also mirrored by the recruitment of RNA Pol II and suggested a functional importance of this \textit{Per2} dependent H3K27 modification (Fig. 6B). The same mechanism is likely to be present in the SCN (Fig. 6C, D). Of note is the strong decrease of the magnitude of H3K27 acetylation in \textit{Per2} KO SCN (Fig. 6D). This can not be accounted for by an event happening only at the \textit{Per1} genomic locus. It is likely, that PER2 has a more widespread function to promote CBP action in order to acetylate additional genomic loci. Interestingly, light has been described to affect other epigenetic changes in the genome. For example, changes in day length affected the methylation pattern on chromatin in the SCN in a reversible manner\textsuperscript{61}. Taken together, it appears that stimuli such as light can affect gene expression via acetylation, methylation, or phosphorylation to modulate transcriptional responses in order to adapt the clock to the current environmental parameters (Fig. 7).

The molecular mechanism that regulates light-dependent responses described here appears to be different from \textit{drosophila}, but similar to the one described in \textit{Neurospora crassa}. In this organism, the transcriptional complex transducing the light signal is composed of WHITE COLLAR 1 (WC-1), WHITE COLLAR-2, and \textit{Neurospora} GCN5 like-1 (NGF-1)\textsuperscript{32,33}. After a light pulse, the large White Collar Complex (WCC) can be rearranged in a similar way as shown in the model we propose here for the mouse (Fig. 7).

The WCC can transiently bind the promoter of target genes in a time-dependent fashion with a peak around 15 min after stimulation\textsuperscript{62}. This dynamic is similar to the one observed here for the PER2: CREB complex (Fig. 2). In our model, the promoter occupancy of forskolin-dependent genes is transient with a peak around 25 min after the stimulus. Similarly, the WCC can be stabilized on the chromatin by the factor Submerged protoperithecia-1 (SUB1)\textsuperscript{63}. This parallels the role of CRTCl in our model (Fig. 7) in stabilizing CREB recruitment to the chromatin. WC-2 can be considered as a functional homolog of CREB. Both are light-associated transcription factors that require a platform for stabilization in order to bind to chromatin. WC-2 undergoes light-dependent phosphorylation steps in a time-dependent fashion\textsuperscript{64}, similar to CREB. However, the phosphorylation of WC-2 is more stable over time than the one of CREB.

Our results highlight many functional similarities between WC-1 and PER2 in the regulation of stimulus-mediated responses. The absence of a functional WC-1 affects the light-mediated gene expression\textsuperscript{33} in
the same way as PER2 does for Per1 (Fig. 1). WC-1 is necessary for WC-2 to be functional. Our results show that PER2 regulates the promoter occupancy of CREB. Without PER2, CREB can recognize the CRE elements to a lesser extent (Fig. 2A). Finally, PER2 modulates the interaction between CREB and CRTC1 to happen only after a stimulus. Interestingly, a similar role for WC-1 has been suggested for light-dependent responses. Lack of the Zinc Finger domain in WC-1 led to the light-independent activation of normally light-dependent pathways.

In many organisms, chromatin modifications can be stimulated after a signal. This usually involves specific co-activators such as histone acetyltransferases (HATs). CBP/P300, PCAF, and GCN5 appear to be the mediators in this process. In Neurospora crassa, the histone H3 acetylation at the lysine 14 was described as the first epigenetic marker associated with light-dependent responses. We observed a similar process in mice in this study. After forskolin/light stimulation, histone H3 was acetylated at lysine 27 (Fig. 6), and this acetylation depended in part on PER2. CBP/p300 targeted this specific lysine in a stimulus dependent manner. Hence, our results suggest that in mammals a similar epigenetic acetylation event occurs as in Neurospora. This view is further supported by our observation that PER2 mediated the physical interaction between CREB and the HAT CBP, which is necessary for CBP to acetylate the target histone (Fig. 4–6). Our observations suggest that even if CREB phosphorylation at Ser-133 is a "condition sine qua non" for CBP recruitment, it seems not to be sufficient if PER2 is not there. Lack of PER2 abolished CREB: CBP dimerization and was associated with less H3 Lys-27 acetylation in vivo (Fig. 6C, D), which mirrors the mechanism observed in Neurospora. Like CBP in mammals, the histone acetyltransferase NGF-1, a homolog of the mammalian GCN5, is involved in the light-dependent chromatin remodeling in Neurospora. After the stimulus, WC-1 starts to be phosphorylated and undergoes a conformational change. This event is responsible for NGF-1 recruitment onto the chromatin of target genes followed by histone H3 acetylation. Lack of WC-1 impairs NGF-1 recruitment to the chromatin, and as a consequence histone H3 acetylation is abolished after a light pulse. Taken together, our data suggest that light-mediated responses are conserved from the invertebrate Neurospora crassa, to vertebrates such as mice.

Materials And Methods

Animals and housing

Mice were housed with food and water ad libitum in transparent plastic cages (267 mm long x 207 mm wide x 140 mm high; Techniplast Makrolon type 2 1264C001) stainless-steel wire lid (Techniplast 1264C116), kept in light- and soundproof ventilated chambers. All mice were entrained to a 12:12 hour light:dark (LD) cycle, and the time of day was expressed as Zeitgeber time (ZT; ZT0 lights on, ZT12 lights off). Two- to four-month-old males were used for the experiments. Housing and experimental procedures were performed following the guidelines of the Schweizer Tierschutzgesetz and the declaration of Helsinki. The state veterinarian of the Canton of Fribourg and the national approved ethics committee approved the protocol. The study was carried out in compliance with the ARRIVE guidelines. The floxed
Per2 mice are available at the European Mouse Mutant Archive (EMMA) strain ID EM: 10599, B6;129P2-Per2tm1Ual/Biat.

**Light pulse and tissue isolation**

Light pulse (LP, circa 500 lux) was given at ZT14, and mice were subsequently sacrificed within 15 min. As a control experiment, mice were sacrificed in the dark at ZT14. Brains were collected and SCN tissue isolated. For immunofluorescence experiments, mice were perfused with 4% PFA.

**Cell culture**

Cell lines described in the paper were maintained in Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal calf serum (FCS) and 100 U/mL penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Forskolin stimulation (10-100M) was used to mimic *in vitro* the molecular pathway activated by light in mice. Samples were collected at specific time points mentioned in the text. The Per2 KO cell line was amplified starting from mouse embryonic fibroblasts (MEFs) and split every three days until immortalization was reached.

