

Supporting information:

Computational model for the mammalian circadian clock

The model is schematized in Fig. 5. It incorporates the following molecular processes (in parentheses we give the symbols denoting the concentrations of the different variables that appear in the equations listed below):

- 1) Transcription of the *Per*, *Cry* and *Bmal1* genes into the corresponding mRNAs (denoted M_P , M_C , M_B , respectively) and degradation of these mRNAs. For simplicity, at this stage we do not distinguish between the *Per1*, *Per2*, *Per3* genes and represent them in the model by a single *Per* gene; similarly *Cry1* and *Cry2* are represented by a single *Cry* gene.
- 2) Translation of these mRNAs into the cytosolic, unphosphorylated proteins PER, CRY and BMAL1 (denoted by P_C , C_C , B_C).
- 3) Reversible phosphorylation of the PER, CRY and BMAL1 proteins (concentrations of the phosphorylated forms are denoted by P_{CP} , C_{CP} , B_{CP}).
- 4) In the cytosol, formation of the unphosphorylated PER–CRY complex (of concentration PC_C) and reversible phosphorylation of this complex (the concentration of the phosphorylated form is denoted by PC_{CP}).
- 5) Reversible entry of the cytosolic PER–CRY complex into the nucleus and reversible phosphorylation of the complex (concentrations of the nuclear forms of the unphosphorylated and phosphorylated complexes are denoted by PC_N and PC_{NP} , respectively).
- 6) Reversible entry of the cytosolic BMAL1 protein into the nucleus and reversible phosphorylation (concentrations of the nuclear forms of unphosphorylated and phosphorylated BMAL1 are denoted by B_N and B_{NP} , respectively).
- 7) In agreement with experimental observations, the expression of *Clock* is considered to be constitutive and to give rise to a high, constant level of cytosolic and nuclear CLOCK protein (1). We will not distinguish between the phosphorylated and unphosphorylated forms of CLOCK and will treat its constant level as a parameter. We assume that once in the nucleus, unphosphorylated BMAL1 immediately forms a complex with CLOCK (the concentration of this complex is that of nuclear BMAL1, i.e. B_N).
- 8) In the nucleus, the CLOCK–BMAL1 activates the transcription of the *Per* and *Cry* genes. By binding to the CLOCK–BMAL1 complex, the PER–CRY complex prevents this activation; such a regulation therefore amounts to indirect repression of the *Per* and *Cry* genes by their protein products (the concentration of the inactive complex between CLOCK–BMAL1 and PER–CRY is denoted I_N).
- 9) Experimental evidence indicates that PER2, and to a lesser degree CRY1 and CRY2, behave as activators of *Bmal1* transcription (1, 2). However, the precise mechanism of this regulation is not yet fully clarified. In analogy with the situation in *Drosophila*, we assume

that the positive feedback occurs indirectly and that CLOCK–BMAL1 represses the transcription of the gene *Bmal1*; the activating effect of PER2, CRY1 and CRY2 would be due to the removal of repression upon formation of the complex between PER–CRY and CLOCK–BMAL1.

10) The negative autoregulation exerted by BMAL1 on the expression of its gene was recently shown to be of indirect nature: BMAL1 promotes the expression of the *Rev-Erb α* gene and the REV-ERB α protein represses the expression of *Bmal1* (3). We shall first consider the regulatory effect of BMAL1 as a direct, negative autoregulation. In a second stage (see below), we shall consider explicitly the action of REV-ERB α in the regulation of *Bmal1* expression.

11) Although the proteins may be multiply phosphorylated (4), we will only consider a single phosphorylated state for PER, CRY, BMAL1 and the complex PER–CRY. We assume that these phosphorylated proteins are subject to degradation in the cytosol and in the nucleus. Degradation is also considered for the nuclear, unphosphorylated form of the complex I_N between PER–CRY and CLOCK–BMAL1; the introduction of a phosphorylation step prior to degradation of I_N would introduce an additional variable but does not change significantly the behavior of the model.

12) The present work deals with the dynamics of the model in conditions corresponding to continuous darkness or to light-dark cycles. The effect of light is to enhance transcription of the *Per* gene and is therefore incorporated into the model through the maximum rate of *Per* expression, denoted by v_{sp} in the model.

