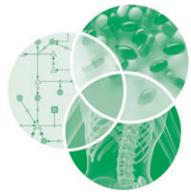
Focus Article



Dynamics of the mammalian cell cycle in physiological and pathological conditions

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A network of cyclin-dependent kinases (Cdks) controls progression along the successive phases G1, S, G2, and M of the mammalian cell cycle. Deregulations in the expression of molecular components in this network often lead to abusive cell proliferation and cancer. Given the complex nature of the Cdk network, it is fruitful to resort to computational models to grasp its dynamical properties. Investigated by means of bifurcation diagrams, a detailed computational model for the Cdk network shows how the balance between quiescence and proliferation is affected by activators (oncogenes) and inhibitors (tumor suppressors) of cell cycle progression, as well as by growth factors and other external factors such as the extracellular matrix (ECM) and cell contact inhibition. Suprathreshold changes in all these factors can trigger a switch in the dynamical behavior of the network corresponding to a bifurcation between a stable steady state, associated with cell cycle arrest, and sustained oscillations of the various cyclin/Cdk complexes, corresponding to cell proliferation. The model for the Cdk network accounts for the dependence or independence of cell proliferation on serum and/or cell anchorage to the ECM. Such computational approach provides an integrated view of the control of cell proliferation in physiological or pathological conditions. Whether the balance is tilted toward cell cycle arrest or cell proliferation depends on the direction in which the threshold associated with the bifurcation is passed once the cell integrates the multiple signals, internal or external to the Cdk network, that promote or impede progression in the cell cycle.

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COMPUTATIONAL APPROACHES TO THE CELL CYCLE: FROM AMPHIBIAN EMBRYOS TO YEAST AND MAMMALIAN CELLS

The cell cycle plays a prominent role in development, from egg fertilization to the adult organism. Such key role also holds in pathological conditions, because deregulation of the cell cycle is associated with aberrant cell proliferation and cancer. In view of the complexity of the regulatory network that governs cell cycle dynamics, it is fruitful to complement its experimental study by computational approaches based on detailed kinetic models. Such models have first been developed for early cell cycles

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in amphibian embryos, which have a duration of about 30 min, and which consist of an alternation between DNA replication (S phase) and mitosis (M phase). Models were subsequently proposed for the cell cycle in yeast and somatic cells, where the M and S phases are separated by the G1 and G2 phases. These more complex cell cycles possess a much longer duration, which can go up to 24 h or more.

In mammals, a network of enzymes known as cyclin-dependent kinases (Cdks) governs the correct ordering of cell cycle phases.[1] A Cdk is active as a protein kinase only when forming a complex with a regulatory subunit, called cyclin. 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. Cdk1 appears to be the major kinase, as it can bind to cyclins D, E, and A and replace Cdk4–6 and Cdk2 for correct progression in the cell cycle.[2]

Models for the Embryonic and Yeast Cell Cycles

Experimental studies in frog embryos and yeast allowed the discovery of the main molecular regulatory mechanisms that drive progression in the cell cycle. The embryonic cell cycle is composed of two phases: interphase, where DNA replication occurs, and mitosis. Progression in the interphase is driven by cyclin accumulation.[3] At the transition between interphase and mitosis, the level reached by cyclin allows to activate a kinase, Cdc2 (also known as Cdk1), which forms with cyclin a complex called MPF (Maturation—or Mitosis—Promoting Factor). During mitosis, MPF activates, by phosphorylation, the APC complex that promotes cyclin degradation.[4] This regulation creates a negative feedback loop, which has the potential of generating oscillations in the levels of cyclin and Cdk. Such oscillations would correspond to the periodic activation of MPF associated with the repetitive passage of the cell through mitosis in the first 12 embryonic cycles.[5]

Early on, theoretical models based on the regulatory interactions between cyclin and Cdc2 were proposed for the dynamics of early cell cycles in amphibian embryos.[6,7] Besides negative feedback, experimental as well as theoretical studies subsequently showed the importance of positive feedback loops in the activation of the kinase Cdc2 for the entry into mitosis.[8–10] Positive feedback produces a bistable switch in the activation of Cdc2, which promotes an all-or-none transition between interphase and mitosis. Oscillations of Cdc2 arise from hysteresis associated with bistability.[9,10] The embryonic cell cycle continues to be studied theoretically, by means of models that incorporate recently discovered regulations.[11]

More complex models, in terms of the number of variables and of the regulations involved, were proposed for the dynamics of the fission and budding yeast cell cycles.[12–14] These models incorporate the control of cell division by cell mass, and again rely on the existence of multiple positive feedback loops. One of these models was able to reproduce the dynamical behavior of more than 100 cell cycle mutants in budding yeast.[13] Related theoretical models were also proposed for the putative dynamics of the cell cycle in primitive cells[15] and for a minimal cell cycle oscillator recently constructed in yeast.[16,17]

Models for the Mammalian Cell Cycle

Several models were proposed to account for the dynamics of specific phases of the mammalian cell cycle, such as the G1 phase[18] and the G1/S transition,[19–21] for the existence of a restriction point in G1 that defines a point of no return beyond which cells do not need the presence of growth factor (GF) to complete a cycle,[22] for the G2/M transition[23] or for the exit from mitosis.[24] Instead of focusing on a single transition between two particular phases of the cell cycle, we previously presented a detailed computational model for the dynamics of the mammalian cell cycle incorporating all phases,[25,26] and later reduced it to a skeleton version that retains the same dynamical properties.[27,28] Theoretical models for the Cdk network were further used to investigate the coupling between the mammalian cell cycle and the circadian clock.[29,30]

In the detailed model for the mammalian cell cycle[25] schematized in Figure 1, exit from the quiescent state is triggered 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, which promotes, and the tumor suppressor pRB, which inhibits cell cycle progression. The kinase Cdk2 in turn regulates, through phosphorylation, the activity of E2F and pRB. 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. Thus, the activity of Cdk4–6 is activated by phosphorylation by the

