

SI Appendix

1. Description and coupling of the four modules of the Cdk network

The model for the Cdk network (see text and simplified scheme in Fig. 1) contains four modules centered, respectively, on cyclin D/Cdk4-6, cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1. A more detailed version of the global model is schematized in Fig. S1. Together with their coupling, the four modules are described below and represented in further detail in Fig. S2.

1.1 Cyclin D/Cdk4-6 module (Module 1)

In the first module (Fig. S2A) controlling progression in G1, a growth factor (GF) promotes the synthesis of the transcription factor AP1. The latter induces the synthesis of cyclin D, which forms a complex with the kinases Cdk4 and Cdk6; these two kinases will be treated as a single molecular entity. In comparison with cyclins E, A and B, fewer details are available for the control of cyclin D degradation. In the absence of any precise indication we assume that cyclin D degradation is not activated by any Cdk. We verified that the dynamics remains largely unchanged when cyclin D degradation is brought about by Skp2, which itself is activated indirectly by cyclin A/Cdk2 and cyclin B/Cdk1 via Cdh1 (see Section 1.2).

Also activating the synthesis of cyclin D —and, even more, cyclins E and A in subsequent modules— is the transcription factor E2F, which is inhibited by the formation of a complex with the active, nonphosphorylated form of the Retinoblastoma protein, pRB (see Figs. S1 and S2A). The cyclin D/Cdk4-6 complex phosphorylates and thereby inhibits the pRB protein (1, 2). Inhibition of pRB activates the transcription factor E2F, because the phosphorylated form of pRB binds less efficiently to E2F. We consider that pRB and, to a lesser degree, pRBp —but not the fully phosphorylated form pRBpp— bind to E2F to form inactive complexes. Accumulation of free E2F results in further transcriptional activation of target genes like cyclin D, cyclin E, and cyclin A (3). Cyclin A/Cdk2 promotes the phosphorylation and subsequent degradation of E2F (see Section 1.2).

1.2 Cyclin E/Cdk2 and Cyclin A/Cdk2 modules (Modules 2 and 3)

In the second module (Fig. S2B), the cyclin E/Cdk2 complex further phosphorylates and inhibits pRB (see also Figs. 1, S1 and S2A). This second phosphorylation of pRB reinforces the partial

inhibition due to prior phosphorylation by cyclin D/Cdk4-6. The peak of activity of the cyclin E/Cdk2 complex brings about the G1/S transition. Cyclin E/Cdk2 forms an inactive complex with the inhibitors p21 and p27, which are considered as a single molecular entity. Phosphorylation of p21/p27 by cyclin E/Cdk2 marks the protein inhibitors for degradation in a process dependent on Skp2, which belongs to the proteasome complex (4). The degradation of Skp2 is itself controlled by Cdh1, which also belongs to the proteasome complex (5). Because Skp2 also controls the degradation of cyclin E, Cdh1 promotes cyclin E accumulation as long as it is not inactivated by cyclin A/Cdk2 (6) in the third module (see Figs. S1 and S2C).

In the third module (Fig. S2C), the synthesis of cyclin A triggered by E2F and the subsequent formation of the cyclin A/Cdk2 complex allow progression in S phase. Cyclin A/Cdk2 inhibits the Cdh1 protein, which degrades the protein Skp2 that promotes degradation of cyclin E (see Fig. S2B). The resulting decrease in cyclin E/Cdk2 allows completion of the G1-S transition. The cyclin A/Cdk2 complex also ensures a correct S-G2 transition. Indeed, this complex promotes the phosphorylation and subsequent degradation of E2F (7, 8) (see Figs. S1 and S2A). At that point, E2F ceases to induce the synthesis of cyclins D, E and A.

1.3 Cyclin B/Cdk1 module (Module 4)

Because Cdh1 also degrades cyclin B (see Fig. S2D), the inhibition of Cdh1 by cyclin A/Cdk2 leads to an increase in the amount of cyclin B in G2. In the fourth and last module (see Figs. 1, S1 and S2D) the accumulation of cyclin B allows the formation of a sufficient amount of cyclin B/Cdk1 complex to bring about the G2/M transition and the entry into mitosis. The cyclin B/Cdk1 complex activates, by phosphorylation, the protein Cdc20 that promotes the degradation of cyclin A as well as cyclin B (see Figs. S1 and S2 C and D) (9-11). This negative feedback loop resets the activity of the corresponding Cdks to a low level, and thereby allows the cell to start a new cycle.

In contrast to cyclin E and cyclin A, the synthesis of cyclin B does not appear to be primarily controlled by E2F or pRB. We therefore considered the simplest case of a constant synthesis of cyclin B. As detailed in Section 6, we verified that the model yields similar results in regard to self-sustained oscillations in the Cdk network when cyclin B synthesis is placed under control of E2F and pRB, like cyclins E and A, or under control of a transcription factor activated by cyclin A/Cdk2, a possibility indicated by some experiments (12).

1.4 Additional regulations and couplings between the Cdk modules

Besides being controlled by variations in the levels of their associated cyclins, the activity of Cdks can be further regulated through reversible association with the Cdk-inhibitor p27 (or p21). While p27 inhibits the activity of Cdk2 and Cdk1, it can also bind to the cyclin D/Cdk4-6 complex, without inhibiting its activity (13). Additional regulation of cyclin/Cdk complexes occurs through phosphorylation-dephosphorylation. Thus, in the fourth module, the kinase Wee1 inhibits Cdk1 (14), while the phosphatase Cdc25 activates it. Two positive feedback loops originate from the concomitant inhibition of Wee1 and activation of Cdc25 through phosphorylation by Cdk1 (15-17). The kinase Wee1 also inhibits Cdk2 (18), but no evidence exists for inhibition of Wee1 by the latter kinase complexed either to cyclin A or cyclin E.