**Plasmids and transfection**

The following plasmids were used for the project:

- **ICAP-NLS**: Vector carrying the two domains KID (CREB) and KIX (CBP) fused with chromophores as described before, kindly provided by Prof. Dominique Glauser (University of Fribourg, CH).

- **Scramble Sh RNA**: vector used as a control for the in vitro silencing of Per2 (Origene cat #TR30021).

- **Sh RNA Per2**: Vector used for silencing Per2 in cell lines. (Origene cat #TL501620).

Sequence: 5'-ATGAGCAGTGGCTCCAGCGGAAACGAGAA-3'

NIH 3T3 cells were co-transfected with either ICAP-NLS/Scramble shRNA or ICAP_NLPS/shRNA Per2 in 2 cm dishes at about 70% of their total confluency using linear polyethyleneimine (LINPEI25; Polysciences Europe). The amounts of expression vectors were adjusted to yield comparable levels of the expressed protein. Thirty hours after transfection, cells were induced with forskolin, and the appropriate experiment was performed.

**RNA extraction from cells**

Cells were grown to confluency on 6 cm Petri dishes and induced with 10 µM forskolin (50 mM stock in dimethyl sulfoxide) for the indicated time. Total RNA was extracted using the Nucleospin RNA II kit (Machery & Nagel) and adjusted to 1 µg/µl with water. An amount of 1 µg was reverse-transcribed using Superscript II with random hexamer primers (Thermo Fisher). Real-time PCR was performed using the KAPA probe fast universal master mix and the indicated primers on a Rotorgene 6000 machine. The
relative expression was calculated compared to the geometric mean of expression of the inert genes Nono, SirT2, Atp5h, and Gsk3β 69. For a complete list of primers used in the paper, please see Table 1.

RNA extraction from the SCN

RNA from SCN samples was isolated using the Macherey-Nagel RNA Plus kit. Subsequently, 500 ng of purified RNA was used for producing cDNA by reverse transcription (Invitrogen SuperScript II). Real-time PCR was performed using the KAPA probe fast universal master mix and the indicated primers on a Rotorgene 6000 machine.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation from cells was performed as described before 70. Briefly, the cells were grown to confluency on 15 cm Petri dishes, induced with 10 µM forskolin (50 mM stock in dimethyl sulfoxide), and fixed at the indicated time with 1% formaldehyde/ 1x phosphate-buffered saline buffer (PBS) for 10 min at 37°C. Then the cells were washed twice with ice-cold 1x PBS and scraped in 1 ml of 10 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl ether)-N N' N'-tetraacetic acid (EGTA), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.6, 0.2% Triton X-100 for 5 min on ice and the obtained nuclei washed with 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl, 10 mM HEPES, pH 7.6. The purified nuclei were sonicated in 2 mM EDTA, 150 mM NaCl, 20 mM Tris, pH 7.5. 1% SDS using a BRANSON SLPe sonicator with a 4C15 tip for 6 cycles of 10 s each kept on ice, and diluted 1:10 with 2 mM EDTA, 150 mM NaCl, 20 mM Tris, pH 7.5, 1.1% Triton X-100. Equal amounts of chromatin were incubated with the indicated antibodies for 1 h at RT. (Table 2) and the immune complexes were captured with protein A agarose fast-flow beads (Sigma-Aldrich) for 1 h at RT. The beads were washed with 2 mM EDTA, 150 mM, 20 mM Tris, pH 7.5, 0.1% SDS, 1% Triton X-100, then 2 mM EDTA, 500 mM, 20 mM Tris, pH 7.5, 0.1% SDS, 1% Triton X-100, then 2 mM, 250 mM LiCl, 20 mM Tris, pH 7.5, 0.5% Na-deoxycholate, 0.5% NP40 substitute, and finally 2 mM EDTA, 150 mM, 20 mM Tris, pH 7.5. DNA fragments were eluted, and the crosslinks reversed in 2 mM EDTA, 150 mM NaCl, 20 mM Tris, pH 7.5, 1% SDS at 65°C overnight. The DNA fragments were purified using a MinElute PCR fragment purification kit (Qiagen). Real-time PCR reactions were performed using the KAPA probe fast universal master mix and the indicated primers on a Rotorgene 6’000 machine. The efficiency of the precipitations was calculated by comparing the amount of precipitated material to 1% of the starting material.

Protein extraction from cells.

Total confluent cells plated in 10 cm dishes were washed two times with 1x PBS (137 mM NaCl, 7.97 mM Na₂HPO₄ × 12 H₂O, 2.68 mM KCl, 1.47 mM KH₂PO₄). Then PBS was added again, and plates were kept for 5 min at 37°C. Cells were detached and collected in tubes and frozen in liquid N₂. They were subsequently resuspended in Ripa buffer (50 mM Tris-HCl pH7.4, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS, 150 mM NaCl, 2 mM EDTA, 50 mM NaF) with freshly added protease or phosphatase inhibitors, and homogenized by using a pellet pestle. Homogenates were kept in ice for 15 min followed by sonication
(10 s, 15% amplitude). Right after, the samples were centrifuged for 15 min at 16,100 g at 4°C. The supernatant was collected in new tubes and pellet discarded.

**Protein extraction from SCN tissue**

Isolated SCNs obtained from 5 different mice were pooled together according to the specific condition (either dark or 15 min after the light pulse). The pooled tissues were frozen in liquid N\textsubscript{2} and resuspended in a brain-specific lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.25% SDS, 0.25% sodium deoxycholate, 1 mM EDTA). They were subsequently homogenized using a pellet pestle kept on ice for 30 min and vortexed five times for 30 s each time. The samples were sonicated (10 s, 15% amplitude) and subsequently centrifuged for 20 min at 16,000 g at 4°C. The supernatant was collected in new tubes and the pellet discarded.

**Immunoprecipitation**

Circa 400 µg of protein extract was diluted with the appropriate protein lysis buffer in a final volume of 250 µl and immunoprecipitated using the indicated antibody (ratio 1:50) at 4°C overnight on a rotary shaker. The day after, samples were captured with 50 µl of 50% (w/v) protein-A agarose beads (Roche) for 3 hr at 4°C on a rotary shaker. Before use, beads were washed three times with the appropriate protein buffer and resuspended in the same buffer (50% w/v). The beads were collected by centrifugation and washed three times with NP-40 buffer (100 mM Tris-HCl pH7.5, 150 mM NaCl, 2 mM EDTA, 0.1% NP-40). After the final wash, beads were resuspended in 2% SDS 10%, glycerol, 63 mM Trish-HCL pH 6.8, and proteins were eluted for 15 min at RT. Laemmli buffer was finally added, samples were boiled 5 min at 95°C and stored at -20°C.