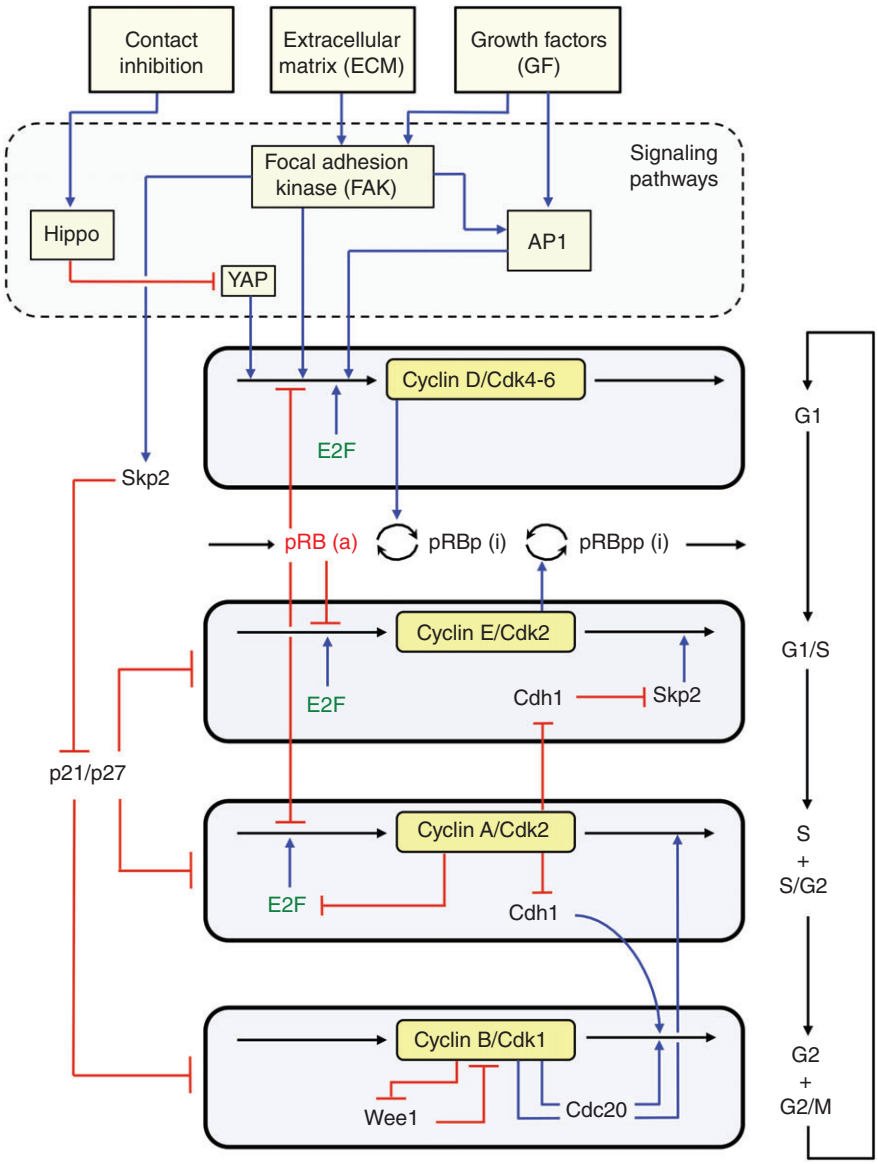

FIGURE 1 | Model for the cyclin-dependent kinase (Cdk) network driving the mammalian cell cycle. The model is composed of four modules centered on the main cyclin/Cdk complexes: cyclin D/Cdk4–6, cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1, which control the successive phases G1, S, G2, and M of the cell cycle. Entry from a quiescent phase G0 (not illustrated) into phase G1 of the cell cycle is controlled by growth factors (GF) and/or sufficient stiffness of the extracellular matrix (ECM). The presence of growth factors elicits the activation of signaling pathways, leading to the synthesis of AP1; this transcription factor in turn promotes the synthesis of cyclin D, which is followed by entry into G1. Moreover, ECM stiffness favors activation of the focal adhesion kinase (FAK), which also leads to the synthesis of cyclin D. Entry in the cell cycle is impeded by contact inhibition at high cell density, via the Hippo/YAP pathway. The transcription factor E2F promotes and the tumor suppressor pRB impedes cell cycle progression. Cyclin D/Cdk4–6 and cyclin E/Cdk2 drive progression in G1 and the G1/S transition by phosphorylating, and thereby inhibiting, pRB. Cyclin A/Cdk2 allows progression in S and G2, while cyclin B/Cdk1 brings about the G2/M transition. The active, unphosphorylated form of pRB inhibits E2F, which promotes cell cycle progression by inducing the synthesis of cyclins D, E, and A. The protein Cdh1, inhibited by cyclin A/Cdk2, promotes the degradation of cyclin B, and inhibits Skp2, which promotes the degradation of cyclin E; activation of cyclin A/Cdk2 thus leads to the activation of cyclin B/Cdk1 and to the inhibition of cyclin E/Cdk2. The protein Cdc20, activated by cyclin B/Cdk1, promotes the degradation of cyclin A and cyclin B, which leads to the decrease in cyclin A/Cdk2 and cyclin B/Cdk1. The roles of the Cdk inhibitor p21/p27 and of the Cdk inhibitory kinase Wee1 are also indicated, together with the positive feedback loop involving Wee1 and Cdk1; the role of the phosphatase Cdc25 that activates Cdk1 and is activated by it, thus creating another positive feedback loop, is not indicated for lack of space (see supporting information in Ref 25 for more detailed schemes of the model for the Cdk network, for a list of kinetic equations and a definition of variables and parameters). The regulatory interactions between the four Cdk modules give rise to sustained Cdk oscillations (see Figures 2 and 3), which allow the repetitive, ordered progression along the successive phases of the cell cycle. (Scheme redrawn from Refs. 25 and 31.)