In mammalian cells, three forms of Cdc25 phosphatases are known, Cdc25A, Cdc25B and Cdc25C. These Cdc25 forms have some overlapping functions in activating the cyclin E,A/Cdk2 complexes and the cyclin B/Cdk1 complex (19, 20), which in turn activate the Cdc25 phosphatases (15, 21, 22). We consider in the model that each Cdc25 phosphatase is associated with one cyclin/Cdk complex, and incorporate the activation of each Cdc25 by the corresponding cyclin/Cdk complex. Recent studies (23) have shown that Cdc25A can replace the function of Cdc25B and C in knockout mice. Although we use different variables for the three different forms of Cdc25, we verified that the Cdk network can still oscillate in the presence of a single Cdc25, in agreement with experimental observations (23).

We will consider that in contrast to Cdk1 and Cdk2, Cdk4-6 is not inhibited through phosphorylation by kinase Wee1 or activated through dephosphorylation by a form of phosphatase Cdc25. Instead, we take into account the activation of cyclin D/Cdk4-6 by the cyclin-dependent activating kinase CAK (24). This kinase also activates the other cyclin/Cdk complexes (24), but we will consider that the transitions between active and inactive forms for the other cyclin/Cdk complexes are primarily controlled by Wee1 and Cdc25. In addition, we consider an implicit inhibition of the cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1 complexes by the kinase Myt1 (25, 26). This additional inhibition is expressed by the terms i_{b1} , i_{b2} and i_{b3} in Eqs. **14**, **15**, **21**, **22**, **31**, and **32**. We assume that the reversal of Myt phosphorylation is carried out, like for Wee1, by phosphatases Cdc25 (25, 26).

We also take into account implicitly the activation of the Cdc25 phosphatases acting on cyclin E/Cdk2 and cyclin A/Cdk2 by the Polo-like kinase 3, Plk3 (27), through the terms a_e and a_a in Eqs. **18**, **19** and **28**, **29**. Similarly, the term a_b in Eqs. **36** and **37** expresses implicitly the

activation of the Cdc25 phosphatase acting on cyclin B/Cdk1 by the Polo-like kinase 1, Plk1 (28, 29). Finally, the inhibition of the kinase Wee1 by kinases other than Cdk1, e.g. Nim1 (30, 31), is also taken into account implicitly through the term i_b in Eqs. **38** and **39**.

In the model several proteins are controlled by reversible phosphorylation. This modification leads to activation of the three Cdc25 phosphatases and of Cdc20, and to inactivation of Wee1, pRB, p27 and Cdh1. We assume that when a protein of the Cdk network is phosphorylated, it is synthesized in its unphosphorylated state, regardless of whether it is then active or inactive.

2. Kinetic equations

The model contains 39 variables, which are listed in Table S1. The system of evolution equations thus consists of 39 ordinary differential equations, listed below as Eqs. **1–39**. Additional variables (see also Table S1) must be incorporated to describe the effect of the ATR/Chk1 checkpoint (5 new variables) and the coupling to the circadian clock (1 new variable). These processes correspond to the additional equations **40–44**, and **45**, respectively.

The 39 variables (see Table S1) are the concentrations of the following proteins involved in cell cycle control (see also Figs. 1 and S2): Cyclins D, E, A, and B; the complexes formed by the cyclin-dependent kinases Cdk4-6, Cdk2, Cdk1 with their corresponding cyclins; E2F; the different forms of pRB; the complexes between pRB and E2F; the inhibitor p27 (or p21) and its complexes with the active cyclin/Cdk complexes; the active and inactive forms of the three phosphatases Cdc25 and of the kinase Wee1; the protein Skp2 involved in the degradation of cyclin E and p27; and the active and inactive forms of the proteins Cdh1 and Cdc20 involved in the degradation of cyclins A and B.

To limit the number of variables in the model, we only consider explicitly the proteins but not the corresponding mRNAs. Thus, induction of gene expression by growth factors and by E2F, as well as repression by pRB, are incorporated directly as functions modulating the synthesis of cyclins. The equations describing the evolution of pRB and E2F as well as their effects are closely related, with some slight modifications, to those considered by Swat et al. (32). Focusing on proteins also allows us to readily incorporate post-translational regulation through phosphorylation-dephosphorylation or through the formation of complexes with p21/p27. For similar reasons of simplicity we will not distinguish between the nuclear and cytosolic compartments of the cell. The model describes the dynamics of the cyclin/Cdk network within one cell. The dynamics of a population of cells can be obtained by considering the

behavior of N cells, each of which is described by the same set of kinetic equations. Taking into account a distribution of parameter values around some mean values would result in progressive desynchronization in such cell populations, as in a study in which the cell cycle is modeled as an automaton switching between the successive phases of the cell cycle (33). Here we restrict our analysis of the model for the mammalian cell cycle to the dynamics of a single cell or to a population of synchronized cells.

In all kinetic equations, except in Eq. 45 for Wee1 mRNA (M_w) where the variable M_w is governed independently by the circadian clock, we multiply the right-hand term by a scaling factor eps . This dimensionless factor allows us to vary the period of the cell cycle oscillator without changing the qualitative nature of the cell cycle dynamics. In all simulations we kept the value of eps constant and set it equal to an arbitrary value that yields a period of the cell cycle oscillator of about 19 h.

The time evolution of the Cdk network model is governed by 39 nonlinear, ordinary differential equations. To improve clarity, these kinetic equations are listed below in sections 2.1–2.6 for the effect of the growth factor GF, the antagonistic effects of E2F and pRB, and the successive modules of the model. The 39 variables of the model are defined in Table S1, and the parameter values used in numerical simulations are listed in Table S2 in alphabetic order, module per module. Incorporating the ATR/Chk1 DNA replication checkpoint and coupling the cell cycle model to the circadian clock (see text) leads to the addition of 5 and 1 more kinetic equations, respectively. These equations are listed in Sections 3 and 5 below. The additional variables are listed in Table S1, while the additional parameters are given, with their numerical value, in Table S2.