**Western blot**

The indicated amount of protein was loaded onto 10% SDS-PAGE gel and run at 100 Volt for two hours. Once the migration was completed, we performed a semidry transfer (40 mA, 1 hour 30 s) using Hybond\textsuperscript{®} ECL™ nitrocellulose membranes followed by red ponceau staining (0,1 % of Ponceau S dye and 5% acetic acid) to validate the success of the transfer. The membrane was subsequently washed with TBS 1x/Tween 0.1% and blocked with TBS 1x/Milk 5%/Tween 0.1% for 1 hour. After washing, the membrane was stained with the appropriate primary antibodies (Table 2) overnight. The day after, membranes were washed three times with TBS 1x/Tween 0.1% followed by secondary antibody immunoblotting for 1 hour at room temperature. The densitometric signal was digitally acquired with the Azure Biosystem.

**Immunofluorescence**

Mice were cardiovascually perfused with 0.9% NaCl followed by 4% PFA and brains were collected. They were subsequently cryoprotected by 30% sucrose solution, and sections of SCN were obtained (30 µm, coronal) using a cryostat. Selected sections were washed three times with 1x TBS (0.1 M Tris/0.15 M
NaCl) followed by 2x SSC (0.3 M NaCl/0.03 M tri-Na-citrate pH 7). Antigen retrieval was performed with 50% formamide/2x SSC by heating to 65°C for 50 min followed by washing 2x SSC and three times in 1x TBS pH 7.5. Slices were blocked for 1.5 hr in 10% fetal bovine serum (Gibco)/0.1% Triton X-100/1x TBS at RT.

After the blocking, the primary antibodies (Table 2) were added to the sections and incubated overnight at 4°C. The next day, sections were washed with 1x TBS and incubated with the appropriate fluorescent secondary antibodies diluted 1:500 in 1% FBS/0.1% Triton X-100/1x TBS for 3 hr at RT. (Lot: 132876, Alexa Fluor647-AffiniPure Donkey Anti-Mouse IgG (H+L) no. 715–605–150, Lot: 131725, Alexa Fluor647-AffiniPure Donkey Anti-Rabbit IgG (H+L) no. 711–602–152, Lot: 136317 and all from Jackson Immuno Research). Tissue sections were stained with DAPI (1:5000 in PBS; Roche) for 10 min. Finally, the tissue sections were rewashed twice in 1x TBS and mounted on glass microscope slides. Fluorescent images were taken using a confocal microscope (Leica TCS SP5), and pictures were taken with a magnification of 10x, 20x, or 63x. Images were processed with the Leica Application Suite Advanced Fluorescence 2.7.3.9723, according to the study by 71.

Live FRET imaging

Transfected NIH3T3 cells were starved for 4 hours with 0.5% FBS DMEM. After starvation (0.5% FBS in DMEM), the medium was removed, and cells were washed twice with 1x HBTS without CaCl$_2$ and MgCl$_2$ (Hepes 25 mM, NaCl 119 mM, Glucose 6gr/L, KCl 5mM) gently to avoid cell disturbance. One plate at the time was imaged. Therefore, the medium was changed shortly before the imaging started. NIH3T3 cells were imaged using an inverted epifluorescence microscope (Leica DMi6000B) with an HCX PL Fluotar 5x/0.15 CORR dry objective, a Leica DFC360FX CCD camera (1.4M pixels, 20 fps), and EL6000 Light Source, and equipped with fast filter wheels for FRET imaging. Excitation filters for CFP and FRET: 427 nm (BP 427/10). Emission filters for CFP: 472 (BP 472/30) and FRET: 542 nm (BP 542/27). Dichroic mirror: RCY 440/520. One frame every 20 seconds was acquired for at least 90 cycles (0.05 Hz frequency), and the recording lasted at least 30 min. This acquisition rate was ideal to avoid cellular photobleaching and phototoxicity during long recordings. The baseline response in the presence of HBTS only was recorded for 2 min and 40 s. At minute 3:00, 100 µM Forskolin, 2 mM CaCl$_2$, and 2 mM MgCl$_2$ were added to the cells. To avoid buffer perturbations due to the addition of the drug stimulus, the latter was added between frames, so it had approximately 20 s to diffuse and activate a response in the cells.

FRET imaging analysis

The time-lapse recordings were analyzed using LAS X software (Leica), adapting a previously described method 72. Briefly, two regions of interest (ROI) were selected for each cell, and 50 cells per plate were chosen randomly. A first ROI delimiting the background and a second ROI including the cell nucleus of NIH3T3 cell expressing NLS KIDKIX were used per cell. For each channel, the ROIbackground values were subtracted from the ROIconcell values. For baseline normalization, the FRET ratio R was expressed as a $\Delta R/R$, where $\Delta R$ is R-R0 and R0 is the average of R over the last 120 s prior stimulus.
Statistical analysis

Statistical analysis of all experiments was performed using GraphPad Prism8 software. Depending on the type of data, either an unpaired t-test with Welch's correction or a paired t-test, when a sample was set to 1, were performed. Two-way ANOVA was performed on time and genotype-dependent experiments. Values considered significantly different are highlighted, *p<0.05, **p<0.01, or ***p<0.001.