CAK (cyclin-activated kinase) protein, while Cdk2 and Cdk1 are activated by phosphatase Cdc25 and inhibited by kinase Wee1. Multiple positive feedback loops control the Cdk network because (1) the phosphatases Cdc25 that activate the Cdks are themselves activated by them, and (2) the Cdks inactivate their inhibitory kinase Wee1. The activity of the Cdks is further regulated through association with the protein inhibitor p21/p27, considered as a single entity in the model (see legend to Figure 1 and Ref 25 for further details). The model for the Cdk network contains 39 variables (see Box 1), while its skeleton version, based on a similar regulatory structure, contains only 5 variables and fewer parameters but displays comparable dynamic behavior.

The computational model proposed for the Cdk network driving the mammalian cell cycle[25,26] allows us to examine its global, integrated dynamics leading to the progression along the different phases of the cell cycle. Moreover, the model sheds light on the nature of the threshold characterizing the transition between cell cycle arrest and cell proliferation. It shows how activators (GFs, oncogenes) and inhibitors (tumor suppressors) of cell cycle progression act in an antagonistic manner to determine the direction of passage through the threshold. The extracellular matrix (ECM) provides further external control of cell proliferation, through integrins and the focal adhesion kinase (FAK).[32,33] To take this additional mode of regulation into account, we extended the model for the Cdk network by including the effect exerted by ECM on the dynamics of the Cdk network via FAK activation. We also included the external control exerted at high cell density by contact inhibition (CI) via cadherins and the Hippo/YAP pathway (see Figure 1). Thus, the extended computational model for the mammalian cell cycle[31] provides a unified description of how changes across threshold values in the levels of growth factors, oncogenes, tumor suppressors, stiffness of ECM or cell CI may tilt the balance toward either cell cycle arrest or cell proliferation, both in normal and pathological conditions.

OSCILLATORY DYNAMICS OF THE Cdk NETWORK

The model for the Cdk network contains four modules centered on cyclin D/Cdk4–6, cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1, respectively. The dynamics of the network can be determined as a function of one or several control parameters (see Box 1). A most natural control parameter is the level of growth factors as the latter can induce exit from cellular quiescence and the subsequent entry into the cell cycle.

Growth Factors Control the Switch from Cell Cycle Arrest to Cell Proliferation

The switch between different modes of dynamic behavior of the Cdk network is illustrated by the

BOX 1

A SET OF DIFFERENTIAL EQUATIONS DESCRIBES THE TIME EVOLUTION OF THE Cdk NETWORK

The time evolution of the variables in the Cdk network is described by a set of kinetic equations, which take the form of ordinary differential equations. The number of these equations is equal to the number of variables in the Cdk network. The detailed version of the model contains 39 variables,[25,26] which represent the concentrations of proteins (cyclins, Cdks, inhibitors such as pRB, transcription factors such as E2F, Cdk inhibitors such as p21, kinases, phosphatases, etc.) involved in the network. Each kinetic equation contains positive and negative terms: the former correspond to processes that increase the concentration of the particular species, while the latter pertain to processes responsible for disappearance of the species, through degradation or, for example, phosphorylation. These various terms, measured by kinetic parameters, also contain the regulatory interactions, activating or inhibiting, between the variables of the system. When incorporating mRNA species, in addition to proteins, the number of variables increases up to about 80.[73] Upon incorporating the DNA replication checkpoint into the basal model (see text), the number of variables increases by 5.[25] To predict the dynamic behavior of the Cdk network, the kinetic equations of the model are integrated numerically so as to determine the temporal evolution of the variables. The kinetic equations of the basal model are listed in the supporting information in Ref 25 (see http://www.pnas.org/content/106/51/21643.long?tab=ds). The kinetic equations and computer code for the extended model incorporating the effect of ECM and CI are given in the supporting information in Ref 31 (see http://rsfs.royalsocietypublishing.org/content/4/3/20130075.figures-only).

