

Supplementary Methods and Information

The positive circadian regulators CLOCK and BMAL1 heterodimer control G2/M cell cycle transition through Cyclin B1

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DETAILED DESCRIPTION OF THE MATHEMATICAL MODELING OF THE CIRCADIAN CLOCK – CELL CYCLE CONNECTION

To study the interaction between the circadian clock and the cell cycle we used two computational models previously proposed for the mammalian circadian clock and for the mammalian cell cycle, respectively. The model for the mammalian circadian clock incorporates the positive and negative regulations involving the PER, CRY, CLOCK, BMAL1 and REV-ERB α proteins (Leloup & Goldbeter, 2003, 2004). For simplicity the PER1 and PER2 proteins, on one hand, and the CRY1 and CRY2 proteins on the other hand, are considered as a single entity referred to as PER and CRY, respectively; moreover, the CLOCK protein is assumed to be expressed constitutively and to instantaneously form a complex with BMAL1. This model accounts for the occurrence of circadian oscillations of the above-mentioned proteins and their mRNAs in a variety of experimental conditions (Leloup & Goldbeter, 2003, 2004).

The model for the mammalian cell cycle is based on the regulatory properties of the CDK network that drives the transitions between the successive phases of the cell cycle (Gérard & Goldbeter, 2009, 2014). The model contains four CDK modules, each of which controls transition to a particular cell cycle phase. Thus, Cyclin D/CDK4-6 and Cyclin E/CDK2 promote progression in G1 and elicit the G1/S transition; the activation of Cyclin A/CDK2 ensures progression in S and G2, while the peak of Cyclin B/CDK1 activity brings about progression into mitosis. Exit from the quiescent state is triggered above a critical level of growth factor by the synthesis of Cyclin D, which allows cells to enter the G1 phase. Synthesis of the various cyclins is regulated through the balance between the antagonistic effects exerted by the transcription factor E2F and the tumor suppressor pRB, which

respectively promote and inhibit cell cycle progression. Additional regulations in this model for the CDK network bear on the control exerted by the proteins SKP2, CDH1, or CDC20 on the degradation of cyclins E, A, and B at the G1/S or G2/M transitions, respectively. Moreover, the activity of each cyclin/CDK complex can itself be regulated through CDK phosphorylation-dephosphorylation. At suprathreshold levels of growth factor sustained oscillations spontaneously occur in the CDK network, which may be associated with cellular proliferation since they correspond to the repetitive, sequential activation of the various cyclin-CDK complexes responsible for the ordered progression along the successive phases of the cell cycle (Gérard & Goldbeter, 2009, 2014).

The circadian clock model is governed by 19 kinetic equations (Leloup & Goldbeter, 2003) while the model for the mammalian cell cycle is described by 39 differential equations (Gérard & Goldbeter, 2012). In a previous study the cell cycle was coupled to the circadian clock via the circadian control of WEE1, p21 or Cyclin E; any one of these three modes of coupling can lead to entrainment of the cell cycle by the circadian clock over a range of cell cycle durations prior to coupling (see Gérard & Goldbeter, 2012 for a computational study of circadian entrainment of the cell cycle resulting from these various modes of coupling, and for a list of kinetic equations for the coupled cell cycle-circadian clock system). Here we focus on the case where the cell cycle is coupled to the circadian control via the induction of *Wee1* gene expression by BMAL1, and also introduce coupling via the induction of *Cyclin B1* gene expression by BMAL1, as suggested by the experiments reported in the present study.

To describe the coupling of the cell cycle to the circadian clock via the kinase WEE1 we incorporate in the CDK network model an additional kinetic equation for the mRNA of the *Wee1* gene, which includes the induction of *Wee1* expression by CLOCK/BMAL1. The

equations [1] and [2] describing the coupling of the cell cycle to the circadian clock through activation of the transcription of the *Wee1* gene by CLOCK/BMAL1 are:

$$\frac{dMw}{dt} = \left(v_{swee1} + v_{sw} \cdot \frac{Bn^{nmw}}{K_{aw}^{nmw} + Bn^{nmw}} - V_{dmw} \cdot \frac{Mw}{K_{dmw} + Mw} \right) \cdot eps \quad (1)$$

$$\frac{dWee1}{dt} = (k_{sw} \cdot Mw - V_{m7b} \cdot (Mb + i_b) \cdot \frac{Wee1}{K_{7b} + Wee1} + V_{m8b} \cdot \frac{Wee1p}{K_{8b} + Wee1p} - k_{dwee1} \cdot Wee1) \cdot eps \quad (2)$$

These equations replace equation [38] in our previous model (Gérard & Goldbeter; 2012). Here, equation [1] describes the time evolution of the *Wee1* mRNA positively regulated by the circadian clock complex, CLOCK/BMAL1 (Bn), with an activation constant K_{aw} , while equation [2] shows the time evolution of the kinase WEE1. The synthesis of this protein kinase combines two terms: the basal rate of WEE1 synthesis (v_{swee1}) is independent from the circadian clock, while the second term, $(k_{sw} \cdot Mw)$, reflects WEE1 synthesis at a rate proportional to the *Wee1* mRNA controlled by CLOCK/BMAL1 in a circadian manner. The total amount of *Wee1* mRNA can therefore be viewed as the sum of two terms, $(v_{swee1}/k_{sw}) + Mw$, which are respectively independent from, and dependent on the circadian clock. The coupling strength is expressed by parameter v_{sw} , which measures the magnitude of the *Wee1* mRNA synthesized under control by the circadian clock. An alternative measure of coupling strength is provided by the parameter K_{aw} , which characterizes the activation of *Wee1* expression by CLOCK/BMAL1; the strength of the coupling to the circadian clock increases as K_{aw} progressively decreases.