Table 1. List of primers used in the paper (5' to 3'; FAM: 6-fluorescin, BHQ1: black hole quencher 1).
| Name of the primer         | Sequence       | Use                                           |
|---------------------------|----------------|-----------------------------------------------|
| Per1_ChIP_CREB_FW         | **FW:** CAG CCT CCC TGC CCC ACA TT            | Amplification of a region within the first intron containing a CRE element |
|                           | **RV:** GAG AGG GAG GTG ACG TCA A AG C     |                                               |
|                           | **TM:** FAM-CCA GCT GCC TCG CCC CG C T-BHQ1 |                                               |
| Per1_ChIP_control         | **FW:** AGG CAC CAG AAA CCT CTT G          | Amplification of a region within the promoter |
|                           | **RV:** GGC GTA GAT CTG ACA GGC TA          |                                               |
|                           | **TM:** FAM-TGC CAG AGT CTC CAA AGT ATG CCC AC-BHQ1 |                                               |
| pPer1                     | **FW:** GGC ATG ATG CTG CTG ACC ACG         | Precursor of *Per1* mRNA used for the R.T.-qPCR |
|                           | **RV:** GGT GGG GAT GGG CTC TGT GA          |                                               |
|                           | **TM:** FAM-TGC CCC TCC CTC ACC TTA GCC TCC T-BHQ1 |                                               |
| pPer2                     | **FW:** CAC CCA CCC ACC CAC GTG AT          | Precursor of *Per2* mRNA used for the R.T.-qPCR |
|                           | **RV:** GGC TGG GAA CTC GCA CTT CCT T      |                                               |
|                           | **TM:** FAM-CCC TCG TGC AGG TAC CTG GAG AGC C BHQ1 |                                               |
| pcFos                     | **FW:** GTG AAG ACC GTG TCA GGA GGC A      | Precursor of *cFos* mRNA used for the R.T.-qPCR |
|                           | **RV:** CCC AGC CCA CAA AGG TCC AGA A      |                                               |
|                           | **TM:** FAM-AGC GCA GAG CAT CGG CAG AAG GGC C-BHQ1 |                                               |
| pBmal1                    | **FW:** GAT CCG AGT GCG GGT GCG            | Precursor of *Bmal1* mRNA used for the R.T.-qPCR |
|                           | **RV:** CGC AGC CAT GCC GAC ACT CA         |                                               |
|                           | **TM:** FAM- CGG GCG CTC GCA GCG AGC CA-BHQ1 |                                               |
| pCry1                     | **FW:** CTG GTT CGC CGG CTC TTC CA         | Precursor of *Cry1* mRNA used for the R.T.-qPCR |
|                           |                                             |                                               |
| Gene          | Forward Primer (FW) | Reverse Primer (RV) | TaqMan Probe (TM) | Description                                             |
|--------------|---------------------|---------------------|-------------------|---------------------------------------------------------|
| Cry2         | GAC ACC CGA CTC GCG CAC A | GGG AGG CTC CAG AGC CAA AGA | FAM-AGG TGG CGG TGA GTC CGA AGC GCT-BHQ1 | Precursor of Cry2 mRNA used for the R.T.-qPCR          |
| Clock        | TGC TAC TGC CCT GTG GGC TT | GTG CCA AGC CAG GTT CTG A | FAM-CGG CCG ACG TAC AGA CCC CAG TGG-BHQ1 | Precursor of Clock mRNA used for the R.T.-qPCR         |
| Gsk3β        | CCA CCT CCT TTG CGG AGA GC | CTG TGG TTA CCT TGC TGC CAT CT | FAM-TGC AAG CCA GTG CAG CAG CCT TCA GCT-BHQ1 | Probe for Gsk3β mRNA used as a normalizer for R.T.-qPCR |
| Atp5h        | TGC CCT GAA GAT TCC TGT GCC T | ACT CAG CAC AGC TCT TCA CAT CCT | FAM-TCT CCT CCT GGT CCA CCA GGG CTG TGT-BHQ1 | Probe for Atp5h mRNA used as a normalizer for R.T.-qPCR |
| Sirt2        | CAG GCC AGA CGG ACC CCT TC | AGG CCA CGT CCC TGT AAG CC | FAM- TGA TGG GCC TGG GAG GTG GCA TGG A-BHQ1 | Probe for Sirt2 mRNA used as a normalizer for R.T.-qPCR |
| Nonos        | TCT TTT CTC GGG ACG GTG GAG | GTC TGC CTC GCA GTC CTC ACT | FAM-TGG GCC TGG GAG GTG GCA TGG A-BHQ1 | Probe for Nonos mRNA used as a normalizer for R.T.-qPCR |
Table 2. List of antibodies used in the paper and relative applications.

| Product name | Catalog reference | Application  | Dilution |
|--------------|-------------------|--------------|----------|
| Rabbit mab Per2 | PER21-A Alpha Diagn. (Lot # 869900A1.2-L) | ChIP | 1:5 |
|               |                   | Western Blot | 1:1000   |
|               |                   | Immunofluorescence | 1:200   |
| Rabbit mab CREB | D76D11 Cell signaling | ChIP | 1:500   |
|               |                   | Western Blot | 1:1000   |
| Rabbit mab CREB (phospho S133) | ab194687 Abcam | ChIP | 1:5 |
|               |                   | Western Blot | 1:1000 |
|               |                   | Immunofluorescence | 1:100 |
| Rabbit mab TORC1 (CRTC1) | ab264144 Abcam | ChIP | 1:5 |
|               |                   | Western Blot | 1:1000 |
| Mouse mab Lamin B1 | sc-374015 SCBT | Western Blot | 1:1000 |
| Rabbit mab CBP | PA5-27369 Thermofish. | ChIP | 1:5 |
|               |                   | Western Blot | 1:1000 |
|               |                   | Immunofluorescence | 1:200 |
| Rabbit mab Acetyl-Histone H3 (Lys27) | D5E4 Cell signaling | ChIP | 1:5 |
|               |                   | Western Blot | 1:1000 |
|               |                   | Immunofluorescence | 1:100 |

Declarations

Acknowledgments

We thank T. Martini for characterizing the shRNA against Per2, A. Hayoz and S. Aebischer for technical support. Support by the Swiss National Science Foundation (310030_184667/1) and the State of Fribourg are gratefully acknowledged.

Author Contributions
Conceived and designed the experiments: AB, JR, UA.

Performed the experiments: AB, GS, JR.

Analyzed the data: AB, GS, JR, UA.

Contributed reagents, materials, analysis tools: DG, ZY, UA

Wrote the paper: AB, UA

Edited the manuscript: GS, JR, DG, ZY

**Competing financial interests**

The authors declare no competing financial interests

**References**

1. Dodd, A. N. *et al.* Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage., *309*, 630–633 https://doi.org/10.1126/science.1115581 (2005).

2. Schmitz, O., Katayama, M., Williams, S. B., Kondo, T. & Golden, S. S. CikA, a bacteriophytochrome that resets the cyanobacterial circadian clock., *289*, 765–768 https://doi.org/10.1126/science.289.5480.765 (2000).

3. McClung, C. R. Circadian Rhythms in Plants. *Annu Rev Plant Physiol Plant Mol Biol*, *52*, 139–162 https://doi.org/10.1146/annurev.arplant.52.1.139 (2001).