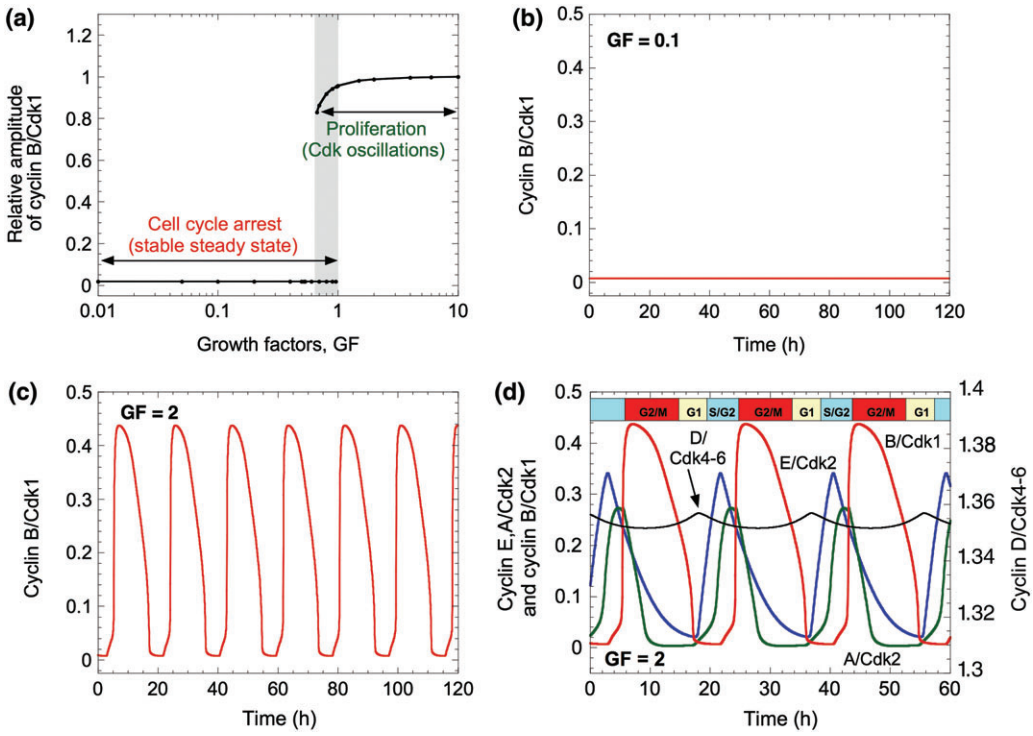

FIGURE 2 | Growth factors (GF) control the dynamical behavior of the cyclin-dependent kinase (Cdk) network. (a) Relative amplitude of cyclin B/Cdk1 shown as a function of GF. Low levels of GF produce a stable steady state corresponding to cell cycle arrest, while high levels of GF elicit sustained oscillations of the different cyclin/Cdk complexes, which corresponds to active cell proliferation. For intermediate levels of GF, a stable steady state coexists with sustained oscillations (grey zone). The time evolution of cyclin B/Cdk1 for subthreshold (GF = 0.1 μM) or suprathreshold amounts of GF (GF = 2 μM) is shown in (b) and (c), respectively. In the sustained oscillatory regime (GF = 2 μM), the sequential, transient activation of the different cyclin/Cdk complexes drives the ordered progression along the different phases of the cell cycle (d). Parameter values are as in Figure 2(a) in Ref 31.

bifurcation diagram established in Figure 2(a) as a function of increasing levels of growth factor (GF).[25] At low levels of GF, this diagram shows the stable steady-state level of one of the 39 variables, cyclin B/Cdk1, representative of the whole Cdk network, as a function of GF. A major prediction of the model is that above a critical level of GF the steady state becomes unstable and sustained oscillations in Cdk activity develop. Instead of the steady state, we then plot in the bifurcation diagram of Figure 2(a) the maximum amplitude of cyclin B/Cdk1 in the course of oscillations. The stable steady state of cyclin B/Cdk1 below the critical level of GF is shown in Figure 2(b), while the oscillatory time course of cyclin B/Cdk1 above the GF threshold is displayed in Figure 2(c). In a narrow range of GF values (grey zone in Figure 2(a)), the stable steady state coexists with stable oscillations. Such a phenomenon, known as hard excitation, is observed in a number of models for periodic behavior in biochemical and cellular systems.[34]

The model thus predicts that increasing the level of growth factors can elicit an abrupt switch in the dynamical behavior of the Cdk network. At subthreshold levels of GF, the network reaches a stable steady state corresponding to low activity of the various cyclin/Cdk complexes; this steady state may be associated with cell cycle arrest. At suprathreshold levels of GF sustained oscillations spontaneously occur, which may be associated with cellular proliferation as they correspond to the repetitive, sequential activation of the various Cdk complexes (see Figure 2(d)) responsible for the ordered progression along the successive phases of the cell cycle.

Design Principle for the Oscillatory Cdk Network

What are the regulatory features of the Cdk network that make it prone to oscillate? We can use the computational model to delineate the design principles underlying the oscillatory dynamics of the Cdk network. The regulatory wiring of the network is such that each of the four Cdk modules is turned on sequentially in a transient manner, because a given

module activates the subsequent modules and inhibits the previous ones. This regulatory design is responsible for the temporal self-organization of the Cdk network in the form of sustained oscillations in the activity of the various cyclin/Cdk complexes.[25–27] Each cyclin/Cdk complex is activated in turn transiently, in a repetitive manner. When plotting the time evolution of the Cdk network in the space of variables, sustained oscillations correspond to the evolution to a closed curve known as limit cycle (see Box 2); this type of oscillatory behavior is highly robust because, for a given set of parameter values, the system always evolves to the same periodic trajectory characterized by a unique period and amplitude, regardless of initial conditions. The modeling approach indicates that the oscillations necessarily arise from the regulatory wiring diagram. Indeed, similar dynamics were found in a reduced, skeletal version of the model for the Cdk network lacking many biochemical details and retaining the same design principle, namely, that each Cdk module activates the next modules in the network while inhibiting the previous ones.[25,27]

The oscillations in Cdk activity predicted by the model possess a large amplitude. This is due to the fact that positive feedback loops, which control most of the Cdk modules, give rise to bistability associated with all-or-none transitions between distinct levels of Cdk activity.[28] Positive feedback loops and bistability render the transitions between successive phases of the cell cycle robust and irreversible.[35–37] This view is corroborated by stochastic simulations of the skeleton model for the cell cycle, which suggest that the multiplicity of positive feedback loops not only provides redundancy in Cdk regulation but also contributes to enhance the robustness of Cdk oscillations with respect to molecular noise,[28] because bistability serves as buffer against fluctuations, and the range of bistability increases with the number of positive feedback loops.

BOX 2

SUSTAINED OSCILLATIONS CORRESPOND TO THE EVOLUTION TO A LIMIT CYCLE

Integration of the kinetic equations describing the time evolution of the Cdk network shows that the latter generally reaches a stable steady state when the level of growth factor (GF) is low. Upon increasing GF above a threshold value, such steady state becomes unstable. Then the system quits the steady state and the Cdk network undergoes sustained oscillations. The trajectory followed in time by the Cdk network can be projected in phase space as a function of two or three variables of the system, e.g., E2F, cyclin A/Cdk2, and cyclin B/Cdk1 (see Figure S1, Supporting Information). This trajectory goes to a closed curve, the limit cycle, which can be reached regardless of initial conditions. For a given set of parameter values, the system always evolves to the same limit cycle, characterized by a given period and amplitude. Limit cycle oscillations are therefore highly robust as the system returns to the same closed trajectory, following a transient perturbation. The threshold parameter value separating the stable steady state from the stable limit cycle in Figure 2(a) corresponds to a bifurcation point. Such bifurcation is said to be subcritical when the transition goes from a stable steady state to limit cycle oscillations through a range in which the two modes of behavior coexist, as in the cases depicted in the bifurcation diagrams established as a function of GF (Figure 2(a)), FAK activation rate (Figure 5(a)), and CI (Figure 5(b)).