A family of closely related models can be built, based on the above assumptions. Here we focus on one particular implementation of this family of models. Alternative versions of the circadian clock model yielding largely similar results indeed exist. Thus, BMAL1 may form a complex with CLOCK before entering the nucleus, and complexes between CRY and PER or between CLOCK and BMAL1 may form when the various proteins are phosphorylated (4). Moreover, the CLOCK–BMAL1 complex seems to remain bound to DNA (4) so that its interaction with PER–CRY occurs on DNA.

Kinetic equations

The time evolution of the model of Fig. 5 is governed by the system of 16 kinetic equations [1]–[16]. For the sake of clarity, we have grouped these equations for the various mRNAs, the phosphorylated and nonphosphorylated proteins PER and CRY in the cytosol, the phosphorylated and nonphosphorylated PER–CRY complex in cytosol and nucleus, the phosphorylated and nonphosphorylated protein BMAL1 in the cytosol and nucleus, and the complex between PER–CRY and CLOCK–BMAL1 in the nucleus:

a) mRNAs of *Per*, *Cry* and *Bmall*:

r

(1)

(2)

(3)

b) Phosphorylated and nonphosphorylated proteins PER and CRY in the cytosol:

(4)

(5)

(6)

(7)

c) Phosphorylated and nonphosphorylated PER–CRY complex in cytosol and nucleus:

(8)

(9)

(10)

(11)

d) Phosphorylated and nonphosphorylated protein BMAL1 in the cytosol and nucleus:

(12)

(13)

(14)

(15)

e) Inactive complex between PER–CRY and CLOCK–BMAL1 in nucleus:

(16)

The definition of the various parameters is indicated in the legend to Fig. 5. In Eqs. (1)–(16), concentrations are defined with respect to the total cell volume. The concentration of every protein species (single protein or complex between two or more proteins) is denoted by a subscript C, N, CP or NP for cytosolic, nuclear, cytosolic phosphorylated or nuclear phosphorylated, respectively. Thus, an expression such as PC_C refers to the concentration of the cytosolic complex between PER and CRY, while the product of the concentrations of PER and CRY in the cytosol is denoted $P_C C_C$.

Sensitivity analysis

Two types of sensitivity are noticeable from the data in Table 1; the first relates to the size of the oscillatory domain, and the other, to the influence on the period. For some parameters – mainly those linked to synthesis and degradation of BMAL1 and its mRNA (see Fig. 5): v_{sb} , v_{mb} , k_{sb} , K_{IB} , and to a lesser degree V_{1B} and V_{3B} – the range of values producing sustained oscillations is quite narrow, less than one order of magnitude, while for other parameters it is much larger. In regard to the second type of sensitivity, the period changes most, by a factor close to 3, from one boundary to the other of the oscillatory domain, for parameters k_1 and k_7 which measure, respectively, the entry of the PER-CRY complex into the nucleus, and the formation of the inactive complex between PER-CRY and CLOCK-BMAL1 in the nucleus. The corresponding change in period is close to 2 for parameters K_{mB} , which relates to degradation of *Bmal1* mRNA, K_{IB} which measures repression by BMAL1 of the expression of its gene, and k_{sp} which measures the rate of PER synthesis. The period changes to a smaller extent with respect to the other parameters. Parameters related to synthesis and degradation of BMAL1 and its mRNA thus possess the narrowest range of values producing sustained oscillations, while the period is most affected by the parameters measuring the entry of the

PER-CRY complex into the nucleus and the formation of the inactive complex between PER-CRY and CLOCK-BMAL1.

A more comprehensive picture of the effect of a parameter is provided by bifurcation diagrams, which show how the period varies over the whole oscillatory range. Such bifurcation diagrams, presented for a selected choice of parameters in Fig. 6, indicate that the period may change monotonously as a function of a parameter or, in contrast, may pass through a maximum or a minimum. One diagram (Fig. 6E) shows the coexistence of two stable rhythms, characterized by distinct periods, over a narrow range of parameter values. The data in Fig. 6G further illustrate the influence of the degrees of cooperativity n and m that characterize the control exerted by BMAL1 on the expression of *Per* and *Cry*, and of *Bmall*, respectively. Degrees of cooperativity larger than unity are often observed in genetic regulatory processes (5). Circadian oscillations can be obtained here when n and m are close to unity, but cooperativity favors oscillations, as the oscillatory domain of the other parameters becomes larger when n and m increase.