We assume mass action or Michaelis-Menten kinetics for each of the reactions. Zero-order ultrasensitivity in phosphorylation-dephosphorylation occurs when the kinase and phosphatase reactions operate near saturation (34). The phenomenon certainly favors but does not play a major role in the occurrence of oscillations here, as it did in simpler models for the mitotic oscillator (35). Indeed, for most of the reactions, the values selected for the Michaelis constants are generally of the same order or ten times smaller than their protein substrate; these values correspond to moderate ultrasensitivity (34).

2.1 Mitotic stimulation by growth factor, GF (Fig. S2A)

$$\frac{dAP1}{dt} = (v_{sap1} \cdot \left(\frac{GF}{K_{agf} + GF}\right) - k_{dap1} \cdot AP1) \cdot eps \quad [1]$$

2.2 Antagonistic regulation exerted by pRB and E2F (Fig. S2 A and B)

$$\begin{aligned} \frac{dpRB}{dt} &= (v_{sprb} - k_{pc1} \cdot pRB \cdot E2F + k_{pc2} \cdot pRBc1 - V_1 \cdot \left(\frac{pRB}{K_1 + pRB}\right)) \cdot (Md + Mdp27) \\ &+ V_2 \cdot \left(\frac{pRBp}{K_2 + pRBp}\right) - k_{dprb} \cdot pRB) \cdot eps \end{aligned} \quad [2]$$

$$\frac{dpRBc1}{dt} = (k_{pc1} \cdot pRB \cdot E2F - k_{pc2} \cdot pRBc1) \cdot eps \quad [3]$$

$$\begin{aligned} \frac{dpRBp}{dt} &= (V_1 \cdot \left(\frac{pRB}{K_1 + pRB}\right) \cdot (Md + Mdp27) - V_2 \cdot \left(\frac{pRBp}{K_2 + pRBp}\right) - V_3 \cdot \left(\frac{pRBp}{K_3 + pRBp}\right) \cdot Me \\ &+ V_4 \cdot \left(\frac{pRBpp}{K_4 + pRBpp}\right) - k_{pc3} \cdot pRBp \cdot E2F + k_{pc4} \cdot pRBc2 - k_{dprbp} \cdot pRBp) \cdot eps \end{aligned} \quad [4]$$

$$\frac{dpRBc2}{dt} = (k_{pc3} \cdot pRBp \cdot E2F - k_{pc4} \cdot pRBc2) \cdot eps \quad [5]$$

$$\frac{dpRBpp}{dt} = (V_3 \cdot \left(\frac{pRBp}{K_3 + pRBp}\right) \cdot Me - V_4 \cdot \left(\frac{pRBpp}{K_4 + pRBpp}\right) - k_{dprbpp} \cdot pRBpp) \cdot eps \quad [6]$$

$$\begin{aligned} \frac{dE2F}{dt} &= (v_{se2f} - k_{pc1} \cdot pRB \cdot E2F + k_{pc2} \cdot pRBc1 - k_{pc3} \cdot pRBp \cdot E2F + k_{pc4} \cdot pRBc2 \\ &- V_{1e2f} \cdot Ma \cdot \left(\frac{E2F}{K_{1e2f} + E2F}\right) + V_{2e2f} \cdot \left(\frac{E2Fp}{K_{2e2f} + E2Fp}\right) - k_{de2f} \cdot E2F) \cdot eps \end{aligned} \quad [7]$$

$$\begin{aligned} \frac{dE2Fp}{dt} &= (V_{1e2f} \cdot Ma \cdot \left(\frac{E2F}{K_{1e2f} + E2F}\right) - V_{2e2f} \cdot \left(\frac{E2Fp}{K_{2e2f} + E2Fp}\right) \\ &- k_{de2fp} \cdot E2Fp) \cdot eps \end{aligned} \quad [8]$$

2.3 Module Cyclin D/Cdk4-6 : G1 phase (Fig. S2A)

$$\begin{aligned} \frac{dCd}{dt} = & (k_{cd1} \cdot AP1 + k_{cd2} \cdot E2F \cdot (\frac{K_{i7}}{K_{i7} + pRB}) \cdot (\frac{K_{i8}}{K_{i8} + pRBp}) \\ & - k_{com1} \cdot Cd \cdot (Cdk4_{tot} - (Mdi + Md + Mdp27)) + k_{decom1} \cdot Mdi \\ & - V_{dd} \cdot (\frac{Cd}{K_{dd} + Cd}) - k_{ddd} \cdot Cd) \cdot eps \end{aligned} \quad [9]$$

$$\begin{aligned} \frac{dMdi}{dt} = & (k_{com1} \cdot Cd \cdot (Cdk4_{tot} - (Mdi + Md + Mdp27)) \\ & - k_{decom1} \cdot Mdi + V_{m2d} \cdot (\frac{Md}{K_{2d} + Md}) - V_{m1d} \cdot (\frac{Mdi}{K_{1d} + Mdi})) \cdot eps \end{aligned} \quad [10]$$

$$\frac{dMd}{dt} = (V_{m1d} \cdot (\frac{Mdi}{K_{1d} + Mdi}) - V_{m2d} \cdot (\frac{Md}{K_{2d} + Md}) - k_{c1} \cdot Md \cdot p27 + k_{c2} \cdot Mdp27) \cdot eps \quad [11]$$

$$\frac{dMdp27}{dt} = (k_{c1} \cdot Md \cdot p27 - k_{c2} \cdot Mdp27) \cdot eps \quad [12]$$