Mechanism of Oscillations in the Cdk Network

To clarify the mechanism of Cdk oscillations, it is useful to determine the dynamic behavior of each Cdk module as a function of its input. Of particular import is the dynamics of the fourth module centered on Cdk1, which controls the G2/M transition. As shown in Figure 1, it receives direct input from cyclin A/Cdk2 in the third module of the network. It is instructive to isolate, in a first step, the Cdk1 module and to determine its dynamic behavior as a function of cyclin A/Cdk2 considered as control parameter. In a second step, letting cyclin A/Cdk2 evolve in time, we may compare the dynamics of the full Cdk network with that of the isolated cyclin B/Cdk1 module.

The bifurcation diagram in Figure 3(b) shows the steady state of cyclin B/Cdk1 in the isolated fourth module of the network, as a function of cyclin A/Cdk2. As long as the latter remains low, Cdk1 reaches a stable steady state corresponding to a low activity. Above a critical level of cyclin A/Cdk2, the steady state becomes unstable and sustained oscillations in Cdk1 set in. The blue curves show the maximum and minimum of Cdk1 oscillations as a function of the level of cyclin A/Cdk2. When cyclin A/Cdk2 exceeds a second, higher threshold,

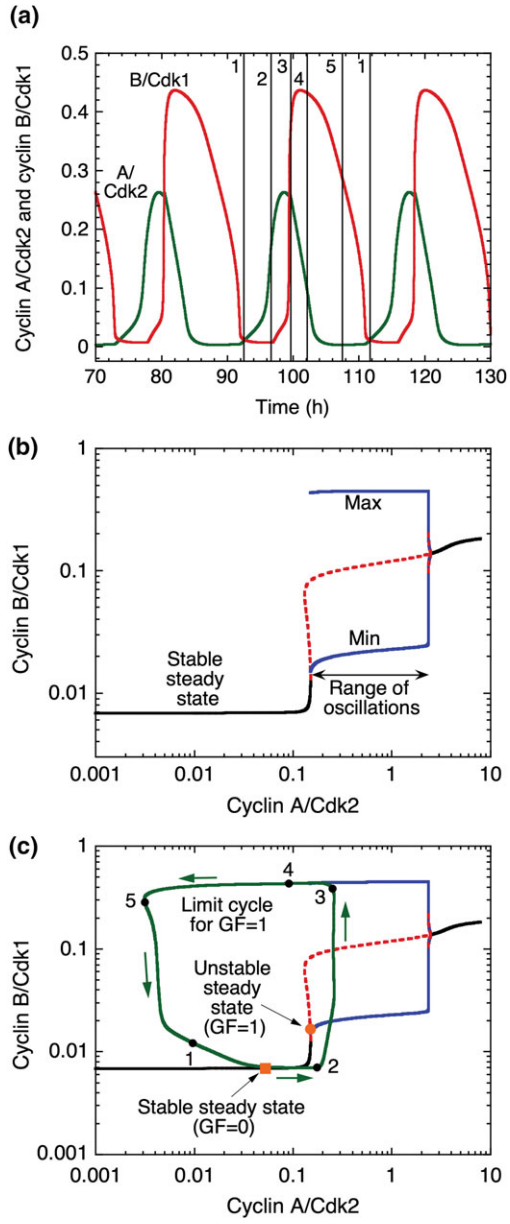

FIGURE 3 | Mechanism of oscillatory behavior in the cyclin-dependent kinase (Cdk) network. (a) Sustained oscillations of cyclin A/Cdk2 (green curve) and cyclin B/Cdk1 (red curve). Bifurcation diagrams illustrating the dynamical behavior of the cyclin B/Cdk1 module as a function of cyclin A/Cdk2, considered as a parameter, are shown in (b) and (c). Black curves correspond to stable steady states, red dashed curves indicate unstable steady states, while blue curves show the envelope, i.e., the maximum (upper curve) and minimum (lower curve) of sustained oscillations. Sustained oscillations in the cyclin B/Cdk1 module occur above a critical level of cyclin A/Cdk2. (c) Superimposed on the bifurcation diagram is the limit cycle trajectory of the full Cdk network (green curve). The black dots 1–5 correspond to the vertical lines 1–5 in the time series of (a). The orange square defines the stable steady state of the Cdk network in the absence of GF (GF = 0), while the orange dot corresponds to the unstable steady state observed when GF = 1 μM. Adapted from Figure 4 in Ref 25.

oscillations disappear as the Cdk1 module again reaches a stable steady state, which now corresponds to a high Cdk1 activity.

If we let cyclin A/Cdk2 evolve in time, as it does in the full model for the entire Cdk network, the latter follows the closed trajectory shown in green in Figure 3(c), which corresponds to the evolution to a limit cycle (see Box 2). This trajectory follows closely the bifurcation diagram of Figure 3(b) on which it is superimposed. Points 1–5 indicated on the green trajectory correspond to the different phases of Cdk1 oscillations in Figure 3(a). Starting from point 1, we first observe a progressive increase in cyclin A/Cdk2, due to the activation of the first and second modules in the Cdk network, as a result of stimulation of cyclin D synthesis by GF, via AP1, as well as by E2F, and inactivation of pRB (see Figure 1). When the system reaches point 2, the level of cyclin A/Cdk2 in the third module is such that the fourth module does not admit a stable steady state and instead enters the range of oscillations in Cdk1 activity. However, cyclin B/Cdk1 undergoes but a single peak of oscillation (point 3), because the sharp increase in Cdk1 leads to cyclin A/Cdk2 inactivation through Cdc20-induced cyclin A degradation (see Figure 1). Thus the trajectory of the full Cdk network quits the oscillatory domain of the Cdk1 module and moves to the left (point 4). The further decrease in cyclin A/Cdk2 (point 5) is followed by a decrease in Cdk1, as the Cdk1 module evolves to a stable steady state of low activity, which is the only attractor accessible in this region of the bifurcation diagram (point 1). A new cycle of oscillations resumes when the first two modules of the Cdk network again produce a rise in cyclin A/Cdk2, as a result of continuing stimulation by GF.