A similar approach was used to describe the coupling of the cell cycle to the circadian clock via Cyclin B1. Thus, we incorporated in the model for the CDK network an additional kinetic equation for the *Cyclin B1* (*CcnB1*) mRNA, which includes the induction of *Cyclin B1* expression by CLOCK/BMAL1. The equations [3] and [4] describing the coupling of the cell cycle to the circadian clock through the activation of the transcription of the *Cyclin B1* gene by CLOCK/BMAL1 are:

$$\frac{dMcb}{dt} = \left(v_{cb} + v_{scb} \cdot \frac{Bn^{ncb}}{K_{acb}^{ncb} + Bn^{ncb}} - V_{dmcb} \cdot \frac{Mcb}{K_{dmcb} + Mcb} \right) \cdot eps \quad (3)$$

$$\begin{aligned} \frac{dCb}{dt} = & \left(k_{cb} \cdot Mcb - k_{com4} \cdot Cb \cdot (Cdk1_{tot} - (Mbi + Mb + Mbp27)) + k_{decom4} \cdot Mbi \right. \\ & \left. - V_{ab} \cdot \frac{Cb}{K_{db} + Cb} \cdot \left(\frac{Cdc20a}{K_{dbc20} + Cdc20a} + \frac{Cdh1a}{K_{dbcdh1} + Cdh1a} \right) - k_{adb} \cdot Cb \right) \\ & \cdot eps \end{aligned} \quad (4)$$

Equations (3) and (4) replace equation [30] in (Gérard & Goldbeter, 2009). The synthesis of the *Cyclin B* mRNA, *Mcb*, is described by two terms: the first term is the basal synthesis rate (v_{cb}), which is independent of CLOCK/BMAL1, and the second term ($v_{scb} \cdot \frac{Bn^{ncb}}{K_{acb}^{ncb} + Bn^{ncb}}$) reflects the induction by CLOCK/BMAL1. The parameter v_{scb} represents the coupling strength, which measures the magnitude of the *Cyclin B* mRNA synthesized under the control by the circadian clock.

To model the impact of knockdown of *Bmal1* gene expression, we reduce the rate of *Bmal1* mRNA synthesis, v_{sB} in the model for the circadian clock. In this model we consider that the level of *Clock* gene expression is constant and that BMAL1 instantaneously forms a

complex with CLOCK. Parameter values used for numerical simulations in Figs 4 and 5 are listed in SI Appendix Table S1. The autonomous period of the cell cycle is 21.1 h, which value is obtained by setting the scaling parameter $eps = 20.45$. The period of the circadian clock is 18 h which is obtained by setting the scaling parameter $delta = 1.336$. Knocking down BMAL1 is achieved in the model by reducing by 70% the rate of *Bmal1* mRNA synthesis, v_{SB} , from 1.55 nM/h in (A) and (C) to 0.465 nM/h in (B) and (D).

The initial values for the 19 concentration variables in the circadian clock model are all equal to 0.1 nM. The initial values for the 41 concentration variables in the cell cycle part of the model are (in μM): AP1=0.01; pRB=1; pRBc1=0.25; pRBp=0.1; pRBc2=0.01; pRBpp=0.01; E2F=0.1; E2Fp=0.05; Cd=0.01; Mdi=0.01; Md=0.01; Mdp27=0.01; Ce=0.01; Mei=0.01; Me=0.01; Skp2=0.01; Mep27=0.01; Pei=0.01; Pe=0.01; Ca=0.01; Mai=0.01; Ma=0.01; Map27=0.01; p27=0.25; p27p=0.01; Cdh1i=0.01; Cdh1a=0.01; Pai=0.01; Pa=0.01; Mcb=0.01; Cb=0.01; Mbi=0.01; Mb=0.01; Mbp27=0.01; Cdc20i=0.01; Cdc20a=0.01; Pbi=0.01; Pb=0.01; Mw=0.01; WEE1=0.1; WEE1p=0.01.

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SUPPLEMENTARY FIGURES

Fig.S1

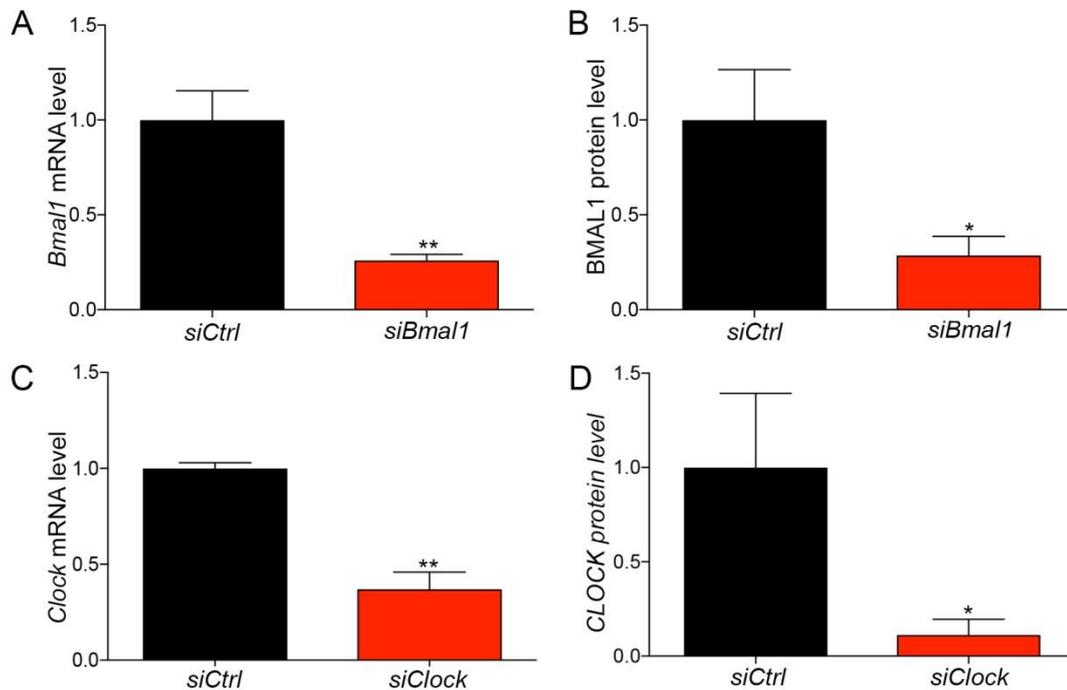


Figure EV1: *Bmal1* and *Clock* mRNA and BMAL1 and CLOCK protein levels in *siBmal1* and *siClock* cells

