Supporting Text

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): (*i*) Transcription of the *Per*, *Cry*, and *Bmal1* genes into the corresponding mRNAs (denoted M_P, M_C, and M_B, respectively) and degradation of these mRNAs. For simplicity, at this stage we do not distinguish between the *Per1*, *Per2*, and *Per3* genes, and we represent them in the model by a single *Per* gene; similarly, *Cry1* and *Cry2* are represented by a single *Cry* gene. (*ii*) Translation of these mRNAs into the cytosolic, unphosphorylated proteins PER, CRY, and BMAL1 (denoted by P_C, C_C, and B_C). (*iii*) Reversible phosphorylation of the PER, CRY, and BMAL1 proteins (concentrations of the phosphorylated forms are denoted by P_{CP}, C_{CP},and B_{CP}). (*iv*) 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}). (*v*) 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). (*vi*) 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). (*vii*) 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). (*viii*) In the nucleus, the CLOCK–BMAL1 complex 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 as I_N). (*ix*) 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. (*x*) 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-Erbα* gene and the REV-ERBα 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-ERBα in the regulation of *Bmal1* expression. (*xi*) 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, formed 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 significantly change the behavior of the model. (*xii*) This article deals with the dynamics of the model in conditions corresponding to DD or to LD 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}.

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 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:

(*i*) mRNAs of *Per*, *Cry*, and *Bmal1*:

$$\frac{dM_P}{dt} = v_{sP}\frac{B_N^n}{K_{AP}^n + B_N^n} - v_{mP}\frac{M_P}{K_{mP} + M_P} - k_{dmp}M_P \qquad \textbf{[1]}$$

$$\frac{dM_C}{dt} = v_{sC}\frac{B_N^n}{K_{AC}^n + B_N^n} - v_{mC}\frac{M_C}{K_{mC} + M_C} - k_{dmc}M_C \qquad \textbf{[2]}$$

$$\frac{dM_B}{dt} = v_{sB}\frac{K_{IB}^m}{K_{IB}^m + B_N^m} - v_{mB}\frac{M_B}{K_{mB} + M_B} - k_{dmb}M_B \qquad \textbf{[3]}$$

(*ii*) Phosphorylated and nonphosphorylated proteins PER and CRY in the cytosol:

$$\frac{dP_C}{dt} = k_{sP}M_P - V_{1P}\frac{P_C}{K_p + P_C} + V_{2P}\frac{P_{CP}}{K_{dp} + P_{CP}} + k_4PC_C - k_3P_CC_C - k_{dn}P_C \qquad \textbf{[4]}$$

$$\frac{dC_C}{dt} = k_{sC}M_C - V_{1C}\frac{C_C}{K_p + C_C} + V_{2C}\frac{C_{CP}}{K_{dp} + C_{CP}} + k_4PC_C - k_3P_CC_C - k_{dnc}C_C \qquad \textbf{[5]}$$

$$\frac{dP_{CP}}{dt} = V_{1P}\frac{P_C}{K_p + P_C} - V_{2P}\frac{P_{CP}}{K_{dp} + P_{CP}} - v_{dPC}\frac{P_{CP}}{K_d + P_{CP}} - k_{dn}P_{CP} \qquad \textbf{[6]}$$

$$\frac{dC_{CP}}{dt} = V_{1C}\frac{C_C}{K_p + C_C} - V_{2C}\frac{C_{CP}}{K_{dp} + C_{CP}} - v_{dCC}\frac{C_{CP}}{K_d + C_{CP}} - k_{dn}C_{CP} \qquad \textbf{[7]}$$

(*iii*) Phosphorylated and nonphosphorylated PER–CRY complex in cytosol and nucleus:

$$\frac{\mathrm{d}PC_{\mathrm{C}}}{\mathrm{d}t} = -V_{1\mathrm{PC}}\frac{PC_{\mathrm{C}}}{K_{\mathrm{p}} + PC_{\mathrm{C}}} + V_{2\mathrm{PC}}\frac{PC_{\mathrm{CP}}}{K_{\mathrm{dp}} + PC_{\mathrm{CP}}} - k_4 PC_{\mathrm{C}} + k_3 P_{\mathrm{C}} C_{\mathrm{C}} + k_2 PC_{\mathrm{N}} - k_1 PC_{\mathrm{C}} - k_{\mathrm{dn}} PC_{\mathrm{C}} \quad \textbf{[8]}$$

$$\frac{\mathrm{d}PC_{\mathrm{N}}}{\mathrm{d}t} = -V_{3\mathrm{PC}}\frac{PC_{\mathrm{N}}}{K_{\mathrm{p}} + PC_{\mathrm{N}}} + V_{4\mathrm{PC}}\frac{PC_{\mathrm{NP}}}{K_{\mathrm{dp}} + PC_{\mathrm{NP}}} - k_2 PC_{\mathrm{N}} + k_1 PC_{\mathrm{C}} - k_7 B_{\mathrm{N}} PC_{\mathrm{N}} + k_8 I_{\mathrm{N}} - k_{\mathrm{dn}} PC_{\mathrm{N}} \quad \textbf{[9]}$$

$$\frac{\mathrm{d}PC_{\mathrm{CP}}}{\mathrm{d}t} = V_{1\mathrm{PC}}\frac{PC_{\mathrm{C}}}{K_{\mathrm{p}} + PC_{\mathrm{C}}} - V_{2\mathrm{PC}}\frac{PC_{\mathrm{CP}}}{K_{\mathrm{dp}} + PC_{\mathrm{CP}}} - v_{\mathrm{dPCC}}\frac{PC_{\mathrm{CP}}}{K_{\mathrm{d}} + PC_{\mathrm{CP}}} - k_{\mathrm{dn}} PC_{\mathrm{CP}} \quad \textbf{[10]}$$

$$\frac{\mathrm{d}PC_{\mathrm{NP}}}{\mathrm{d}t} = V_{3\mathrm{PC}}\frac{PC_{\mathrm{N}}}{K_{\mathrm{p}} + PC_{\mathrm{N}}} - V_{4\mathrm{PC}}\frac{PC_{\mathrm{NP}}}{K_{\mathrm{dp}} + PC_{\mathrm{NP}}} - v_{\mathrm{dPCN}}\frac{PC_{\mathrm{NP}}}{K_{\mathrm{d}} + PC_{\mathrm{NP}}} - k_{\mathrm{dn}} PC_{\mathrm{NP}} \quad \textbf{[11]}$$