The bifurcation diagram of Figure 3(c) shows that when the level of growth factor (GF) is too low, the full Cdk network remains in a stable steady state. When the level of GF exceeds a threshold value, the steady state becomes unstable and sustained oscillations develop. These oscillations can also occur in the absence of GF if the balance of internal and external controlling factors is sufficiently tilted toward cell proliferation, e.g., at high values of E2F relative to pRB activity. What controls the dynamics of the Cdk network is indeed the relative rather than absolute levels of the factors that promote or impede cell cycle progression.

Cell Cycle Checkpoints and Cdk Oscillations

A comprehensive understanding of the dynamics of the cell cycle requires consideration of checkpoint

mechanisms, which ensure that progress to the next phase occurs only if the preceding phase of the cell cycle has been completed.[38] To implement such mechanisms into the computational model for the mammalian cell cycle requires the inclusion of additional variables. This was done for the DNA replication checkpoint, by incorporating the role of DNA polymerase and of the kinase ATR, which activates the kinase Chk1; the latter inhibits, through phosphorylation, the Cdc25 phosphatases that activate the various Cdks. As long as DNA replication proceeds, Cdk2 and Cdk1 are blocked, which prevents the transit to G2 and the occurrence of mitosis before DNA replication is completed. Incorporation of this checkpoint mechanism into the model for the Cdk network shows (see Box 1) that the Cdk network retains its oscillatory dynamics; in these conditions, however, the peaks in cyclin E/Cdk2, cyclin A/Cdk2 and cyclin B/Cdk1 are better separated.[25] This is clear from the comparison of the time evolution of the various cyclin/Cdk complexes without and with the DNA replication checkpoint (Figure 4(a) and (b)). The limit cycle trajectories reflect the better separation of the different Cdk peaks in the presence of the checkpoint (Figure 4(c) and (d)).

THE BALANCE BETWEEN CELL CYCLE ARREST AND CELL PROLIFERATION

Control by Factors Intrinsic to the Cdk Network: Oncogenes and Tumor Suppressors

The analysis of the computational model shows that the Cdk network driving the mammalian cell cycle can operate in either one of two states: a stable steady state corresponding to cell cycle arrest, and a regime of sustained oscillations associated with cell proliferation. The two states are separated by a

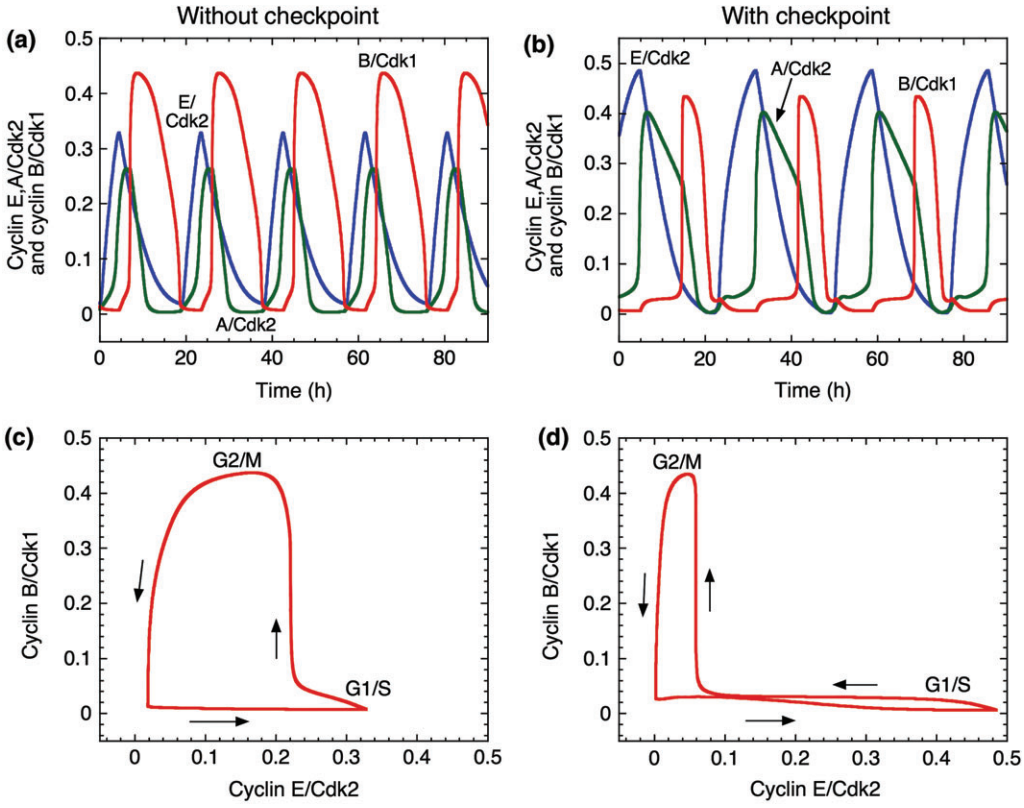

FIGURE 4 | Effect of the DNA replication checkpoint on the oscillatory behavior of the cyclin-dependent kinase (Cdk) network. The checkpoint is mediated by the kinases ATR and Chk1. Time series (a, b) for sustained oscillations of cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1, and the corresponding projection of the limit cycle oscillations (c, d) into the phase plane defined by cyclin B/Cdk1 and cyclin E/Cdk2 are shown in the absence (a and c) or presence (b and d) of the DNA replication checkpoint. The checkpoint slows down the progression in the cell cycle (compare time series in (a) and (b)). Moreover, it improves the separation between the peak of cyclin B/Cdk1 defining the G2/M transition and the peak of cyclin E/Cdk2 corresponding to G1/S (compare time series, and the corresponding limit cycles in (c) and (d)). In (a) and (c), $k_{aatr} = 0$, while in (b) and (d), $k_{aatr} = 0.02\ \mu M^{-1}\ h^{-1}$; other parameter values are as in Ref 25.