4. Corrochano, L. M. Fungal photoreceptors: sensory molecules for fungal development and behaviour. *Photochem Photobiol Sci*, *6*, 725–736 https://doi.org/10.1039/b702155k (2007).

5. Ivleva, N. B., Bramlett, M. R., Lindahl, P. A. & Golden, S. S. LdpA: a component of the circadian clock senses redox state of the cell. *EMBO J*, *24*, 1202–1210 https://doi.org/10.1038/sj.emboj.7600606 (2005).

6. Shigeyoshi, Y. *et al.* Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript., *91*, 1043–1053 https://doi.org/10.1016/s0092-8674(00)80494-8 (1997).

7. Bedrosian, T. A. & Nelson, R. J. Timing of light exposure affects mood and brain circuits. *Transl Psychiatry*, *7*, e1017 https://doi.org/10.1038/tp.2016.262 (2017).

8. Sikka, G. *et al.* Melanopsin mediates light-dependent relaxation in blood vessels. *Proc Natl Acad Sci U S A*, *111*, 17977–17982 https://doi.org/10.1073/pnas.1420258111 (2014).

9. Hannibal, J., Moller, M., Ottersen, O. P. & Fahrenkrug, J. PACAP and glutamate are co-stored in the retinohypothalamic tract. *J Comp Neurol*, *418*, 147–155 (2000).

10. Meijer, J. H. & Schwartz, W. J. In search of the pathways for light-induced pacemaker resetting in the suprachiasmatic nucleus. *J Biol Rhythms*, *18*, 235–249
11. Ginty, D. D. et al. Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock. *Science*, **260**, 238–241 https://doi.org/10.1126/science.8097062 (1993).

12. Gau, D. et al. Phosphorylation of CREB Ser142 regulates light-induced phase shifts of the circadian clock. *Science*, **34**, 245–253 https://doi.org/10.1016/s0896-6273(02)00656-6 (2002).

13. Lonze, B. E. & Ginty, D. D. Function and regulation of CREB family transcription factors in the nervous system. *Science*, **35**, 605–623 https://doi.org/10.1126/science.1082396 (2002).

14. Travnickova-Bendova, Z., Cermakian, N., Reppert, S. M. & Sassone-Corsi, P. Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. *Proc Natl Acad Sci USA*, **99**, 7728–7733 https://doi.org/10.1073/pnas.102075599 (2002).

15. Saura, C. A. & Cardinaux, J. R. Emerging Roles of CREB-Regulated Transcription Coactivators in Brain Physiology and Pathology. *Trends Neurosci*, **40**, 720–733 https://doi.org/10.1016/j.tins.2017.10.002 (2017).

16. Parra-Damas, A., Rubio-Ferrarons, L., Shen, J. & Saura, C. A. CRTC1 mediates preferential transcription at neuronal activity-regulated CRE/TATA promoters. *Sci Rep*, **7**, 18004 https://doi.org/10.1038/s41598-017-18215-y (2017).

17. Mair, W. et al. Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. *Nature*, **470**, 404–408 https://doi.org/10.1038/nature09706 (2011).

18. Mayr, B. & Montminy, M. Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol*, **2**, 599–609 https://doi.org/10.1038/35085068 (2001).

19. Kim, T. K., Kim, T. H. & Maniatis, T. Efficient recruitment of TFIIB and CBP-RNA polymerase II holoenzyme by an interferon-beta enhanceosome in vitro. *Proc Natl Acad Sci USA*, **95**, 12191–12196 https://doi.org/10.1073/pnas.95.21.12191 (1998).

20. Kee, B. L., Arias, J. & Montminy, M. R. Adaptor-mediated recruitment of RNA polymerase II to a signal-dependent activator. *J Biol Chem*, **271**, 2373–2375 https://doi.org/10.1074/jbc.271.5.2373 (1996).

21. Jagannath, A. et al. The CRTC1-SIK1 pathway regulates entrainment of the circadian clock. *Science*, **154**, 1100–1111 https://doi.org/10.1016/j.cell.2013.08.004 (2013).

22. Tischkau, S. A., Mitchell, J. W., Tyan, S. H., Buchanan, G. F. & Gillette, M. U. Ca2+/cAMP response element-binding protein (CREB)-dependent activation of Per1 is required for light-induced signaling in the suprachiasmatic nucleus circadian clock. *J Biol Chem*, **278**, 718–723 https://doi.org/10.1074/jbc.M209241200 (2003).

23. Rusak, B., Robertson, H. A., Wisden, W. & Hunt, S. P. Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus. *Science*, **248**, 1237–1240 https://doi.org/10.1126/science.2112267 (1990).

24. Kako, K., Wakamatsu, H. & Ishida, N. c-fos CRE-binding activity of CREB/ATF family in the SCN is regulated by light but not a circadian clock. *Neurosci Lett*, **216**, 159–162 https://doi.org/10.1016/0304-3940(96)13018-4 (1996).
25. Arpaia, G., Loros, J. J., Dunlap, J. C., Morelli, G. & Macino, G. Light induction of the clock-controlled gene ccg-1 is not transduced through the circadian clock in Neurospora crassa. *Mol Gen Genet*, **247**, 157–163 https://doi.org/10.1007/BF00705645 (1995).

26. Harding, R. W. & Turner, R. V. Photoregulation of the Carotenoid Biosynthetic Pathway in Albino and White Collar Mutants of Neurospora crassa. *Plant Physiol*, **68**, 745–749 https://doi.org/10.1104/pp.68.3.745 (1981).

27. Harding, R. W. & Melles, S. Genetic Analysis of Phototropism of Neurospora crassa Perithecial Beaks Using White Collar and Albino Mutants. *Plant Physiol*, **72**, 996–1000 https://doi.org/10.1104/pp.72.4.996 (1983).

28. Lauter, F. R. & Russo, V. E. Blue light induction of conidiation-specific genes in Neurospora crassa. *Nucleic Acids Res*, **19**, 6883–6886 https://doi.org/10.1093/nar/19.24.6883 (1991).

29. Ballario, P. et al. White collar-1, a central regulator of blue light responses in Neurospora, is a zinc finger protein. *EMBO J*, **15**, 1650–1657 (1996).

30. Ballario, P., Talora, C., Galli, D., Linden, H. & Macino, G. Roles in dimerization and blue light photoresponse of the PAS and LOV domains of Neurospora crassa white collar proteins. *Mol Microbiol*, **29**, 719–729 https://doi.org/10.1046/j.1365-2958.1998.00955.x (1998).