(*iv*) Phosphorylated and nonphosphorylated protein BMAL1 in the cytosol and nucleus:

$$\frac{\mathrm{d}B_{\mathrm{C}}}{\mathrm{d}t} = k_{\mathrm{sB}} M_{\mathrm{B}} - V_{1\mathrm{B}}\frac{B_{\mathrm{C}}}{K_{\mathrm{p}} + B_{\mathrm{C}}} + V_{2\mathrm{B}}\frac{B_{\mathrm{CP}}}{K_{\mathrm{dp}} + B_{\mathrm{CP}}} - k_5 B_C + k_6 B_N - k_{\mathrm{dn}} B_{\mathrm{C}} \quad \textbf{[12]}$$

$$\frac{\mathrm{d}B_{\mathrm{CP}}}{\mathrm{d}t} = V_{1\mathrm{B}}\frac{B_{\mathrm{C}}}{K_{\mathrm{p}} + B_{\mathrm{C}}} - V_{2\mathrm{B}}\frac{B_{\mathrm{CP}}}{K_{\mathrm{dp}} + B_{\mathrm{CP}}} - v_{\mathrm{dBC}}\frac{B_{\mathrm{CP}}}{K_{\mathrm{d}} + B_{\mathrm{CP}}} - k_{\mathrm{dn}} B_{\mathrm{CP}} \quad \textbf{[13]}$$

$$\frac{\mathrm{d}B_{\mathrm{N}}}{\mathrm{d}t} = -V_{3\mathrm{B}}\frac{B_{\mathrm{N}}}{K_{\mathrm{p}} + B_{\mathrm{N}}} + V_{4\mathrm{B}}\frac{B_{\mathrm{NP}}}{K_{\mathrm{dp}} + B_{\mathrm{NP}}} + k_5 B_{\mathrm{C}} - k_6 B_{\mathrm{N}} - k_7 B_{\mathrm{N}} PC_{\mathrm{N}} + k_8 I_{\mathrm{N}} - k_{\mathrm{dn}} B_{\mathrm{N}} \quad \textbf{[14]}$$

$$\frac{\mathrm{d}B_{\mathrm{NP}}}{\mathrm{d}t} = V_{3\mathrm{B}}\frac{B_{\mathrm{N}}}{K_{\mathrm{p}} + B_{\mathrm{N}}} - V_{4\mathrm{B}}\frac{B_{\mathrm{NP}}}{K_{\mathrm{dp}} + B_{\mathrm{NP}}} - v_{\mathrm{dBN}}\frac{B_{\mathrm{NP}}}{K_{\mathrm{d}} + B_{\mathrm{NP}}} - k_{\mathrm{dn}} B_{\mathrm{NP}} \quad \textbf{[15]}$$

(*v*) Inactive complex between PER–CRY and CLOCK–BMAL1 in nucleus:

$$\frac{dI_N}{dt} = -k_8 I_N + k_7 B_N PC_N - v_{dIN}\frac{I_N}{K_d + I_N} - k_{dn} I_N \qquad \textbf{[16]}$$

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. We have investigated the sensitivity of the oscillations predicted by the model, by varying one parameter at a time (Table 1). 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. 6*E*) shows the coexistence of two stable rhythms, characterized by distinct periods, over a narrow range of parameter values. The data in Fig. 6*G* 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 *Bmal1*, 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-ERBα. The model explicitly incorporating the role of REV-ERBα in the repression of *Bmal1* is shown by the full scheme in Fig. 5 (the direct repression of *Bmal1* by BMAL1 should then be disregarded). We consider the following additional steps, indicated in gray in Fig. 5: (*i*) BMAL1-activated transcription of *Rev-Erbα* into the corresponding mRNA (M_R) and degradation of this mRNA. (*ii*) Translation of the mRNA into cytosolic REV-ERBα protein (R_C) and degradation of this protein. (*iii*) Reversible entry of the cytosolic protein into the nucleus and degradation of the nuclear form of REV-ERBα (R_N).

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

$$\frac{dM_R}{dt} = v_{sR}\frac{B_N^h}{K_{AR}^h + B_N^h} - v_{mR}\frac{M_R}{K_{mR} + M_R} - k_{dmr}M_R \qquad \textbf{[17]}$$

$$\frac{dR_C}{dt} = k_{sR}M_R - k_9R_C + k_{10}R_N - v_{dRC}\frac{R_C}{K_d + R_C} - k_{dn}R_C \qquad \textbf{[18]}$$

$$\frac{dR_N}{dt} = k_9 R_C - k_{10} R_N - v_{dRN} \frac{R_N}{K_d + R_N} - k_{dn} R_N \qquad \textbf{[19]}$$

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

$$\frac{dM_B}{dt} = v_{sB} \frac{K_{IB}^m}{K_{IB}^m + R_N^m} - v_{mB} \frac{M_B}{K_{mB} + M_B} - k_{dmb} M_B \qquad \textbf{[3']}$$

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