critical switch point corresponding to a bifurcation. We have already seen that an increase in growth factor suffices to induce the switch from cell cycle arrest to cell proliferation. The computational approach indicates that multiple factors are capable of triggering the transition between the quiescent and proliferative states, in one or the other direction. Thus, within the Cdk network, overexpression of factors that promote cell cycle progression can lead to the onset of proliferation. Such factors, exemplified by E2F and Cdc25 phosphatases, behave as oncogenes. As demonstrated experimentally for Cdk inhibitors, the same transition may be brought about by a decrease in the activity of factors that impede cell cycle progression.[39,40] Belonging to this class of tumor suppressors are pRB, p53, which induces the Cdk inhibitors p21/p27, and the kinase Wee1. The reverse transition to cell cycle arrest, which is a prerequisite for cell differentiation, is often achieved by a rise in the level of Cdk inhibitors such as p21.[41] Increasing or decreasing the levels of Cdk inhibitors tilts in one or the other direction, across a critical threshold, the balance between cell cycle arrest and cell proliferation and illustrates well the logic of cell cycle exit and reentry.[31,39]

Some factors, depending on conditions and cell type, may exert opposite effects on cell cycle progression. A case in point is Skp2, which sometimes promotes cell cycle progression by inhibiting p27, and generally impedes progression in the cell cycle, by promoting cyclin E degradation. If its inhibitory effect on p27 predominates, Skp2 will behave as an oncogene, and in the opposite case as tumor suppressor.

Control by Extrinsic Factors: Extracellular Matrix (ECM) and Contact Inhibition (CI)

Besides these factors intrinsic to the Cdk network, which control its dynamic behavior, factors extrinsic to the network can also govern the transition between cell cycle arrest and cell proliferation. The role of growth factors present in the extracellular medium has already been emphasized. Additional external influences are those of the ECM to which cells bind, and of the density of surrounding cells.

Mammalian cells are embedded in a cellular microenvironment composed of a complex protein network, the ECM, which exerts control over cell growth. Such control is mediated by integrins, cell surface proteins that transduce mechanical and chemical signals from the matrix into the cell and allow its binding to the support. In turn, these signals regulate the cytoskeleton as well as a complex cascade of intracellular kinases, which can promote different types of cellular responses such as quiescence, proliferation, cell motility, differentiation, or apoptosis.[42] Sensing of stiffness and anchorage of the cell to ECM are mediated by integrin signaling pathways,[42] which involve numerous molecular components among which the FAK is a key actor.[42,43] Activation of FAK leads to the activation of ERK and MEK kinases, which ultimately promote cyclin D synthesis through activation of AP1, thereby allowing entry into the G1 phase of the cell cycle (see Figure 1). We incorporated phenomenologically the regulation of cyclin D synthesis by ECM stiffness,[31] the increase of which is reflected in the model by the progressive activation of the kinase FAK, which is assumed to elicit AP1 activation and the ensuing synthesis of cyclin D.

The effect of increasing the rate of FAK activation as a result of a rise in ECM stiffness is shown in the bifurcation diagram in Figure 5(a), which bears a striking resemblance to the bifurcation diagram established as a function of growth factors (Figure 2 (a)). The rise in GF or ECM stiffness (via FAK) both lead to the switch from a stable steady state to sustained Cdk oscillations, via a narrow range in which these two states coexist. Upon increasing progressively the rate of FAK activation, sustained oscillations in Cdk activity develop spontaneously once the control parameter exceeds a critical value (Figure 5(c)).

Besides FAK, which serves to integrate proliferation signals transduced from ECM into the cell, other pathways play a role in modulating cell proliferation as a function of the extracellular environment. An important physiological process in this regard is contact inhibition (CI) of cell proliferation at high cell density, which is mediated by cadherin adhesion molecules via the Hippo signaling pathway. This pathway plays a crucial role in organ size control, and cancer development.[44–47] It inhibits cell growth through a kinase cascade that leads to the phosphorylation and nuclear exclusion of the growth-promoting transcriptional coactivator YAP (see Figure 1). The role of the Hippo pathway controlled by cell density was incorporated into the model for the Cdk network[31] by assuming that Hippo is activated by CI, which increases with cell density and is mediated by cadherin molecules present at the cell surface, while YAP is phosphorylated downstream of Hippo, and thereby kept in the cytosol in a form unable to elicit synthesis of cyclin D.[44–47]

Much as for FAK, but in the opposite direction, an increase in cell density above a critical threshold