31. Linden, H., Ballario, P. & Macino, G. Blue light regulation in Neurospora crassa. *Fungal Genet Biol*, **22**, 141–150 https://doi.org/10.1006/fgbi.1997.1013 (1997).

32. Grimaldi, B. et al. The Neurospora crassa White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by NGF-1. *Mol Biol Cell*, **17**, 4576–4583 https://doi.org/10.1091/mbc.e06-03-0232 (2006).

33. Brenna, A., Grimaldi, B., Filetici, P. & Ballario, P. Physical association of the WC-1 photoreceptor and the histone acetyltransferase NGF-1 is required for blue light signal transduction in Neurospora crassa. *Mol Biol Cell*, **23**, 3863–3872 https://doi.org/10.1091/mbc.E12-02-0142 (2012).

34. Savkur, R. S. & Burris, T. P. The coactivator LXXLL nuclear receptor recognition motif. *J Pept Res*, **63**, 207–212 https://doi.org/10.1111/j.1399-3011.2004.00126.x (2004).

35. Proietto, M., Bianchi, M. M., Ballario, P. & Brenna, A. Epigenetic and Posttranslational Modifications in Light Signal Transduction and the Circadian Clock in Neurospora crassa. *Int J Mol Sci*, **16**, 15347–15383 https://doi.org/10.3390/ijms160715347 (2015).

36. Schmutz, I., Ripperger, J. A., Baeriswyl-Aebischer, S. & Albrecht, U. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. *Genes Dev*, **24**, 345–357 https://doi.org/10.1101/gad.564110 (2010).

37. Nam, H. J. et al. Phosphorylation of LSD1 by PKCalpha is crucial for circadian rhythmicity and phase resetting. *Mol Cell*, **53**, 791–805 https://doi.org/10.1016/j.molcel.2014.01.028 (2014).

38. Daan, S. & Pittendrigh, C. S. A functional analysis of circadian pacemakers in nocturnal rodents II. The variability of phase response curves. *J. Comp. Physiol*, **106**, 253–266 (1976).

39. Albrecht, U., Sun, Z. S., Eichele, G. & Lee, C. C. A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light., **91**, 1055–1064 https://doi.org/10.1016/s0092-
40. Yagita, K. & Okamura, H. Forskolin induces circadian gene expression of rPer1, rPer2 and dbp in mammalian rat-1 fibroblasts. *FEBS Lett.*, **465**, 79–82 https://doi.org/10.1016/s0014-5793(99)01724-x (2000).

41. Albrecht, U., Zheng, B., Larkin, D., Sun, Z. S. & Lee, C. C. MPer1 and mper2 are essential for normal resetting of the circadian clock. *J Biol Rhythms*, **16**, 100–104 https://doi.org/10.1177/074873001129001791 (2001).

42. Chavan, R. *et al.* Liver-derived ketone bodies are necessary for food anticipation. *Nat Commun*, **7**, 10580 https://doi.org/10.1038/ncomms10580 (2016).

43. Motzkus, D., Albrecht, U. & Maronde, E. The human PER1 gene is inducible by interleukin-6. *J Mol Neurosci*, **18**, 105–109 https://doi.org/10.1385/JMN:18:1-2 (2002).

44. Wheaton, K. L. *et al.* The Phosphorylation of CREB at Serine 133 Is a Key Event for Circadian Clock Timing and Entrainment in the Suprachiasmatic Nucleus. *J Biol Rhythms*, **33**, 497–514 https://doi.org/10.1177/0748730418791713 (2018).

45. Altarejos, J. Y. & Montminy, M. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol*, **12**, 141–151 https://doi.org/10.1038/nrm3072 (2011).

46. Sakamoto, K. *et al.* Clock and light regulation of the CREB coactivator CRTC1 in the suprachiasmatic circadian clock. *J Neurosci*, **33**, 9021–9027 https://doi.org/10.1523/JNEUROSCI.4202-12.2013 (2013).

47. Garg, A. *et al.* Structural and mechanistic insights into the interaction of the circadian transcription factor BMAL1 with the KIX domain of the CREB-binding protein. *J Biol Chem*, **294**, 16604–16619 https://doi.org/10.1074/jbc.RA119.009845 (2019).

48. Friedrich, M. W., Aramuni, G., Mank, M., Mackinnon, J. A. & Griesbeck, O. Imaging CREB activation in living cells. *J Biol Chem*, **285**, 23285–23295 https://doi.org/10.1074/jbc.M110.124545 (2010).

49. De Cesare, D., Fimia, G. M. & Sassone-Corsi, P. Signaling routes to CREM and CREB: plasticity in transcriptional activation. *Trends Biochem Sci*, **24**, 281–285 https://doi.org/10.1016/s0968-0004(99)01414-0 (1999).

50. Jin, Q. *et al.* Distinct roles of GCN5/PCAF-mediated H3K9ac and CBP/p300-mediated H3K18/27ac in nuclear receptor transactivation. *EMBO J*, **30**, 249–262 https://doi.org/10.1038/emboj.2010.318 (2011).

51. Crosio, C., Cermakian, N., Allis, C. D. & Sassone-Corsi, P. Light induces chromatin modification in cells of the mammalian circadian clock. *Nat Neurosci*, **3**, 1241–1247 https://doi.org/10.1038/81767 (2000).

52. Akashi, M. *et al.* A positive role for PERIOD in mammalian circadian gene expression. *Cell Rep*, **7**, 1056–1064 https://doi.org/10.1016/j.celrep.2014.03.072 (2014).

53. Chiou, Y. Y. *et al.* Mammalian Period represses and de-represses transcription by displacing CLOCK-BMAL1 from promoters in a Cryptochrome-dependent manner. *Proc Natl Acad Sci U S A*, **113**, E6072–E6079 https://doi.org/10.1073/pnas.1612917113 (2016).
54. Honrado, G. I. et al. The circadian system of c-fos deficient mice. *J Comp Physiol A*, **178**, 563–570 https://doi.org/10.1007/BF00190186 (1996).

55. Hirota, T. & Fukada, Y. Resetting mechanism of central and peripheral circadian clocks in mammals. *Zoolog Sci*, **21**, 359–368 https://doi.org/10.2108/zsj.21.359 (2004).

56. Abarca, C., Albrecht, U. & Spanagel, R. Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proc Natl Acad Sci U S A*, **99**, 9026–9030 https://doi.org/10.1073/pnas.142039099 (2002).