NIH3T3^{3C} cells were transiently transfected with Silencer® Select Pre-designed control, *Bmal1* or *Clock* siRNA to generate *siCtrl*, *siBmal1* cells and *siClock* cells, respectively. (A) Quantitative RT-PCR analysis of *Bmal1* mRNA level in proliferating *siCtrl* and *siBmal1* cells 72 hours after transient siRNA transfection (n=3). mRNA levels were normalized to that of the *B2M* housekeeping gene and expressed relative to the mRNA level in *siCtrl* cells, which was set as 1. Error bars indicate SE. ** p<0.01. (B) Western blot analysis of BMAL1 protein levels in *siCtrl* and *siBmal1* cell lysates 72 hours after transient siRNA transfection (n=3). Protein levels were normalized to β -actin protein levels. Error bars indicate SE. * p=0.03. (C) Quantitative RT-PCR analysis of *Clock* mRNA level in proliferating *siCtrl* and *siClock* cells 72

hours after transient siRNA transfection (n=3). mRNA levels were normalized to that of the *B2M* housekeeping gene and expressed relative to the mRNA level in *siCtrl* cells, which was set as 1. Error bars indicate SE. ** p<0.01. (D) Western blot analysis of CLOCK protein levels in *siCtrl* and *siClock* cell lysates 72 hours after transient siRNA transfection (n=3). Protein levels were normalized to β -actin protein levels. Error bars indicate SE. * p=0.03.

Fig. S2

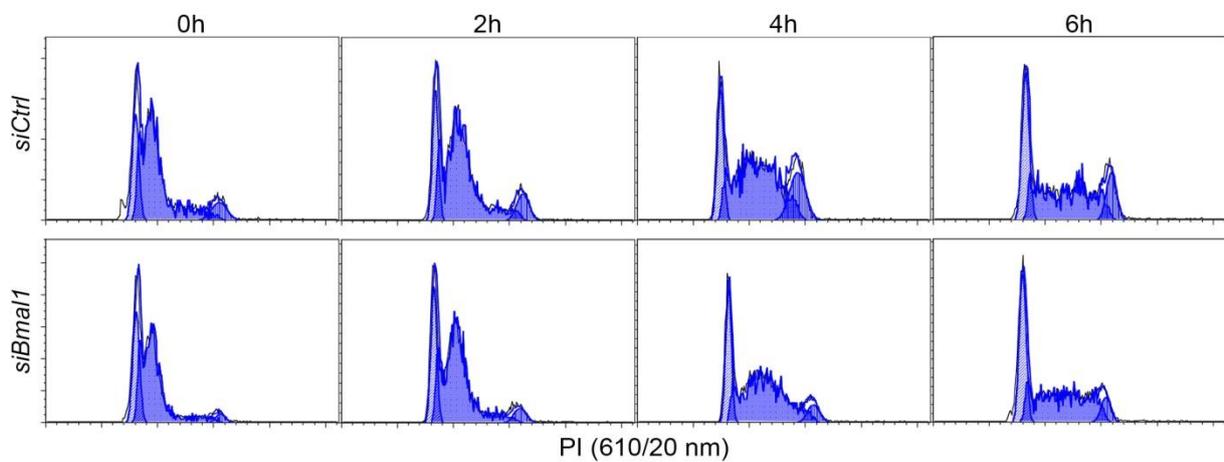


Figure EV2: S-phase progression and G2 phase entry of *siCtrl* and *siBmal1* cells after thymidine block release.

Flow cytometric analysis of cell cycle phases in *siCtrl* and *siBmal1* cells after release from a thymidine block. Shown are representative examples of propidium iodide (PI) stained *siCtrl* and *siBmal1* cells, analysed for DNA content (n = 3 experiments). The vertical axis indicates the relative number of cells and the horizontal axis indicates the relative PI fluorescence. The 2N and 4N peaks and intermediate region correspond to G1, G2/M and S phase, respectively. Note that *siCtrl* and *siBmal1* cells proceed through S phase and enter G2 phase with comparable kinetics.

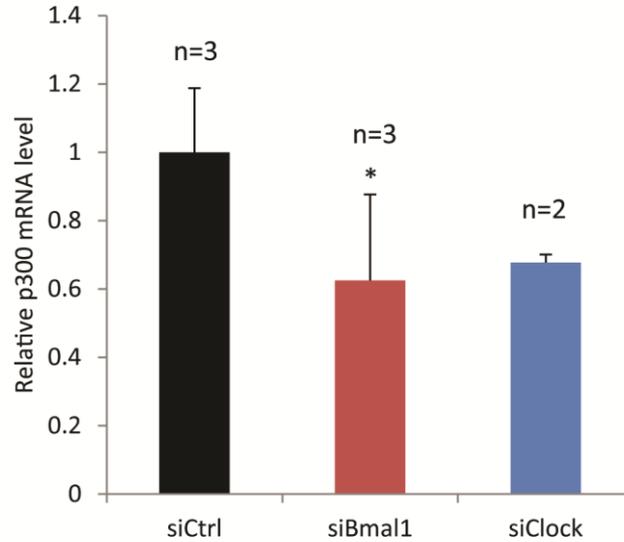


Figure EV3: Expression of p300 in *siCtrl* and *siBmal1* cells.

NIH3T3^{3C} cells were transiently transfected with Silencer® Select Pre-designed control, *Bmal1* or *Clock* siRNA to generate *siCtrl*, *siBmal1* cells and *siClock* cells, respectively. Quantitative RT-PCR analysis of *p300* mRNA level in proliferating *siCtrl* and *siBmal1* cells 72 hours after transient siRNA transfection. mRNA levels were normalized to that of the *B2M* housekeeping gene and expressed relative to the mRNA level in *siCtrl* cells, which was set as 1. Error bars indicate StDev. * $p < 0.05$.

SUPPLEMENTARY TABLES

Table S1: Parameters of the model

Parameter values used for the numerical simulations as presented in Figures 4 and 5. Indicated are the parameter values for the circadian clock, the cell cycle, and the coupling of the circadian clock and cell cycle through Wee1 (Gérard and Goldbeter, 2009, 2012). The last section of the table lists the parameter values used to extend the aforementioned coupling model to circadian control of Cyclin B1 (see equations 3 and 4 in the detailed description of the mathematical modeling of the circadian clock - cell cycle connection).