Model incorporating REV-ERB α

The model incorporating explicitly the role of REV-ERB α in the repression of *Bmall* is shown by the full scheme in Fig. 5 (the direct repression of *Bmall* by BMAL1 should then be disregarded). We consider the following additional steps, indicated in grey in Fig. 5:

- 1) BMAL1-activated transcription of *Rev-Erb α* into the corresponding mRNA (M_R) and degradation of this mRNA.
- 2) Translation of the mRNA into cytosolic REV-ERB α protein (R_C) and degradation of this protein.
- 3) Reversible entry of the cytosolic protein into the nucleus and degradation of the nuclear form of REV-ERB α (R_N).

The full model is governed by Eqs. (1)-(16) and by the additional Eqs. (17)-(19):

$$(17)$$

$$(18)$$

$$(19)$$

Moreover, because repression of *Bmal1* is now exerted by nuclear REV-ERB α instead of nuclear BMAL1, Eq. (3) should be replaced by Eq. (3'):

(3')

References

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Table 1: Sensitivity analysis showing for each parameter the range of values producing sustained oscillations and the effect on the period. The lower and upper values bounding the oscillatory domain are indicated in columns 3 and 4, while the value of the period in these points is listed in columns 5 and 6, respectively. Parameters were varied one at a time, while holding other parameters at their basal values (column 2) used in Fig. 2A,B. The full variation of the period over the whole oscillatory range is shown in Fig. 6 for a selected choice of parameters.

Parameter	Basal value	Lower boundary	Upper boundary	Period at lower boundary (h)	Period at upper boundary (h)
k_1 (h ⁻¹)	0.4	0.008	> 100	46.4	14.8
k_2 (h ⁻¹)	0.2	0	3.8	25.3	30.3
k_3 (nM ⁻¹ h ⁻¹)	0.4	0.000015	> 100	41.2	22.3
k_4 (h ⁻¹)	0.2	0	> 100	24.2	27.3
k_5 (h ⁻¹)	0.4	0.008	> 100	17.7	23.0
k_6 (h ⁻¹)	0.2	0	8.6	23.7	38
k_7 (nM ⁻¹ h ⁻¹)	0.5	0.033	> 100	44	14.7
k_8 (h ⁻¹)	0.1	0	11.7	23.4	39.5
K_{AP} (nM)	0.7	0	0.99	17.5	19.9
K_{AC} (nM)	0.6	0	1.06	22.3	14.3
K_{JB} (nM)	2.2	1.46	6.4	20.3	44.9
k_{dmb} (h ⁻¹)	0.01	0	0.023	26.6	20.7
k_{dmc} (h ⁻¹)	0.01	0	0.222	23.8	16.7
k_{dmp} (h ⁻¹)	0.01	0	0.232	24.4	18.8
k_{dn} (h ⁻¹)	0.01	0	0.18	24.8	17.7
k_{dnc} (h ⁻¹)	0.12	0	> 100	22.3	25
K_d (nM)	0.3	0.001	> 100	22.3	30.8
K_{dp} (nM)	0.1	0	> 100	23.5	22.5
K_p (nM)	0.1	0.0007	> 100	25.9	32.8
K_{mB} (nM)	0.4	0	4	22.7	43.3
K_{mC} (nM)	0.4	0.0004	> 100	27.6	22.3
K_{mP} (nM)	0.31	0.002	> 100	24.6	17.6
k_{sB} (h ⁻¹)	0.12	0.06	0.66	20.7	23.5
k_{sC} (h ⁻¹)	1.6	0.15	> 100	33.2	22.2
k_{sP} (h ⁻¹)	0.6	0.06	> 100	35	17.6
m	2	1.38	> 100	21.8	25.4
n	4	1.07	> 100	25.7	23.5