2.4 Module Cyclin E/Cdk2: G1 phase and G1/S transition (Fig. S2B)

$$\begin{aligned} \frac{dCe}{dt} = & (k_{ce} \cdot E2F \cdot (\frac{K_{i9}}{K_{i9} + pRB}) \cdot (\frac{K_{i10}}{K_{i10} + pRBp}) \\ & - k_{com2} \cdot Ce \cdot (Cdk2_{tot} - (Mei + Me + Mep27 + Mai + Ma + Map27)) \\ & + k_{decom2} \cdot Mei - V_{de} \cdot (\frac{Skp2}{K_{deskp2} + Skp2}) \cdot (\frac{Ce}{K_{de} + Ce}) - k_{dde} \cdot Ce) \cdot eps \end{aligned} \quad [13]$$

$$\begin{aligned} \frac{dMei}{dt} = & (k_{com2} \cdot Ce \cdot (Cdk2_{tot} - (Mei + Me + Mep27 + Mai + Ma + Map27)) \\ & - k_{decom2} \cdot Mei + Vm2e \cdot (Wee1 + i_{b1}) \cdot (\frac{Me}{K_{2e} + Me}) - V_{m1e} \cdot Pe \cdot (\frac{Mei}{K_{1e} + Mei})) \cdot eps \end{aligned} \quad [14]$$

$$\begin{aligned} \frac{dMe}{dt} = & (V_{m1e} \cdot Pe \cdot (\frac{Mei}{K_{1e} + Mei}) - V_{m2e} \cdot (Wee1 + i_{b1}) \cdot (\frac{Me}{K_{2e} + Me}) \\ & - k_{c3} \cdot Me \cdot p27 + k_{c4} \cdot Mep27) \cdot eps \end{aligned} \quad [15]$$

$$\frac{dSkp2}{dt} = (v_{sskp2} - V_{dskp2} \cdot (\frac{Skp2}{K_{dskp2} + Skp2}) \cdot (\frac{Cdh1a}{K_{cdh1} + Cdh1a}) - k_{dskp2} \cdot Skp2) \cdot eps \quad [16]$$

$$\frac{dMep27}{dt} = (k_{c3} \cdot Me \cdot p27 - k_{c4} \cdot Mep27) \cdot eps \quad [17]$$

$$\begin{aligned} \frac{dPei}{dt} = & (v_{spei} + V_{6e} \cdot (x_{e1} + x_{e2} \cdot Chk1) \cdot (\frac{Pe}{K_{6e} + Pe}) \\ & - V_{m5e} \cdot (Me + a_e) \cdot (\frac{Pei}{K_{5e} + Pei}) - k_{dpei} \cdot Pei) \cdot eps \end{aligned} \quad [18]$$

$$\begin{aligned} \frac{dPe}{dt} = & (V_{m5e} \cdot (Me + a_e) \cdot (\frac{Pei}{K_{5e} + Pei}) \\ & - V_{6e} \cdot (x_{e1} + x_{e2} \cdot Chk1) \cdot (\frac{Pe}{K_{6e} + Pe}) - k_{dpe} \cdot Pe) \cdot eps \end{aligned} \quad [19]$$

2.5 Module Cyclin A/Cdk2 : S phase and S/G2 transition (Fig. S2C)

$$\begin{aligned} \frac{dCa}{dt} = & (k_{ca} \cdot E2F \cdot (\frac{K_{i11}}{K_{i11} + pRB}) \cdot (\frac{K_{i12}}{K_{i12} + pRBp}) \\ & - k_{com3} \cdot Ca \cdot (Cdk2_{tot} - (Mei + Me + Mep27 + Mai + Ma + Map27)) \\ & + k_{decom3} \cdot Mai - V_{da} \cdot (\frac{Ca}{K_{da} + Ca}) \cdot (\frac{Cdc20a}{K_{acdc20} + Cdc20a}) - k_{dda} \cdot Ca) \cdot eps \end{aligned} \quad [20]$$

$$\begin{aligned} \frac{dMai}{dt} = & (k_{com3} \cdot Ca \cdot (Cdk2_{tot} - (Mei + Me + Mep27 + Mai + Ma + Map27)) \\ & - k_{decom3} \cdot Mai + V_{m2a} \cdot (Wee1 + i_{b2}) \cdot (\frac{Ma}{K_{2a} + Ma}) - V_{m1a} \cdot Pa \cdot (\frac{Mai}{K_{1a} + Mai})) \cdot eps \end{aligned} \quad [21]$$

$$\begin{aligned} \frac{dMa}{dt} &= (V_{m1a} \cdot Pa \cdot (\frac{Mai}{K_{1a} + Mai}) - V_{m2a} \cdot (Wee1 + i_{b2}) \cdot (\frac{Ma}{K_{2a} + Ma}) \\ &- k_{c5} \cdot Ma \cdot p27 + k_{c6} \cdot Map27) \cdot eps \end{aligned} \quad [22]$$

$$\frac{dMap27}{dt} = (k_{c5} \cdot Ma \cdot p27 - k_{c6} \cdot Map27) \cdot eps \quad [23]$$

$$\begin{aligned} \frac{dp27}{dt} &= (v_{s1p27} + v_{s2p27} \cdot E2F \cdot (\frac{K_{i13}}{K_{i13} + pRB}) \cdot (\frac{K_{i14}}{K_{i14} + pRBp}) - k_{c1} \cdot Md \cdot p27 + k_{c2} \cdot Mdp27 \\ &- k_{c3} \cdot Me \cdot p27 + k_{c4} \cdot Mep27 - k_{c5} \cdot Ma \cdot p27 + k_{c6} \cdot Map27 - k_{c7} \cdot Mb \cdot p27 \\ &+ k_{c8} \cdot Mbp27 - V_{1p27} \cdot Me \cdot (\frac{p27}{K_{1p27} + p27}) + V_{2p27} \cdot (\frac{p27p}{K_{2p27} + p27p}) - k_{ddp27} \cdot p27) \cdot eps \end{aligned} \quad [24]$$

$$\begin{aligned} \frac{dp27p}{dt} &= (V_{1p27} \cdot Me \cdot (\frac{p27}{K_{1p27} + p27}) - V_{2p27} \cdot (\frac{p27p}{K_{2p27} + p27p}) \\ &- V_{dp27p} \cdot (\frac{Skp2}{K_{dp27skp2} + Skp2}) \cdot (\frac{p27p}{K_{dp27p} + p27p}) - k_{ddp27p} \cdot p27p) \cdot eps \end{aligned} \quad [25]$$