Symbol	Definition	Value in model
Circadian clock		
Parameters are (with some modifications) based on those provided in Supporting Figure 8 in Leloup and Goldbeter (2003; see http://www.pnas.org/content/100/12/7051/suppl/DC1) and on Table 1 in Leloup and Goldbeter (2004).		
δ	Scaling factor used to modify the time scale of circadian clock	1.336
k_1	Rate constant for entry of the PER/CRY complex into the nucleus	0.8 h^{-1}
k_2	Rate constant for exit of the PER/CRY complex from the nucleus	0.4 h^{-1}
k_3	Rate constant for the formation of the PER/CRY complex	$0.8 \text{ nM}^{-1} \text{ h}^{-1}$
k_4	Rate constant for dissociation of the PER/CRY complex	0.4 h^{-1}
k_5	Rate constant for entry of the BMAL1 protein into the nucleus	0.8 h^{-1}
k_6	Rate constant for exit of the BMAL1 protein from the nucleus	0.4 h^{-1}
k_7	Rate constant for the formation of the inactive PER/CRY/CLOCK/BMAL1 complex	$1 \text{ nM}^{-1} \text{ h}^{-1}$
k_8	Rate constant for the dissociation of the PER/CRY/CLOCK/BMAL1 complex	0.2 h^{-1}
k_9	Rate constant for entry of the REV-ERB α protein into the nucleus	0.8 h^{-1}
k_{10}	Rate constant for exit of the REV-ERB α protein from the nucleus	0.4 h^{-1}
K_{AP}	Constant for activation by CLOCK-BMAL1 (Bn) of <i>Per</i> mRNA synthesis	0.6 nM
K_{AC}	Constant for activation by CLOCK-BMAL1 (Bn) of <i>Cry</i> mRNA synthesis	0.6 nM
K_{AR}	Constant for activation by CLOCK-BMAL1 (Bn) of <i>Rev-erba</i> mRNA synthesis	0.6 nM
K_{IB}	Constant of inhibition by Rev-ERB α protein of <i>Bmal1</i> mRNA synthesis	1 nM
k_{dmb}	Nonspecific degradation rate constant for <i>Bmal1</i> mRNA	0.02 h^{-1}
k_{dmc}	Nonspecific degradation rate constant for <i>Cry</i> mRNA	0.02 h^{-1}
k_{dmp}	Nonspecific degradation rate constant for <i>Per</i> mRNA	0.02 h^{-1}

k_{dmr}	Nonspecific degradation rate constant for <i>Rev-erba</i> mRNA	0.02 h ⁻¹
k_{dn}	Nonspecific degradation rate constant for other proteins	0.02 h ⁻¹
k_{dnc}	Nonspecific degradation rate constant for cytosolic non-phosphorylated CRY	0.02 h ⁻¹
K_{d}	Michaelis constant for protein degradation	0.3 nM
K_{dp}	Michaelis constant for protein dephosphorylation	0.1 nM
K_{p}	Michaelis constant for protein phosphorylation	1.006 nM
K_{mB}	Michaelis constant for degradation of <i>Bmall</i> mRNA	0.4 nM
K_{mC}	Michaelis constant for degradation of <i>Cry</i> mRNA	0.4 nM
K_{mP}	Michaelis constant for degradation of <i>Per</i> mRNA	0.3 nM
K_{mR}	Michaelis constant for degradation of <i>Rev-erba</i> mRNA	0.4 nM
k_{sB}	Rate constant for synthesis of BMAL1 protein	0.32 h ⁻¹
k_{sC}	Rate constant for synthesis of CRY protein	3.2 h ⁻¹
k_{sP}	Rate constant for synthesis of PER protein	1.2 h ⁻¹
k_{sR}	Rate constant for synthesis of REV-ERB α protein	1.7 h ⁻¹
n	Degree of cooperativity of activation of <i>Per</i> and <i>Cry</i> expression by BMAL1	2
m	Degree of cooperativity of repression of <i>Bmall</i> expression by REV-ERB α	2
h	Degree of cooperativity of activation of <i>Rev-erba</i> expression by BMAL1	2
V_{1B}	Maximum rate of cytosolic BMAL1 phosphorylation	0.07 nM h ⁻¹
V_{1C}	Maximum rate of cytosolic CRY phosphorylation	1.2 nM h ⁻¹
V_{1P}	Maximum rate of cytosolic PER phosphorylation	9.6 nM h ⁻¹
V_{1PC}	Maximum rate of phosphorylation of cytosolic PER-CRY complex	2.4 nM h ⁻¹
V_{2B}	Maximum rate of cytosolic BMAL1 dephosphorylation	2 nM h ⁻¹
V_{2C}	Maximum rate of cytosolic CRY dephosphorylation	0.2 nM h ⁻¹
V_{2P}	Maximum rate of cytosolic PER dephosphorylation	0.6 nM h ⁻¹
V_{2PC}	Maximum rate of cytosolic PER-CRY complex dephosphorylation	0.2 nM h ⁻¹
V_{3B}	Maximum rate of nuclear BMAL1 phosphorylation	0.07 nM h ⁻¹
V_{3PC}	Maximum rate of phosphorylation of nuclear PER/CRY complex	2.4 nM h ⁻¹
V_{4B}	Maximum rate of nuclear BMAL1 dephosphorylation	4 nM h ⁻¹
V_{4PC}	Maximum rate of dephosphorylation of nuclear PER/CRY complex	0.2 nM h ⁻¹
V_{phos}	Phosphorylation rate	0.4 nM h ⁻¹
v_{dBC}	Maximum rate of degradation of cytosolic phosphorylated BMAL1	3 nM h ⁻¹
v_{dBN}	Maximum rate of degradation of nuclear phosphorylated BMAL1	3 nM h ⁻¹
v_{dCC}	Maximum rate of degradation of cytosolic phosphorylated CRY	1.4 nM h ⁻¹
v_{dIN}	Maximum rate of degradation of nuclear PER/CRY/CLOCK/BMAL1 complex	1.6 nM h ⁻¹
v_{dPC}	Maximum rate of degradation of cytosolic phosphorylated PER	3.4 nM h ⁻¹
v_{dPCC}	Maximum rate of degradation of cytosolic phosphorylated PER/CRY complex	1.4 nM h ⁻¹
v_{dPCN}	Maximum rate of degradation of nuclear phosphorylated PER/CRY complex	1.4 nM h ⁻¹
v_{dRC}	Maximum rate of degradation of cytosolic REV-ERB α	4.4 nM h ⁻¹
v_{dRN}	Maximum rate of degradation of nuclear REV-ERB α	0.8 nM h ⁻¹
v_{mB}	Maximum rate of <i>Bmall</i> mRNA degradation	1.3 nM h ⁻¹
v_{mC}	Maximum rate of <i>Cry</i> mRNA degradation	2.0 nM h ⁻¹
v_{mR}	Maximum rate of <i>Rev-erba</i> mRNA degradation	1.6 nM h ⁻¹
v_{mP}	Maximum rate of <i>Per</i> mRNA degradation	2.2 nM h ⁻¹
v_{sB}	Maximum rate of <i>Bmall</i> mRNA synthesis	1.55 nM h ⁻¹