57. Spanagel, R. et al. The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. *Nat Med*, **11**, 35–42 https://doi.org/10.1038/nm1163 (2005).

58. Dong, L. et al. Effects of the circadian rhythm gene period 1 (per1) on psychosocial stress-induced alcohol drinking. *Am J Psychiatry*, **168**, 1090–1098 https://doi.org/10.1176/appi.ajp.2011.10111579 (2011).

59. Gamsby, J. J. et al. The circadian Per1 and Per2 genes influence alcohol intake, reinforcement, and blood alcohol levels. *Behav Brain Res*, **249**, 15–21 https://doi.org/10.1016/j.bbr.2013.04.016 (2013).

60. Chappuis, S. et al. Role of the circadian clock gene Per2 in adaptation to cold temperature. *Mol Metab*, **2**, 184–193 https://doi.org/10.1016/j.molmet.2013.05.002 (2013).

61. Azzi, A. et al. Circadian behavior is light-reprogrammed by plastic DNA methylation. *Nat Neurosci*, **17**, 377–382 https://doi.org/10.1038/nn.3651 (2014).

62. He, Q. & Liu, Y. Molecular mechanism of light responses in Neurospora: from light-induced transcription to photoadaptation. *Genes Dev*, **19**, 2888–2899 https://doi.org/10.1101/gad.1369605 (2005).

63. Sancar, C. et al. Combinatorial control of light induced chromatin remodeling and gene activation in Neurospora. *PLoS Genet*, **11**, e1005105 https://doi.org/10.1371/journal.pgen.1005105 (2015).

64. Schwerdtfeger, C. & Linden, H. Localization and light-dependent phosphorylation of white collar 1 and 2, the two central components of blue light signaling in Neurospora crassa. *Eur J Biochem*, **267**, 414–422 https://doi.org/10.1046/j.1432-1327.2000.01016.x (2000).

65. Cheng, P., Yang, Y., Wang, L., He, Q. & Liu, Y. WHITE COLLAR-1, a multifunctional neurospora protein involved in the circadian feedback loops, light sensing, and transcription repression of wc-2. *J Biol Chem*, **278**, 3801–3808 https://doi.org/10.1074/jbc.M209592200 (2003).

66. Brenna, A. & Talora, C. WC-1 and the Proximal GATA Sequence Mediate a Cis-/Trans-Acting Repressive Regulation of Light-Dependent Gene Transcription in the Dark. *Int J Mol Sci*, **20**, https://doi.org/10.3390/ijms20122854 (2019).

67. Hebbar, P. B. & Archer, T. K. Chromatin remodeling by nuclear receptors., **111**, 495–504 https://doi.org/10.1007/s00412-003-0232-x (2003).

68. Gallwitz, D. & Sures, I. Histone acetylation. Purification and properties of three histone-specific acetyltransferases from rat thymus nuclei. *Biochim Biophys Acta*, **263**, 315–328 https://doi.org/10.1016/0005-2795(72)90085-2 (1972).
69. Fonseca Costa, S. S., Wegmann, D. & Ripperger, J. A. Normalisation against Circadian and Age-Related Disturbances Enables Robust Detection of Gene Expression Changes in Liver of Aged Mice. *PLoS One*, **12**, e0169615 https://doi.org/10.1371/journal.pone.0169615 (2017).

70. Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A. & Brown, M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *J. Cell Biol.*, **103**, 843–852 https://doi.org/10.1016/s0092-8674(00)00188-4 (2000).

71. Schnell, A. *et al.* The nuclear receptor REV-ERBalpha regulates Fabp7 and modulates adult hippocampal neurogenesis. *PLoS One*, **9**, e99883 https://doi.org/10.1371/journal.pone.0099883 (2014).

72. Saro, G. *et al.* Specific Ion Channels Control Sensory Gain, Sensitivity, and Kinetics in a Tonic Thermonociceptor. *Cell Rep* **30**, 397–408 e394, doi:10.1016/j.celrep.2019.12.029 (2020).

**Figures**
Figure 1

Induction of Per1, Per2, and cFos gene expression after light or forskolin stimulation. The diagram top left indicates the light treatment protocol of mice (A, C, E) and the top right diagram shows the forskolin treatment of cells (B, D, F). (A) Fold change of Per1 (black bars) and Per2 (grey bars) pre-mRNA expression in the SCN after a 15 min light pulse (LP) applied at ZT14. Values are the mean ± SEM. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. (B) Fold change of Per1 (black bars)
and Per2 (grey bars) pre-mRNA expression in NIH 3T3 cells after forskolin treatment. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. (C) Fold change of Per1 (top panel) and Per2 (bottom panel) pre-mRNA expression in the SCN of wild type (wt, black bars) and Per2 knock-out (KO, grey bars) mice after a 15 min. light pulse (LP) applied at ZT14. Values are the mean ± SEM. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. (D) Fold change of Per1 (top panel) and Per2 (bottom panel) pre-mRNA expression in immortalized wt or Per2 KO fibroblast cells after forskolin treatment. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. (E) Fold change of cFos expression in the SCN of wt (black bars) and Per2 KO (grey bars) mice after a 15 min light pulse (LP) applied at ZT14. Values are the mean ± SEM. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. (F) Fold change of cFos expression in immortalized wt or Per2 KO fibroblast cells after forskolin treatment. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05.
Figure 2

Binding of CREB and PER2 to the CRE-element of the Per1 promoter and interaction between CREB and PER2 (A) Top panel: Chromatin immunoprecipitation (ChIP) of CREB on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Bottom panel: Control ChIP on an unrelated promoter region of Per1. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. Two-way ANOVA indicates a significantly different
time profile between the two genotypes, ** p < 0.01. (B) Top panel: ChIP of pSer-133 CREB on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Bottom panel: Control ChIP on an unrelated promoter region of Per1. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05. Two-way ANOVA indicates a significantly different time profile between the two genotypes, ** p < 0.01. (C) Top panel: ChIP of PER2 on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Bottom panel: Control ChIP on an unrelated promoter region of Per1. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05. Two-way ANOVA indicates a significantly different time profile between the two genotypes, ** p < 0.01. (D) Western blot (left panel) was performed on 10% of the total input used for the immunoprecipitation using a CREB antibody as bait (right panel). PER2 co-precipitates with CREB in NIH 3T3 cell extracts, the pSer-133 antibody was used to confirm the forskolin induction. kD = kilo Dalton. (E) IP with an antibody recognizing CREB in SCN extracts, which co-precipitated with PER2 before (D) and after a 15 min light pulse (LP) at ZT14. The pSer-133 antibody was used to confirm light induction. Input control with lamin B (bottom panel), kD = kilo Dalton. * unspecific band recognized by the αPER2 antibody in tissue extracts.
Figure 3