$$\begin{aligned} \frac{dCdh1i}{dt} &= (V_{2cdh1} \cdot (\frac{Cdh1a}{K_{2cdh1} + Cdh1a}) \cdot (Ma + Mb) - V_{1cdh1} \cdot (\frac{Cdh1i}{K_{1cdh1} + Cdh1i}) \\ &- k_{dcdh1i} \cdot Cdh1i) \cdot eps \end{aligned} \quad [26]$$

$$\begin{aligned} \frac{dCdh1a}{dt} &= (v_{scdh1a} + V_{1cdh1} \cdot (\frac{Cdh1i}{K_{1cdh1} + Cdh1i}) - V_{2cdh1} \cdot (\frac{Cdh1a}{K_{2cdh1} + Cdh1a}) \cdot (Ma + Mb) \\ &- k_{dcdh1a} \cdot Cdh1a) \cdot eps \end{aligned} \quad [27]$$

$$\begin{aligned} \frac{dPai}{dt} &= (v_{spai} + V_{6a} \cdot (x_{a1} + x_{a2} \cdot Chk1) \cdot (\frac{Pa}{K_{6a} + Pa}) \\ &- V_{m5a} \cdot (Ma + a_a) \cdot (\frac{Pai}{K_{5a} + Pai}) - k_{dpai} \cdot Pai) \cdot eps \end{aligned} \quad [28]$$

$$\begin{aligned} \frac{dPa}{dt} &= (V_{m5a} \cdot (Ma + a_a) \cdot \left(\frac{Pai}{K_{5a} + Pai}\right) \\ &- V_{6a} \cdot (x_{a1} + x_{a2} \cdot Chk1) \cdot \left(\frac{Pa}{K_{6a} + Pa}\right) - k_{dpa} \cdot Pa) \cdot eps \end{aligned} \quad [29]$$

2.6 Module Cyclin B/Cdk1 : G2 phase and G2/M transition (Fig. S2D)

$$\begin{aligned} \frac{dCb}{dt} &= (v_{cb} - k_{com4} \cdot Cb \cdot (Cdk1_{tot} - (Mbi + Mb + Mbp27)) + k_{decom4} \cdot Mbi \\ &- V_{db} \cdot \left(\frac{Cb}{K_{db} + Cb}\right) \cdot \left(\left(\frac{Cdc20a}{K_{dbcdc20} + Cdc20a}\right) + \left(\frac{Cdh1a}{K_{dbcdh1} + Cdh1a}\right)\right) - k_{ddb} \cdot Cb) \cdot eps \end{aligned} \quad [30]$$

$$\begin{aligned} \frac{dMbi}{dt} &= (k_{com4} \cdot Cb \cdot (Cdk1_{tot} - (Mbi + Mb + Mbp27)) - k_{decom4} \cdot Mbi \\ &+ V_{m2b} \cdot (Wee1 + i_{b3}) \cdot \left(\frac{Mb}{K_{2b} + Mb}\right) - V_{m1b} \cdot Pb \cdot \left(\frac{Mbi}{K_{1b} + Mbi}\right)) \cdot eps \end{aligned} \quad [31]$$

$$\begin{aligned} \frac{dMb}{dt} &= (V_{m1b} \cdot Pb \cdot \left(\frac{Mbi}{K_{1b} + Mbi}\right) - V_{m2b} \cdot (Wee1 + i_{b3}) \cdot \left(\frac{Mb}{K_{2b} + Mb}\right) \\ &- k_{c7} \cdot Mb \cdot p27 + k_{c8} \cdot Mbp27) \cdot eps \end{aligned} \quad [32]$$

$$\frac{dMbp27}{dt} = (k_{c7} \cdot Mb \cdot p27 - k_{c8} \cdot Mbp27) \cdot eps \quad [33]$$

$$\begin{aligned} \frac{dCdc20i}{dt} &= (v_{scdc20i} - V_{m3b} \cdot Mb \cdot \left(\frac{Cdc20i}{K_{3b} + Cdc20i}\right) + V_{m4b} \cdot \left(\frac{Cdc20a}{K_{4b} + Cdc20a}\right) \\ &- k_{dc20i} \cdot Cdc20i) \cdot eps \end{aligned} \quad [34]$$

$$\begin{aligned} \frac{dCdc20a}{dt} &= (V_{m3b} \cdot Mb \cdot \left(\frac{Cdc20i}{K_{3b} + Cdc20i}\right) - V_{m4b} \cdot \left(\frac{Cdc20a}{K_{4b} + Cdc20a}\right) \\ &- k_{dc20a} \cdot Cdc20a) \cdot eps \end{aligned} \quad [35]$$

$$\begin{aligned} \frac{dPbi}{dt} &= (v_{spbi} + V_{6b} \cdot (x_{b1} + x_{b2} \cdot Chk1)) \cdot \left(\frac{Pb}{K_{6b} + Pb}\right) \\ &- V_{m5b} \cdot (Mb + a_b) \cdot \left(\frac{Pbi}{K_{5b} + Pbi}\right) - k_{dpbi} \cdot Pbi \cdot eps \end{aligned} \quad [36]$$

$$\begin{aligned} \frac{dPb}{dt} &= (V_{m5b} \cdot (Mb + a_b)) \cdot \left(\frac{Pbi}{K_{5b} + Pbi}\right) \\ &- V_{6b} \cdot (x_{b1} + x_{b2} \cdot Chk1) \cdot \left(\frac{Pb}{K_{6b} + Pb}\right) - k_{dpb} \cdot Pb \cdot eps \end{aligned} \quad [37]$$

$$\begin{aligned} \frac{dWeel}{dt} &= (v_{sweel} + k_{sw} \cdot Mw - V_{m7b} \cdot (Mb + i_b)) \cdot \left(\frac{Weel}{K_{7b} + Weel}\right) \\ &+ V_{m8b} \cdot \left(\frac{Weelp}{K_{8b} + Weelp}\right) - k_{dweel} \cdot Weel \cdot eps \end{aligned} \quad [38]$$

$$\begin{aligned} \frac{dWeelp}{dt} &= (V_{m7b} \cdot (Mb + i_b)) \cdot \left(\frac{Weel}{K_{7b} + Weel}\right) - V_{m8b} \cdot \left(\frac{Weelp}{K_{8b} + Weelp}\right) \\ &- k_{dweelp} \cdot Weelp \cdot eps \end{aligned} \quad [39]$$