v_{sC}	Maximum rate of <i>Cry</i> mRNA synthesis	2.2 nM h ⁻¹
v_{sP}	Maximum rate of <i>Per</i> mRNA synthesis	2.4 nM h ⁻¹
v_{sR}	Maximum rate of <i>Rev-erba</i> mRNA synthesis	1.6 nM h ⁻¹
Cell cycle		
Parameters are (with some modifications) based on those provided in Supporting Table S2 in Gérard and Goldbeter (2009; see http://www.pnas.org/content/106/51/21643/suppl/DCSupplemental).		
GF	Growth factor	1
K_{agf}	Michaelis constant for synthesis of AP1 induced by growth factor	0.1 μM
k_{dap1}	Apparent first-order rate constant for AP1 transcription factor degradation	0.15 h ⁻¹
eps	Scaling factor used to modify the time scale of cell cycle	20.45
v_{sap1}	Rate of synthesis of the transcription factor AP1 depending on growth factor GF	1 μM h ⁻¹
k_{de2f}	Apparent first-order rate constant for non-specific E2F degradation	0.002 h ⁻¹
k_{de2fp}	Apparent first-order rate constant for E2Fp degradation	1.1 h ⁻¹
k_{dprb}	Apparent first-order rate constant for pRB degradation	0.01 h ⁻¹
k_{dprbp}	Apparent first-order rate constant for pRBp degradation	0.06 h ⁻¹
k_{dprbpp}	Apparent first-order rate constant for pRBpp degradation	0.04 h ⁻¹
k_{pc1}	Bimolecular rate constant for binding of pRB to E2F	0.05 μM ⁻¹ h ⁻¹
k_{pc2}	Rate constant for dissociation of complex between pRB and E2F	0.5 h ⁻¹
k_{pc3}	Bimolecular rate constant for binding of pRBp to E2F	0.025 μM ⁻¹ h ⁻¹
k_{pc4}	Rate constant for dissociation of complex between pRBp and E2F	0.5 h ⁻¹
K_1	Michaelis constant for pRB phosphorylation	0.1 μM
K_2	Michaelis constant for pRBp dephosphorylation	0.1 μM
K_3	Michaelis constant for pRBp phosphorylation	0.1 μM
K_4	Michaelis constant for pRBpp dephosphorylation	0.1 μM
V_1	Rate constant for phosphorylation of pRB	2.2 h ⁻¹
V_2	Maximum rate of dephosphorylation of pRBp	2 μM h ⁻¹
V_3	Rate constant for phosphorylation of pRBp	1 h ⁻¹
V_4	Maximum rate of dephosphorylation of pRBpp	2 μM h ⁻¹
K_{1e2f}	Michaelis constant for E2F phosphorylation by Cyclin A/Cdk2	5 μM
K_{2e2f}	Michaelis constant for E2F dephosphorylation	5 μM
V_{1e2f}	Rate constant for phosphorylation of E2F by Cyclin A/Cdk2	4 h ⁻¹
V_{2e2f}	Maximum rate of dephosphorylation of E2F	0.75 μM h ⁻¹
v_{se2f}	Basal rate of synthesis of E2F	0.15 μM h ⁻¹
v_{sprb}	Basal rate of synthesis of pRB	0.8 μM h ⁻¹
$Cdk4_{tot}$	Total concentration of Cdk4-6 kinase	1.5 μM
K_{i7}	Constant of inhibition by pRB of Cyclin D synthesis	0.1 μM
K_{i8}	Constant of inhibition by pRBp of Cyclin D synthesis	2 μM
k_{cd1}	Rate constant for Cyclin D synthesis induced by AP1	0.4 h ⁻¹
k_{cd2}	Rate constant for Cyclin D synthesis induced by E2F	0.005 h ⁻¹
k_{decom1}	Rate constant for dissociation of complex between Cyclin D and Cdk4-6	0.1 h ⁻¹
k_{com1}	Bimolecular rate constant for binding of Cyclin D to Cdk4-6	0.175 μM ⁻¹ h ⁻¹
k_{c1}	Bimolecular rate constant for binding of Cyclin D/Cdk4-6 to p27	0.15 μM ⁻¹ h ⁻¹
k_{c2}	Rate constant for dissociation of complex between Cyclin D/Cdk4-6 and p27	0.05 h ⁻¹