Binding of CRTC1 to the Per1 promoter and interaction with PER2 and CREB (A) Left panel: Chromatin immunoprecipitation (ChIP) of CRTC1 on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Right panel: Control ChIP on an unrelated promoter region of Per1. Values are the mean ± SD. Student’s t-test with Welch’s correction, n = 3, * p < 0.05, ** p < 0.01. Two-way ANOVA indicates a significantly different time profile between the two
genotypes, ** p < 0.01. (B) Western blot of wt and Per2-/- cells before and 25 min. after forskolin treatment. CRTC1 is induced by forskolin independently of Per2. CREB is phosphorylated (pSer133) in both genotypes after the stimulus. (C) Immunoprecipitation (IP) of CRTC1 pulls down PER2 independently of forskolin treatment. (D) IP of CREB pulls down CRTC1 after forskolin treatment in wt cells, but in Per2-/- cells, the interaction between CREB and CRTC1 is forskolin independent. (E) IP of CRTC1 in SCN extracts before and after a 15 min light pulse (LP) at ZT14. Co-IP of PER2 is independent of LP. (F) Western blot of wt and Per2-/- SCN tissue performed as in B on samples collected before and after LP; * unspecifc band recognized by the αPER2 antibody in tissue extracts. CREB is phosphorylated in both genotypes after the light pulse. (G) IP of CREB pulls down CRTC1 in wt SCN extracts after LP, but in Per2-/- SCN the CREB: CRTC1 interaction is light-independent. kD = kilo Dalton.
Figure 4

Modulation of the interaction between CREB and CBP by PER2 (A) Left panel: Chromatin immunoprecipitation (ChIP) of CBP on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Right panel: Control ChIP on a promoter region of Per1 without a CRE-element. Values are the mean ± SD. Student’s t-test with Welch’s correction, n = 3, * p < 0.05, ** p < 0.01. Two-way ANOVA indicates a significantly different time profile between the
two genotypes, left panel: **** p < 0.0001, right panel: ** p < 0.01. (B) Immunohistochemistry (IHC) of wt and Per2/-/- cells before and after forskolin treatment. CBP (red signal) is induced after forskolin treatment in both genotypes. Scale bar: 17 µm. (C) IP of CREB pulls down CBP in wt but not Per2/-/- cells after forskolin treatment, kD = kilo Dalton. (D) IHC of wt and Per2/-/- SCN before and after a light pulse (LP) at ZT14. CBP (red signal) is induced after LP in both genotypes. Scale bar: 7 µm. (E) IP of CREB pulls down CBP in wt SCN extracts after LP, but not in Per2/-/- SCN extracts. kD = kilo Dalton.

Figure 5
FRET analysis of the interaction between PER2 and the KID/KIX domains of CREB/CBP (A) Top panel: Schematic diagram of the ICAP construct that is an indicator of CREB: CBP dimerization due to phosphorylation on the KID (CREB) domain at serine 133. Bottom panel: Proposed model of PER2 mediated interaction between the KID and KIX domains. (B) % of cellular response to forskolin after 3 min. in NIH 3T3 cells transfected either with scrambled (scr) shRNA or an shRNA directed against Per2 (shPer2). Values are the mean ± SD. Student’s t-test with Welch’s correction, n = 3, *** p < 0.001. (C) The FRET/CFP signal ratio changes in response to forskolin treatment in cells transfected with either an scr control (red) or shPer2 (blue) expressing construct. Values are the mean ± SD. Two-way ANOVA revealed a significant difference between the curves, n = 50, **** p < 0.0001. (D) Time elapsed to reach half of the total FRET/CFP signal is significantly shorter in scr control transfected cells than shPer2 transfected cells. Values are the mean ± SD. Student’s t-test with Welch’s correction, n = 50, * p< 0.05. (E) KID/KIX dimerization is significantly lower in shPer2 cells than scr control cells. Values are the mean ± SD. Two-way ANOVA, n = 50, * p < 0.05.
Modulation of CBP-mediated chromatin acetylation of histone H3K27 by PER2 (A) Chromatin immunoprecipitation (ChIP) of acetylated histone H3 (AcH3K27) on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, * p < 0.05, ** p < 0.01. Two-way ANOVA indicates a significantly different time profile between the two genotypes, ** p < 0.01. (B) The ChIP of RNA polymerase II (RNA Pol II) on the CRE-element of the Per1 promoter in wt (black bars) and Per2 KO (grey bars) fibroblast cell lines after forskolin treatment. Values are the mean ± SD. Student's t-test with Welch's correction, n = 3, ** p < 0.01. Two-way ANOVA indicates a significantly different time profile between the
two genotypes, *** p < 0.001. (C) Immunohistochemistry (IHC) on wt and Per2 KO SCN before and after a light pulse (LP) at ZT14. AcH3K27 (red signal) is stronger after LP in wt compared to Per2 KO SCN. Scale bar: 121 µm. (D) Quantification of 3 independent Western blot experiments of wt and Per2 KO SCN before and after an LP at ZT14. Acetylation of H3K27 is increased after an LP with a tendentially lower increase in Per2 KO. Two-way ANOVA, p = 0.059.

Figure 7

Model of PER2 modulating the assembly of the CREB/CRTC1/CBP transcriptional complex Left: In the early dark phase at ZT14, PER2 interacts with CREB and CRTC1. On the other hand, CREB and CRTC1 do not dimerize at this stage, and all the elements do not bind to the CRE element in the Per1 promoter. CBP is not recruited to the regulatory region of Per1 and the histone H3K27 is not acetylated. Per1 is not transcribed through this pathway. Right: In the SCN or in cells a light pulse or forskolin treatment initiates a kinase cascade that leads to CREB phosphorylation and assembly of the CREB: CRTC1: CBP complex which is facilitated by PER2. As a consequence of this assembly, histone H3K27 is acetylated (grey star) and the Per1 gene is transcribed. The hatched oval with question mark indicates that most likely additional factors contribute to the initiation of transcription by the RNA polymerase II (POLII).

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- SUPPLEMENTARYINFORMATION.docx