3. Incorporating the ATR/Chk1 DNA replication checkpoint

Cyclin E/Cdk2 activates by phosphorylation the anchor factor Cdc45, which allows DNA polymerase α to initiate replication. The kinase ATR is activated upon binding the RNA primer synthesized by DNA polymerase α . Then ATR phosphorylates, and thereby activates, the kinase Chk1. The latter phosphorylates and inhibits the Cdc25 phosphatases, thus preventing the activation of Cdk2 and Cdk1 as long as DNA replication proceeds. Eventually the decrease in Cdk2 activity, inherent to the oscillatory dynamics of the Cdk network, inhibits DNA polymerase, owing to the inactivation of the Cdc45 switch (36). The subsequent inhibition of ATR and Chk1 relaxes the inhibition of the phosphatases Cdc25 and thereby permits the rise in the activity of cyclin B/Cdk1 that will elicit the G2/M transition (see main text and Fig. S5).

To take into account the intrinsic checkpoint mediated by kinases ATR and Chk1 on DNA replication, we add the following kinetic equations **40–44**:

$$\begin{aligned} \frac{dCdc45}{dt} = & (V_{1cdc45} \cdot Me \cdot (\frac{Cdc45_{tot} - Cdc45}{K_{1cdc45} + (Cdc45_{tot} - Cdc45)}) \\ & - V_{2cdc45} \cdot (\frac{Cdc45}{K_{2cdc45} + Cdc45}) - k_{spol} \cdot (Pol_{tot} - Pol) \cdot Cdc45 + k_{dpol} \cdot Pol) \cdot eps \end{aligned} \quad [40]$$

$$\frac{dPol}{dt} = (k_{spol} \cdot (Pol_{tot} - Pol) \cdot Cdc45 - k_{dpol} \cdot Pol) \cdot eps \quad [41]$$

$$\begin{aligned} \frac{dPrimer}{dt} = & (k_{sprim} \cdot Pol - k_{dprim} \cdot Primer \\ & - k_{aatr} \cdot (ATR_{tot} - ATR) \cdot Primer + k_{datr} \cdot ATR) \cdot eps \end{aligned} \quad [42]$$

$$\frac{dATR}{dt} = (k_{aatr} \cdot (ATR_{tot} - ATR) \cdot Primer - k_{datr} \cdot ATR) \cdot eps \quad [43]$$

$$\frac{dChk1}{dt} = (V_{1chk} \cdot ATR \cdot (\frac{Chk1_{tot} - Chk1}{K_{1chk} + (Chk1_{tot} - Chk1)}) - V_{2chk} \cdot (\frac{Chk1}{K_{2chk} + Chk1})) \cdot eps \quad [44]$$

Oscillations in the presence of the ATR/Chk1 checkpoint are shown in Fig. 2C in main text where the effects of the checkpoint are discussed. As the rate of activation of ATR increases, the model predicts that the cell cycle slows down and eventually stops at the entrance in G2, as observed in the experiments (37).

4. Oscillations in the presence of only Cdk1

As explained in the main text, experiments indicate that oscillations can still occur in the presence of only Cdk1. This suggests that Cdk1 can form complexes with all cyclins, and that the resulting complexes substitute for the regular cyclin/Cdk complexes. To test this possibility we considered the situation where Cdk1 forms complexes with cyclins D, E, A and B. Moreover we assumed that the first three of these complexes perform the same functions as the cyclin D/Cdk4-6, cyclin E/Cdk2, cyclin A/Cdk2 complexes, respectively. Then the evolution equations **1-39** remain formally the same except that the term yielding the amount of free Cdk4-6 in Eqs. **9** and **10**, free Cdk2 in Eqs. **13, 14, 20**, and **21**, and free Cdk1 in Eqs. **30** and **31** must all be replaced by the amount of free Cdk1, given by the expression:

$$(Cdk1_{tot} - (Mdi + Md + Mdp27 + Mei + Me + Mep27 + Mai + Ma + Map27 + Mbi + Mb + Mbp27))$$

where the variables retain the same definition as in Table S1, with Cdk1 substituting for the other Cdks in the various cyclin/Cdk complexes. In such a case numerical simulations show (see Fig. S6A) that self-sustained oscillations can occur in the presence of only Cdk1, much as in the original set of kinetic equations based on the four different cyclin/Cdk complexes.

5. Coupling the mammalian cell cycle to the circadian clock

A direct link exists between the cell cycle and the circadian clock. The kinase Wee1, which inhibits Cdk1 and Cdk2, is indeed induced by the transcriptional regulatory complex CLOCK-BMAL1 that plays a central role in the mammalian circadian clock (38, 39) (see Fig. S7A). To test the effect of a circadian induction of the Wee1 gene we use a computational model proposed for the mammalian circadian clock (40) to generate the periodic variation of CLOCK-BMAL1. In addition, we incorporate an equation describing the synthesis of Wee1 mRNA induced by CLOCK-BMAL1, and consider that the synthesis of the Wee1 protein occurs at a rate proportional to the amount of Wee1 mRNA, which varies in a circadian manner as a result of its coupling to CLOCK-BMAL1.