k_{dd}	Apparent first-order rate constant for non-specific Cyclin D protein degradation	0.005 h^{-1}
K_{dd}	Michaelis constant for Cyclin D degradation	$0.1 \text{ }\mu\text{M}$
K_{1d}	Michaelis constant for Cyclin D/Cdk4-6 (inactive) activation	$0.1 \text{ }\mu\text{M}$
K_{2d}	Michaelis constant for Cyclin D/Cdk4-6 (active) inactivation	$0.1 \text{ }\mu\text{M}$
V_{dd}	Maximum rate of degradation of Cyclin D	$5 \text{ }\mu\text{M}\cdot\text{h}^{-1}$
V_{m1d}	Maximum rate of activation of Cyclin D/Cdk4-6 (inactive)	$1 \text{ }\mu\text{M}\cdot\text{h}^{-1}$
V_{m2d}	Maximum rate of inactivation of Cyclin D/Cdk4-6 (active)	$0.2 \text{ }\mu\text{M}\cdot\text{h}^{-1}$
a_e	Factor measuring the contribution of Polo-like kinase 3 (Plk3) to phosphorylation and activation of Cdc25 phosphatase (Pei)	$0.25 \text{ }\mu\text{M}$
$Cdk2_{tot}$	Total concentration of Cdk2 kinase	$2 \text{ }\mu\text{M}$
i_{b1}	Factor measuring the contribution of kinase Myt1 to phosphorylation and inhibition of Cyclin E/Cdk2	$0.5 \text{ }\mu\text{M}$
K_{i9}	Constant of inhibition by pRB of Cyclin E synthesis	$0.1 \text{ }\mu\text{M}$
K_{i10}	Constant of inhibition by pRBp of Cyclin E synthesis	$2 \text{ }\mu\text{M}$
k_{ce}	Rate constant for Cyclin E synthesis induced by E2F	0.29 h^{-1}
k_{c3}	Bimolecular rate constant for binding of Cyclin E/Cdk2 to p27	$0.2 \text{ }\mu\text{M}^{-1} \text{ h}^{-1}$
k_{c4}	Rate constant for dissociation of complex between Cyclin E/Cdk2 and p27	0.1 h^{-1}
k_{decom2}	Rate constant for dissociation of complex between Cyclin E and Cdk2	0.1 h^{-1}
k_{com2}	Bimolecular rate constant for binding of Cyclin E to Cdk2	$0.2 \text{ }\mu\text{M}^{-1} \text{ h}^{-1}$
k_{dde}	Apparent first-order rate constant for non-specific Cyclin E degradation	0.005 h^{-1}
k_{dskp2}	Apparent first-order rate constant for non-specific Skp2 degradation	0.005 h^{-1}
k_{dpe}	Apparent first-order rate constant for degradation of active Cdc25 phosphatase (Pe)	0.075 h^{-1}
k_{dpei}	Apparent first-order rate constant for degradation of inactive Cdc25 phosphatase (Pei)	0.15 h^{-1}
K_{de}	Michaelis constant for Cyclin E degradation	$0.1 \text{ }\mu\text{M}$
$K_{dceskp2}$	Michaelis constant for activation by Skp2 of Cyclin E degradation	$2 \text{ }\mu\text{M}$
K_{dskp2}	Michaelis constant for Skp2 degradation	$0.5 \text{ }\mu\text{M}$
K_{cdh1}	Michaelis constant for activation by Cdh1 of Skp2 degradation	$0.4 \text{ }\mu\text{M}$
K_{1e}	Michaelis constant for Cyclin E/Cdk2 (inactive) activation through dephosphorylation by phosphatase Cdc25 (Pe)	$0.1 \text{ }\mu\text{M}$
K_{2e}	Michaelis constant for Cyclin E/Cdk2 (active) inactivation through phosphorylation by kinases WEE1 and Myt1	$0.1 \text{ }\mu\text{M}$
K_{5e}	Michaelis constant for Cdc25 (Pei) activation through phosphorylation by Cyclin E/Cdk2 and Plk3	$0.1 \text{ }\mu\text{M}$
K_{6e}	Michaelis constant for Cdc25 (Pe) inactivation	$0.1 \text{ }\mu\text{M}$
V_{de}	Maximum rate of Cyclin E degradation	$3 \text{ }\mu\text{M h}^{-1}$
V_{dskp2}	Maximum rate of Skp2 degradation	$1.1 \text{ }\mu\text{M h}^{-1}$
V_{m1e}	Rate constant for activation of Cyclin E/Cdk2 (inactive) through dephosphorylation by phosphatase Cdc25 (Pe)	2 h^{-1}
V_{m2e}	Rate constant for inactivation of Cyclin E/Cdk2 (active) through phosphorylation by kinases WEE1 and Myt1	1.4 h^{-1}
V_{m5e}	Rate constant for activation of phosphatase Cdc25 through phosphorylation by Cyclin E/Cdk2 and Polo-like kinase 3	5 h^{-1}
V_{6e}	Maximum rate of inactivation of phosphatase Cdc25 through phosphorylation	$0.8 \text{ }\mu\text{M h}^{-1}$
v_{spei}	Rate of synthesis of phosphatase Cdc25 acting on Cyclin E/Cdk2	$0.13 \text{ }\mu\text{M h}^{-1}$