V_{1B} (nMh ⁻¹)	0.5	0	1.3	25.0	20.7
V_{1C} (nMh ⁻¹)	0.6	0	> 100	23.5	30.6
V_{1P} (nMh ⁻¹)	0.4	0	> 100	23.2	28.9
V_{1PC} (nMh ⁻¹)	0.4	0	22	24.5	23.5
V_{2B} (nMh ⁻¹)	0.1	0	> 100	23.4	25.2
V_{2C} (nMh ⁻¹)	0.1	0	> 100	23.8	23.5
V_{2P} (nMh ⁻¹)	0.3	0	> 100	23.8	23.3
V_{2PC} (nMh ⁻¹)	0.1	0	> 100	23.7	24.5
V_{3B} (nMh ⁻¹)	0.5	0	1.3	25.6	18.5
V_{3PC} (nMh ⁻¹)	0.4	0	16	24.9	22.6
V_{4B} (nMh ⁻¹)	0.2	0	> 100	23.1	24.7
V_{4PC} (nMh ⁻¹)	0.1	0	> 100	23.6	24.9
V_{phos} (nMh ⁻¹)	0.4	0	3.2	22.4	26.5
v_{dBC} (nMh ⁻¹)	0.5	0	> 100	23.9	23.7
v_{dBN} (nMh ⁻¹)	0.6	0	> 100	23.6	23.7
v_{dCC} (nMh ⁻¹)	0.7	0	> 100	23.8	23.8
v_{dIN} (nMh ⁻¹)	0.8	0	> 100	34	23.4
v_{dPC} (nMh ⁻¹)	0.7	0	> 100	23.6	23.8
v_{dPCC} (nMh ⁻¹)	0.7	0	> 100	23.9	23.8
v_{dPCN} (nMh ⁻¹)	0.7	0	> 100	23.6	23.7
v_{mB} (nMh ⁻¹)	0.8	0.65	0.89	27.1	21.0
v_{mC} (nMh ⁻¹)	1.0	0	1.4	22.2	16.7
v_{mP} (nMh ⁻¹)	1.1	0	1.5	17.6	19.8
v_{sB} (nMh ⁻¹)	1.0	0.91	1.11	20.7	26.8
v_{sC} (nMh ⁻¹)	1.1	0.76	> 100	18.4	22.2
v_{sP} (nMh ⁻¹)	1.5	1.06	> 100	21.6	17.6