To incorporate the coupling of the Cdk network to the circadian clock we thus add kinetic equation **45**, which reflects the activation by the circadian clock complex CLOCK-BMAL1 of the expression of Wee1 mRNA (Mw). The circadian variation of CLOCK-BMAL1 is generated by the 16-variable model for the mammalian circadian clock (40) in the conditions of Fig. 2C of ref. 40.

$$\frac{dMw}{dt} = v_{sw} \cdot \left(\frac{Bn^n}{K_{iw}^n + Bn^n} \right) - v_{dw} \cdot \left(\frac{Mw}{K_{dw} + Mw} \right) \quad [45]$$

In the term $(v_{swee1} + k_{sw} \cdot Mw)$ expressing synthesis of the Wee1 protein in Eq. **38**, the first part refers to a constant basal value independent of the circadian clock, and the second part pertains to the synthesis of Wee1 protein at a rate proportional to the amount of *Wee1* mRNA controlled by the circadian clock according to Eq. **45**. Thus, in Eq. **38** we can express the rate of synthesis of the protein Wee1 as $k_{sw}[(v_{swee1}/k_{sw}) + Mw]$ where the first and second terms in brackets refer to the basal amount of mRNA present in the absence of coupling to the circadian clock, and to the amount of *Wee1* mRNA controlled by the circadian clock.

Numerical simulations of the model indicate that the circadian clock acting through Wee1 can readily entrain the cell cycle (Fig. S7B). In the case considered, the period of the cell cycle oscillator before entrainment is close to 19h. As soon as Wee1 mRNA begins to vary in a circadian manner, the cell cycle entrains to it so that its duration increases up to 24h. Entrainment can occur over a large domain of parameter values yielding periods of the cell cycle well below or above 24h. Similar results are obtained when the coupling to the circadian clock occurs through other components of the cell cycle machinery such as p21/p27 (41), or c-Myc (42), which drives the synthesis of cyclins A and E.

6. Regulation of cyclin B synthesis

In writing Eq. 30 we considered the simplest case of a constant synthesis of cyclin B. We have verified that when cyclin B synthesis is placed under control of E2F and pRB, like cyclins E and A, the model yields similar results in regard to self-sustained oscillations in the Cdk network. On the other hand, experimental observations point to the activation by cyclin A/Cdk2 of several transcription factors that enhance cyclin B transcription (12). When the synthesis of cyclin B is controlled by a transcription factor X activated through reversible phosphorylation by cyclin A/Cdk2, the time evolution of the concentration of the active form X of the transcription factor is given by Eq. 46, while the evolution equation 30 for the concentration of cyclin B (*Cb*) must be replaced by equation 30a.

$$\frac{dX}{dt} = (V_{1x} \cdot Ma \cdot (\frac{X_{tot} - X}{K_{1x} + (X_{tot} - X)}) - V_{2x} \cdot (\frac{X}{K_{2x} + X})) \cdot eps \quad [46]$$

$$\begin{aligned} \frac{dCb}{dt} = & (v_{cb} \cdot X - k_{com4} \cdot Cb \cdot (Cdk1_{tot} - (Mbi + Mb + Mbp27))) + k_{decom4} \cdot Mbi \\ & - V_{db} \cdot (\frac{Cb}{K_{db} + Cb}) \cdot ((\frac{Cdc20a}{K_{dbcdc20} + Cdc20a}) + (\frac{Cdh1a}{K_{dbcdh1} + Cdh1a})) - k_{ddb} \cdot Cb) \cdot eps \end{aligned} \quad [30a]$$

Self-sustained oscillations in the Cdk network readily occur in these conditions, much as when the synthesis of cyclin B is assumed to be constant. For obtaining oscillations, typical values of the new parameters that appear in Eq. 46 are: $K_{1x}=0.1 \mu\text{M}$, $K_{2x}=0.1 \mu\text{M}$, $V_{1x}=10 \text{ h}^{-1}$, $V_{2x}=2 \mu\text{Mh}^{-1}$, $X_{tot}=1 \mu\text{M}$.

The nature of the oscillations obtained when the synthesis of cyclin B is constant (Eq. 30) or when it is controlled by cyclin A/Cdk2 (Eq. 30a) is similar : in both cases, the Cdk network behaves as a self-sustained oscillator since cyclical behavior occurs spontaneously, in the absence of any external periodic forcing, solely as a result of the regulatory structure of the cyclin/Cdk network.

7. Further comparison with experimental observations and model predictions

Besides accounting for the possibility of oscillations in the presence of only Cdk1 (Fig. S6A) or in the absence of pRB (see main text and Fig. S6B), the model further compares with experimental observations. Thus, as shown by Fig. S4B, when the rates of synthesis of pRB and p27 are sufficiently low, oscillations can occur in the absence of cyclin E, as observed in the experiments (43, 44) or in the absence of cyclin D. The latter result holds with the fact that cyclin E/Cdk2 can substitute for cyclin D/Cdk4 and renders it dispensable for cell cycling (45). The results of Fig. S4A nevertheless predict that oscillations can still occur in the absence of cyclin D but not of cyclin E when the levels of pRB or p27 become sufficiently large.

As shown in Fig. S4C, at very large values of the rate of synthesis of Cdh1 oscillations in Cdk2 occur in the absence of oscillations in Cdk1, a situation known as endoreplication (see Discussion in main text). This result fits with experimental observations (46). The diagram of Fig. S4C also compares with experimental observations showing that mutations that reduce the activity of Cdh1 or increase the activity of Cdc25 possess tumorigenic effects (see main text and refs. 47-49). Two arrows in Fig. S4C illustrate how such mutations can bring the Cdk network from a stable steady state to self-sustained oscillation; such a transition corresponds to the switch from quiescence to cell proliferation.

In regard to the restriction point, the model remains to be compared in a systematic manner with the experimental findings of Zetterberg and Larsson (50) on the mitotic delay that follows transient growth factor depletion. Preliminary results obtained in the present model are similar to those obtained in the model proposed by Novak and Tyson for the restriction point (51).

The prediction that in the absence of both cyclin B and Cdk1, oscillations associated with endoreplication might occur in the activity of cyclin A/Cdk2 could be tested by means of RNAi experiments. Because cyclin A/Cdk2 is involved in all four oscillatory circuits, the suppression of cyclin A should abolish any oscillatory behavior in the network, except if other cyclin/Cdk complexes can substitute for cyclin A/Cdk2 in at least one of the oscillatory circuits in Fig. 5.