v_{sskp2}	Maximum rate of synthesis of Skp2	$0.15 \mu\text{M h}^{-1}$
x_{e1}	Factor measuring basal, Chk1-independent contribution to the rate of inactivation through phosphorylation of phosphatase Cdc25 (Pe)	1
a_a	Factor measuring the contribution of Polo-like kinase 3 (Plk3) to phosphorylation and activation of phosphatase Cdc25 (Pa)	$0.2 \mu\text{M}$
i_{b2}	Factor measuring the contribution of kinase Myt1 to phosphorylation and inhibition of Cyclin A/Cdk2	$0.5 \mu\text{M}$
K_{i11}	Constant of inhibition by pRB of Cyclin A synthesis	$0.1 \mu\text{M}$
K_{i12}	Constant of inhibition by pRBp of Cyclin A synthesis	$2 \mu\text{M}$
K_{i13}	Constant of inhibition by pRB of p27 synthesis	$0.1 \mu\text{M}$
K_{i14}	Constant of inhibition by pRBp of p27 synthesis	$2 \mu\text{M}$
k_{ca}	Rate constant for Cyclin A synthesis induced by E2F	0.0375h^{-1}
k_{decom3}	Rate constant for dissociation of complex between Cyclin A and Cdk2	0.1h^{-1}
k_{com3}	Bimolecular rate constant for binding of Cyclin A to Cdk2	$0.2 \mu\text{M}^{-1} \text{h}^{-1}$
k_{c5}	Bimolecular rate constant for binding of active Cyclin A/Cdk2 to p27	$0.15 \mu\text{M}^{-1} \text{h}^{-1}$
k_{c6}	Rate constant for dissociation of complex between Cyclin A/Cdk2 and p27	0.125h^{-1}
k_{dda}	Apparent first-order rate constant for non-specific Cyclin A degradation	0.005h^{-1}
k_{ddp27}	Apparent first-order rate constant for non-specific p27 degradation	0.06h^{-1}
k_{ddp27p}	Apparent first-order rate constant for non-specific p27p degradation	0.01h^{-1}
k_{dcdh1a}	Apparent first-order rate constant for degradation of active Cdh1	0.1h^{-1}
k_{dcdh1i}	Apparent first-order rate constant for degradation of inactive Cdh1	0.2h^{-1}
k_{dpa}	Apparent first-order rate constant for degradation of active phosphatase Cdc25 (Pa)	0.075h^{-1}
k_{dpai}	Apparent first-order rate constant for degradation of inactive phosphatase Cdc25 (Pai)	0.15h^{-1}
K_{da}	Michaelis constant for Cyclin A degradation	$1.1 \mu\text{M}$
K_{dp27p}	Michaelis constant for p27p degradation	$0.1 \mu\text{M}$
$K_{dp27skp2}$	Michaelis constant for activation by Skp2 of p27p degradation	$0.1 \mu\text{M}$
K_{acdc20}	Michaelis constant for activation by Cdc20 of Cyclin A degradation	$2 \mu\text{M}$
K_{1a}	Michaelis constant for Cyclin A/Cdk2 (inactive) activation through dephosphorylation by Cdc25 (Pa)	$0.1 \mu\text{M}$
K_{2a}	Michaelis constant for Cyclin A/Cdk2 (active) inactivation through phosphorylation by kinases WEE1 and Myt1	$0.1 \mu\text{M}$
K_{1cdh1}	Michaelis constant for Cdh1 (inactive) activation through dephosphorylation	$0.01 \mu\text{M}$
K_{2cdh1}	Michaelis constant for Cdh1 (active) inactivation through phosphorylation by Cyclin A/Cdk2 and Cyclin B/Cdk1	$0.01 \mu\text{M}$
K_{5a}	Michaelis constant for Cdc25 (Pai) activation through phosphorylation by Cyclin A/Cdk2 and kinase Plk3	$0.1 \mu\text{M}$
K_{6a}	Michaelis constant for Cdc25 (Pa) inactivation	$0.1 \mu\text{M}$
K_{1p27}	Michaelis constant for p27 phosphorylation by Cyclin E/Cdk2	$0.5 \mu\text{M}$
K_{2p27}	Michaelis constant of p27p dephosphorylation	$0.5 \mu\text{M}$
V_{dp27p}	Maximum rate of p27p degradation	$5 \mu\text{M h}^{-1}$
V_{da}	Maximum rate of Cyclin A degradation	$2.5 \mu\text{M h}^{-1}$
V_{m1a}	Rate constant for activation of Cyclin A/Cdk2 (inactive) through dephosphorylation by phosphatase Cdc25 (Pa)	2h^{-1}
V_{m2a}	Rate constant for inactivation of Cyclin A/Cdk2 (active) through phosphorylation by kinases WEE1 and Myt1	1.85h^{-1}

V_{m5a}	Rate constant for activation of phosphatase Cdc25 (Pai) through phosphorylation by Cyclin A/Cdk2 and kinase Plk3	4 h^{-1}
V_{6a}	Maximum rate of phosphatase Cdc25 (Pa) inactivation	$1 \mu\text{M h}^{-1}$
v_{scdh1a}	Rate of synthesis of active Cdh1	$0.11 \mu\text{M h}^{-1}$
v_{spai}	Rate of synthesis of phosphatase Cdc25 (Pai) acting on Cyclin A/Cdk2	$0.105 \mu\text{M h}^{-1}$
v_{s1p27}	Basal rate of synthesis of p27	$0.8 \mu\text{M h}^{-1}$
v_{s2p27}	Rate constant for synthesis of p27 induced by E2F	0.1 h^{-1}
V_{1cdh1}	Maximum activation rate of Cdh1 (inactive) through dephosphorylation	$1.25 \mu\text{M h}^{-1}$
V_{2cdh1}	Rate constant for inactivation of Cdh1 (active) through phosphorylation by Cyclin A/Cdk2 and Cyclin B/Cdk1	8 h^{-1}
V_{1p27}	Rate constant for inactivation of p27 through phosphorylation by Cyclin E/Cdk2	100 h^{-1}
V_{2p27}	Maximum rate of activation of p27p through dephosphorylation	$0.1 \mu\text{M h}^{-1}$
x_{a1}	Factor measuring basal, Chk1-independent contribution to the inactivation rate of phosphatase Cdc25 (Pa)	1
a_b	Factor measuring the contribution of Polo-like kinase 1 (Plk1) to phosphorylation and activation of Cdc25 phosphatase (Pbi)	$0.11 \mu\text{M}$
$Cdk1_{tot}$	Total concentration of the Cdk1 kinase	$0.5 \mu\text{M}$
i_b	Factor measuring the contribution of kinase(s) other than Cdk1 to phosphorylation and inactivation of kinase WEE1	$0.75 \mu\text{M}$
i_{b3}	Factor measuring the contribution of kinase Myt1 to phosphorylation and inhibition of Cyclin B/Cdk1	$0.5 \mu\text{M}$
k_{c7}	Bimolecular rate constant for binding of Cyclin B/Cdk1 (active) to p27	$0.12 \mu\text{M}^{-1} \text{ h}^{-1}$
k_{c8}	Rate constant for dissociation of complex between Cyclin B/Cdk1 and p27	0.2 h^{-1}
k_{decom4}	Rate constant for dissociation of complex between Cyclin B and Cdk1	0.1 h^{-1}
k_{com4}	Bimolecular rate constant for binding of Cyclin B to Cdk1	$0.25 \mu\text{M}^{-1} \text{ h}^{-1}$
$k_{dcdc20a}$	Apparent first-order rate constant for degradation of active Cdc20	0.05 h^{-1}
$k_{dcdc20i}$	Apparent first-order rate constant for degradation of inactive Cdc20	0.14 h^{-1}
k_{ddb}	Apparent first-order rate constant for non-specific Cyclin B degradation	0.005 h^{-1}
k_{dpb}	Apparent first-order rate constant for degradation of active phosphatase Cdc25 (Pb)	0.1 h^{-1}
k_{dpbi}	Apparent first-order rate constant for degradation of inactive phosphatase Cdc25 (Pbi)	0.2 h^{-1}
k_{dwee1}	Apparent first-order rate constant for degradation of active kinase WEE1	0.1 h^{-1}
k_{dwee1p}	Apparent first-order rate constant for degradation of inactive kinase WEE1	0.2 h^{-1}
K_{db}	Michaelis constant for Cyclin B degradation	$0.005 \mu\text{M}$
$K_{dbcdc20}$	Michaelis constant for activation by Cdc20 (active) of Cyclin B degradation	$0.2 \mu\text{M}$
K_{dbcdh1}	Michaelis constant for activation by Cdh1 (active) of Cyclin B degradation	$0.1 \mu\text{M}$
K_{1b}	Michaelis constant for Cyclin B/Cdk1 (inactive) activation through dephosphorylation by phosphatase Cdc25 (Pb)	$0.1 \mu\text{M}$
K_{2b}	Michaelis constant for Cyclin B/Cdk1 (active) inactivation through phosphorylation by kinases WEE1 and Myt1	$0.1 \mu\text{M}$
K_{3b}	Michaelis constant for Cdc20 (inactive) activation through phosphorylation by Cyclin B/Cdk1	$0.1 \mu\text{M}$
K_{4b}	Michaelis constant for Cdc20 (active) inactivation through dephosphorylation	$0.1 \mu\text{M}$
K_{5b}	Michaelis constant for Cdc25 (Pbi) activation through phosphorylation by Cyclin B/Cdk1 and kinase Plk1	$0.1 \mu\text{M}$