Fig. 5: Model for circadian oscillations in mammals involving interlocked negative and positive regulations of *Per*, *Cry*, *Bmal1* and *Rev-Erb α* genes by their protein products. The figure is identical to Fig. 1, with indication of the kinetic parameters. In the article we focus on the case where BMAL1 exerts a direct negative feedback on the expression of its gene. The role of the *Rev-Erb α* gene product in the indirect regulation of *Bmal1* expression by BMAL1 (indicated in grey) is considered in a second stage. *Per* (M_P), *Cry* (M_C), *Bmal1* (M_B) and *Rev-Erb α* (M_R) mRNAs are synthesized in the nucleus and transferred into the cytosol where they accumulate at a maximum rate v_{sP} , v_{sC} , v_{sB} and v_{sR} , respectively. There they are degraded by an enzyme of maximum rate v_{mP} , v_{mC} , v_{mB} or v_{mR} , with the Michaelis constants K_{mP} , K_{mC} , K_{mB} and K_{mR} , respectively. The rates of synthesis of the PER, CRY, BMAL1 and REV-ERB α proteins, proportional to M_P , M_C , M_B or M_R , are characterized by the apparent first-order rate constants k_{sP} , k_{sC} , k_{sB} and k_{sR} . Parameters V_{iP} , V_{iC} ($i=1,2$), V_{jB} , V_{jPC} ($j=1,\dots,4$) denote the maximum rates and Michaelis constants of the kinase and phosphatase involved in the reversible phosphorylation of P_C into P_{CP} , C_C into C_{CP} , B_C into B_{CP} , B_N into B_{NP} , PC_C into PC_{CP} , and PC_N into PC_{NP} , respectively, while K_p and K_{dp} represent the Michaelis constants of the kinase and phosphatase involved in these processes. One of the kinases acting on PER is casein kinase I ϵ (CKI ϵ) (6). The fully phosphorylated forms P_{CP} , C_{CP} , B_{CP} , B_{NP} , PC_{CP} , PC_{NP} , are degraded by enzymes of maximum rate v_{dPC} , v_{dCC} , v_{dBC} , v_{dBN} , v_{dPCC} , v_{dPCN} , respectively, and of Michaelis constant K_d . The cytosolic (R_C) and nuclear (R_N) forms of REV-ERB α are degraded by Michaelian enzymes of maximum rate v_{dRC} and v_{dRN} , with Michaelis constant K_d . To avoid accumulation of any of the intermediates, we also consider nonspecific degradation of mRNAs (with apparent first-order rate constants k_{dmp} , k_{dmc} , k_{dmb} , k_{dmr}) and of proteins (with apparent first-order rate constants which may differ for each protein species, but in the simulations are taken equal to k_{dn} , except for the nonspecific degradation of cytosolic CRY that is characterized by rate constant k_{dmc}). The cytosolic unphosphorylated forms of PER and CRY reversibly form a complex, with the association and dissociation rate constants k_3 and k_4 . The cytoplasmic PER-CRY complex as well as cytosolic BMAL1 protein are transported into the nucleus at rates characterized by the apparent first-order rate constant k_1 and k_5 . Transport of the nuclear form of PER-CRY and BMAL1 into the cytosol are characterized by the apparent first-order rate constant k_2 and k_6 . As the expression of *Clock* is considered to be constitutive and to give rise to a high, constant level of cytosolic and nuclear CLOCK protein, we assume that once in the nucleus, unphosphorylated BMAL1 immediately forms a complex with CLOCK (the concentration of this complex is that of BMAL1, i.e. B_N). The nuclear unphosphorylated PER-CRY and CLOCK–BMAL1 complexes reversibly form an inactive complex, I_N (association and dissociation are characterized by the rate constants k_7 and k_8 , respectively). The positive regulation exerted by nuclear BMAL1 on *Per* and *Cry* transcription is described by an equation of the Hill type, in which n denotes the degree of cooperativity, and K_{AP} and K_{AC} the threshold constant for activation. The positive regulation

exerted by nuclear BMAL1 on *Rev-Erb α* transcription is also described by a Hill equation, with a degree of cooperativity h , and an activation constant K_{AR} . The negative feedback exerted by nuclear CLOCK–BMAL1 (grey arrow) or REV-ERB α on *Bmal1* transcription is described by a Hill function in which m denotes the degree of cooperativity and K_{IB} the threshold constant for repression. The effect of light is to increase the rate of expression of the *Per* gene, v_{sP} .

Fig. 6: Bifurcation diagrams showing the variation of the period as a function of a selected parameters of the model for the mammalian circadian clock: k_1 (A), k_7 (B), k_8 (C), k_{sB} (D), K_{IB} (E), K_{AP} (F), m and n (G), and v_{dPCC} (H). These parameters are defined in the legend of Fig. 5. The curves were obtained by numerical integration of Eqs. (1)-(16) for the model without REV-ERB α . On each curve the dot relates to the basal parameter value considered in Fig. 2A and listed in Table 1. Other parameters were given the basal values listed in Table 1. In panel E, two stable rhythms coexist over a small range of K_{IB} values. This phenomenon, referred to as birhythmicity, has also been observed in a 10-variable model for circadian rhythms in *Drosophila* (7).

Fig. 7: Damped oscillations (A) can transform into sustained oscillations (B) under periodic forcing. The curves, showing the time evolution of *Per*, *Cry* and *Bmal1* mRNAs, were obtained as in Fig. 2A, for the same parameter values except $K_{AC} = 0.4$ nM, $v_{mB} = 0.9$ nM/h. In (B), the maximum rate of *Per* expression (v_{sP}) varies in a sinusoidal manner with a period equal to 24 h. This periodic variation could represent the periodic influence exerted on peripheral damped oscillators by circadian rhythms produced by suprachiasmatic nuclei. The peak in *Per* mRNA upon entrainment follows by several hours the peak in the SCN signal, in agreement with experimental observations on circadian oscillations in peripheral tissues in mammals (see Discussion).