Treatments perturbing the normal operation of the Cdk network have already revealed the oscillatory dynamics of a sub-network involving cyclin B/Cdk1 (52).

8. Role of bistability in the Cdk network

Bistability can occur in multiple parts of the Cdk network because of the abundance of positive feedback loops. Thus, cyclin E/Cdk2, cyclin A/Cdk2 and cyclin B/Cdk1 all activate the Cdc25 phosphatase that activates the corresponding Cdk. Moreover, cyclin B/Cdk1 inhibits the kinase Wee1 that inhibits Cdk1. All these positive feedback loops can give rise to bistability associated with all-or-none transitions and hysteresis (16, 17). We discuss in the text the role of these transitions in the all-or-none nature of the rise in the amplitude of the peak in Cdk1 after the restriction point (Fig. 3A).

Bistability can also occur in the E2F/pRB regulatory module as a result of a positive feedback loop: E2F activates cyclin E/Cdk2, which, through phosphorylation, inhibits pRB, leading to increased activation of E2F and cyclin E/Cdk2. In agreement with experimental observations (53) and with previous theoretical studies (32, 53), bistability originating from the interactions between E2F and pRB can readily occur in the present model. To observe bistability, it is sufficient, for example, to change only two parameter values with respect to the basal values listed in Table S2. Thus, bistability occurs in the E2F/pRB module as a function of GF, in the absence of regulation of E2F by cyclin A/Cdk2 (to prevent the negative feedback exerted by the latter Cdk), when increasing the pRB phosphorylation rates measured by parameters V_1 and V_3 — i.e., when raising the rates of pRB inactivation by cyclin D/Cdk4-6 and cyclin E/Cdk2 — from the basal values of 2.2 h^{-1} and 1 h^{-1} both to the value of 4 h^{-1} . Abrupt transitions associated with bistability in various modules contribute to the global oscillatory behavior of the Cdk network. Their role might not be required for the establishment of self-sustained oscillatory behavior, but they contribute to ensure the occurrence of sharp, large-amplitude oscillations in the various cyclin/Cdk complexes and to enhance robustness of oscillatory behavior.

9. Self-sustained oscillations and the dominoes versus clock views of the cell cycle

The question arises as to whether the cell cycle can be viewed as a clock or as a succession of switches, each of which induces the occurrence of the next switch in the network, as a series of falling dominoes, according to the distinction introduced by Murray and Kirschner (54). We showed that the Cdk network behaves as a self-sustained oscillator because the oscillations occur spontaneously, in the absence of any periodic external cue, and remain sustained whether the

synthesis of cyclin B/Cdk1 is constant or subjected to regulation within the Cdk network (see Section 6 above). As previously illustrated on an abstract model of a cyclical network of phosphorylation-dephosphorylation reactions (55) the distinction between the two contrasting views —dominoes versus clock—becomes blurred, and the two views can be reconciled, in the case where the successive cyclin/Cdk complexes are activated beyond a threshold in an all-or-none manner, in such a way that the transient activation of the last cyclin/Cdk complex brings about the transient activation of the first cyclin/Cdk complex in the cyclically organized network.

Positive feedback loops strengthen the all-or-none nature of the activation of the cyclin/Cdk complexes (see Section 8). Thereby the dynamics of the network approaches that of a relaxation oscillator. The global oscillatory behavior of the Cdk network thus results from a combination of a variety of negative and positive feedback loops, and feed-forward activations. Negative feedback loops confer a transient character to the activation of the cyclin/Cdk complexes, or prevent (as in the case of the ATR/Chk1 checkpoint) the activation of these complexes at an inappropriate time in the cell cycle. By giving rise to bistability, positive feedback loops contribute to the all-or-none nature of many cell cycle processes, e.g., Cdk activation. Feed-forward activations allow one cyclin/Cdk complex to activate the next one in the network; once activated, a given cyclin/Cdk will turn off the preceding cyclin/Cdk via negative feedback, by increasing cyclin degradation, or decreasing cyclin synthesis through inhibition of E2F.

10. Initial conditions for numerical simulations

For Figure 2A, initial conditions correspond to the stable steady state obtained in the absence of growth factor, i.e. for $GF=0$. For Figures 2B, 3, 4, S3, S4, S6, and S7B, initial values for the 39 concentration variables (in μM) are: $AP1=0.01$, $pRB=1$, $pRBp=0.25$, $pRBpp=0.1$, $E2F=0.01$, $E2Fp=0.01$, $pRBc1=0.1$, $pRBc2=0.05$, $Cd=0.01$, $Mdi=0.01$, $Md=0.01$, $Mdp27=0.01$, $Ce=0.01$, $Mei=0.01$, $Me=0.01$, $Skp2=0.01$, $Mep27=0.01$, $Pei=0.01$, $Pe=0.01$, $Ca=0.01$, $Mai=0.01$, $Ma=0.01$, $Map27=0.01$, $p27=0.25$, $p27p=0.01$, $Cdh1i=0.01$, $Cdh1a=0.01$, $Pai=0.01$, $Pa=0.01$, $Cb=0.01$, $Mbi=0.01$, $Mb=0.01$, $Mbp27=0.01$, $Cdc20i=0.01$, $Cdc20a=0.01$, $Pbi=0.01$, $Pb=0.01$, $Wee1=0.1$, $Wee1p=0.01$. For Figure 2C, initial conditions for the additional variables are (in μM): $Pol=0.01$, $Cdc45=0.01$, $Primer=0.01$, $Chk1=0.01$, $ATR=0.01$; other values are as for Fig. 2B. For Figure S7B, the initial value of the additional variable is $Mw=0$; other initial values are the same as in Figs. 2B, 3, and 4 (see above). Limit cycle oscillations do not depend on initial conditions. However, the periodic trajectory is reached asymptotically after some transients. In Figs. 2B and

2C the curves begin after 150h of transients while in Fig. S6A the curves begin after 600h of transients.

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