K_{6b}	Michaelis constant for Cdc25 (Pb) inactivation	0.1 μM
K_{7b}	Michaelis constant for Wee1 inactivation through phosphorylation by Cyclin B/Cdk1 and kinase Plk1	0.1 μM
K_{8b}	Michaelis constant for Wee1 activation through dephosphorylation	0.1 μM
V_{db}	Maximum rate of Cyclin B degradation	0.06 $\mu\text{M h}^{-1}$
V_{m1b}	Rate constant for activation of Cyclin B/Cdk1 (inactive) through dephosphorylation by phosphatase Cdc25 (Pb)	3.9 h^{-1}
V_{m2b}	Rate constant for inactivation of Cyclin B/Cdk1 (active) through phosphorylation by kinases WEE1 and Myt1	2.1 h^{-1}
v_{sdc20i}	Rate of synthesis of Cdc20 (inactive)	0.1 $\mu\text{M h}^{-1}$
V_{m3b}	Rate constant for activation of Cdc20 (inactive) through phosphorylation by Cyclin B/Cdk1	8 h^{-1}
V_{m4b}	Maximum rate of Cdc20 (active) inactivation through dephosphorylation	0.7 $\mu\text{M h}^{-1}$
V_{m5b}	Rate constant for activation of phosphatase Cdc25 (Pbi) through phosphorylation by Cyclin B/Cdk1 and kinase Plk1	5 h^{-1}
V_{6b}	Maximum inactivation rate of phosphatase Cdc25 (Pb)	1 $\mu\text{M h}^{-1}$
V_{m7b}	Rate constant for inactivation of kinase WEE1 through phosphorylation by Cyclin B/Cdk1 and kinase Plk1	1.2 h^{-1}
V_{m8b}	Maximum rate of kinase Wee1 activation through dephosphorylation	1 $\mu\text{M h}^{-1}$
v_{spbi}	Rate of synthesis of phosphatase Cdc25 (Pbi) acting on Cyclin B/Cdk1	0.12 $\mu\text{M h}^{-1}$
x_{b1}	Factor measuring basal, Chk1-independent contribution to the rate of phosphatase Cdc25 (Pb) inactivation	1

Coupling the mammalian cell cycle to the circadian clock through Wee1

Parameters are (with some modifications) based on those given in Supporting Information to Gérard and Goldbeter (2009; see <http://www.pnas.org/content/106/51/21643/suppl/DCSupplemental>) and in Figure 2 in Gérard and Goldbeter (2012).

K_{dmw}	Michaelis constant for the degradation of <i>Wee1</i> mRNA (Mw)	0.5 μM
K_{aw}	Threshold constant for activation by CLOCK/BMAL1 (Bn) of <i>Wee1</i> mRNA (Mw) synthesis	4 nM
nmw	Degree of cooperativity for the activation by CLOCK/BMAL1 (Bn) of <i>Wee1</i> mRNA (Mw) synthesis	4
V_{dmw}	Maximum rate of degradation of <i>Wee1</i> mRNA (Mw)	$2.445 \times 10^{-2} \mu\text{M h}^{-1}$
v_{swee1}	Rate of synthesis of <i>Wee1</i> mRNA (Mw) independent from coupling to the circadian clock	$5.721 \times 10^{-4} \mu\text{M h}^{-1}$
v_{sw}	Rate of synthesis of Mw depending on CLOCK/BMAL1 (Bn)	$6.846 \times 10^{-3} \mu\text{M h}^{-1}$
k_{sw}	Rate constant for synthesis of WEE1 protein	5 h^{-1}

Coupling the mammalian cell cycle to the circadian clock through Cyclin B

This study

K_{dmcb}	Michaelis constant for the degradation of <i>Cyclin B</i> mRNA (Mcb)	0.5 μM
K_{acb}	Threshold constant for activation by CLOCK/BMAL1 (Bn) of <i>Cyclin B</i> mRNA (Mcb) synthesis	4 nM
ncb	Degree of cooperativity for the activation by CLOCK/BMAL1 (Bn) of <i>Cyclin B</i> mRNA (Mcb) synthesis	4

V_{dmcb}	Maximum rate of degradation of <i>cyclin B</i> mRNA (Mcb)	2.445×10^{-2} $\mu\text{M h}^{-1}$
v_{cb}	Rate of synthesis of <i>cyclin B</i> mRNA independent from coupling to the circadian clock	5.263×10^{-4} $\mu\text{M h}^{-1}$
v_{scb}	Rate of synthesis of <i>cyclin B</i> mRNA depending on CLOCK/BMAL1 (Bn)	9.78×10^{-5} $\mu\text{M h}^{-1}$
k_{cb}	Rate of synthesis of Cyclin B protein	5 h^{-1}

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