Fig. 8: Circadian oscillations in constant darkness (A) and entrainment by LD cycles (B) in the model incorporating REV-ERB α . (A) The mRNA of *Bmal1* oscillates out of phase with respect to the mRNAs of *Per*, *Cry* and *Rev-Erb α* . (B) Oscillations of the mRNAs after entrainment by 24 h light-dark (LD) cycles. The peak in *Per* mRNA occurs in the light phase. The maximum value of the rate of *Per* gene expression, v_{sP} , varies in a square-wave manner such that it remains at a constant low value of 2.4 nM/h during the 12h-long dark phase (black rectangle), and is raised up to the high value of 3 nM/h during the 12h-long light phase (white rectangle). (C) Phase response curve (PRC) showing the phase shift resulting from a single pulse in the light-sensitive parameter, corresponding to a 2h-increase in v_{sP} from 2.4 nM/h to 3.4 nM/h. Also indicated is the time evolution of *Per* mRNA (M_P) in DD in the absence of

perturbation; phase 0 corresponds to the minimum in M_p . The curves in panels A-C have been obtained by numerical integration of Eqs. (1)-(19), with Eq. (3) replaced by Eq. (3'). Parameter values, selected so as to yield a period close to 24 h, are: $k_1 = k_5 = k_9 = 0.8 \text{ h}^{-1}$, $k_2 = k_4 = k_6 = k_{10} = 0.4 \text{ h}^{-1}$, $k_8 = 0.2 \text{ h}^{-1}$, $k_3 = 0.8 \text{ nM}^{-1}\text{h}^{-1}$, $k_7 = 1 \text{ nM}^{-1}\text{h}^{-1}$, $K_{AP} = K_{AC} = K_{AR} = 0.6 \text{ nM}$, $K_{IB} = 2.2 \text{ nM}$, $k_{dmB} = k_{dmc} = k_{dmp} = k_{dmr} = k_{dnC} = k_{dn} = 0.02 \text{ h}^{-1}$, $K_d = 0.3 \text{ nM}$, $K_{dp} = 0.1 \text{ nM}$, $K_p = 1.006 \text{ nM}$, $K_{mP} = 0.3 \text{ nM}$, $K_{mC} = K_{mB} = K_{mR} = 0.4 \text{ nM}$, $k_{sP} = 1.2 \text{ h}^{-1}$, $k_{sC} = 3.2 \text{ h}^{-1}$, $k_{sB} = 0.32 \text{ h}^{-1}$, $k_{sR} = 1.7 \text{ h}^{-1}$, $h = m = n = 2$, $V_{1P} = 9.6 \text{ nM/h}$, $V_{1PC} = V_{3PC} = 2.4 \text{ nM/h}$, $V_{2P} = 0.6 \text{ nM/h}$, $V_{1C} = 1.2 \text{ nM/h}$, $V_{2C} = V_{2B} = V_{2PC} = V_{4PC} = 0.2 \text{ nM/h}$, $V_{1B} = V_{3B} = 1.4 \text{ nM/h}$, $V_{4B} = 0.4 \text{ nM/h}$, $v_{dPC} = 3.4 \text{ nM/h}$, $v_{dCC} = v_{dPCC} = v_{dPCN} = 1.4 \text{ nM/h}$, $v_{dBC} = v_{dBN} = 3 \text{ nM/h}$, $v_{dIN} = 1.6 \text{ nM/h}$, $v_{dRC} = 4.4 \text{ nM/h}$, $v_{dRN} = 0.8 \text{ nM/h}$, $v_{mP} = 2.2 \text{ nM/h}$, $v_{mC} = 2 \text{ nM/h}$, $v_{mB} = 1.3 \text{ nM/h}$, $v_{mR} = 1.6 \text{ nM/h}$, $v_{sP} = 2.4 \text{ nM/h}$, $v_{sC} = 2.2 \text{ nM/h}$, $v_{sB} = 1.8 \text{ nM/h}$, $v_{sR} = 1.6 \text{ nM/h}$.