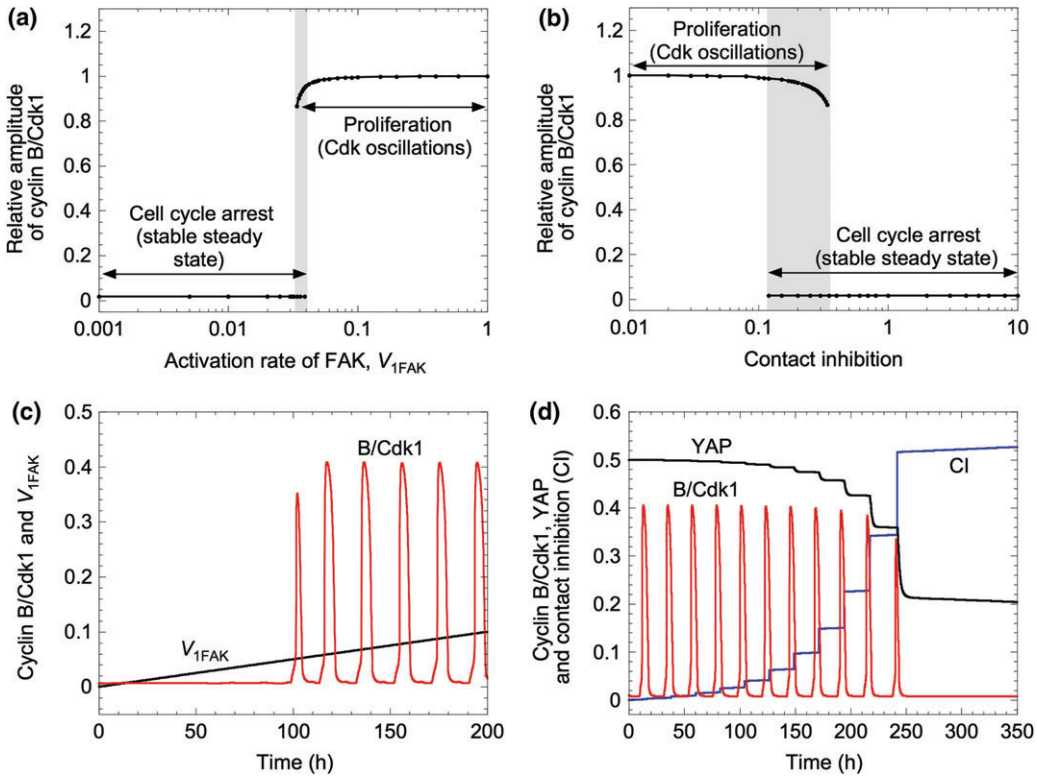

FIGURE 5 | Control of the cell cycle by factors external to the cyclin-dependent kinase (Cdk) network. Relative amplitude of cyclin B/Cdk1 is represented as a function of the activation rate of focal adhesion kinase (FAK), V_{1FAK}, in (a) and of contact inhibition (CI) in (b). Increasing the activity of FAK promotes the transition from a stable steady state to sustained oscillations of the Cdk network, while increasing the level of CI elicits the switch from proliferation (Cdk oscillations) to cell cycle arrest (stable steady state). The grey zone denotes a region of coexistence between a stable steady state and a stable oscillatory regime. (c) The time evolution of cyclin B/Cdk1, in the presence of a constant increase in FAK activity ($V_{1FAK}(t) = 0.0005 \times t$), illustrates the switch from cell cycle arrest to cell proliferation. (d) Plotting the time evolution of cyclin B/Cdk1, the active form of YAP and the level of CI indicate that cell proliferation is abolished when CI exceeds a critical level. In the latter simulation, we considered that CI is multiplied by an arbitrary factor of 1.5 after each cell division (peak of cyclin B/Cdk1). In (d), $V_{s1p27} = 0.6\ \mu M\ h^{-1}$, $V_{2cdh1} = 14\ h^{-1}$, while other parameter values are as in Figure 2 in Ref 31.

tilts the balance between quiescence and proliferation toward the stable steady state corresponding to an arrest of cell proliferation (Figure 5(b)). When considering that the phenomenological parameter (CI) measuring contact inhibition increases stepwise with cell density at each cell division, i.e., following each peak in cyclin B/Cdk1, the oscillations in Cdk activity eventually die out and the Cdk network reaches a stable steady state. This occurs once active YAP has decreased below a critical bifurcation value, owing to the suprathreshold increase in cell density and CI (Figure 5(d)).

CELL CYCLE REGULATION IN NORMAL AND PATHOLOGICAL CONDITIONS

To understand the dynamics of the cell cycle, we need to characterize the balance between cell cycle arrest and cell proliferation, which plays a key role in normal developmental conditions. On the other hand, spurious activation of cell proliferation can often lead to cancer. The computational approach to the Cdk network provides insight into the nature of the transition from cell cycle arrest to cell proliferation and into the factors capable of tilting the balance toward either one of these states, which represent two distinct modes of dynamic behavior of the Cdk network.

The effect of parameters intrinsic to the Cdk network is illustrated in Figure 6(a) where the domain of oscillatory behavior is shown as a function of the phosphatase Cdc25, which activates the Cdks and thereby behaves as an oncogene, and the protein Cdh1 that impedes progression in the cell cycle by acting as cofactor for the degradation of cyclins A and B. As shown in Figure 6(a), if the Cdk network operates initially in the region of stable

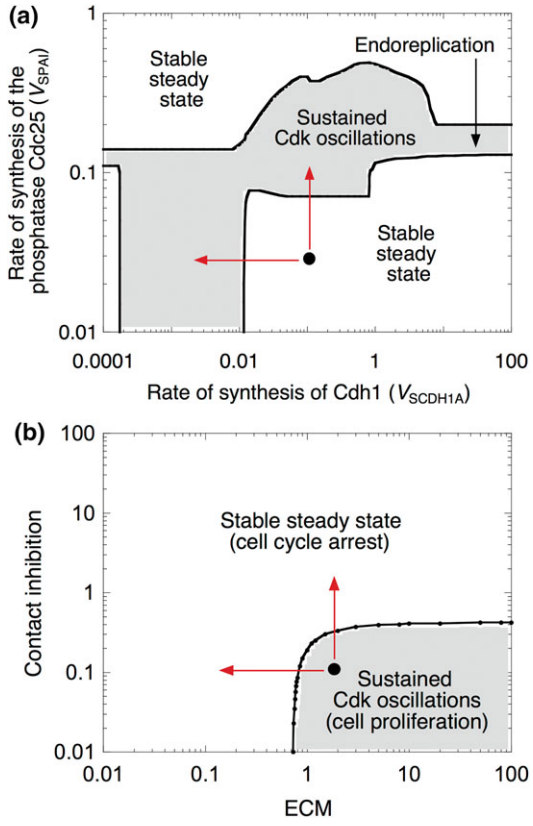

FIGURE 6 | Controlling the dynamics of the cell cycle by factors intrinsic or extrinsic to the cyclin-dependent kinase (Cdk) network. Sustained oscillatory regime (cell proliferation) and stable steady states domain (cell cycle arrest) are represented in a two-parameter plane defined toward the rates of synthesis of Cdc25, V_{SPAI}, and Cdh1, V_{SCDH1A} in (a), as well as toward the level of contact inhibition (CI) and the stiffness of the extracellular matrix (ECM) in (b). From the condition illustrated by the black dot in (a), the model indicates that cell proliferation is elicited by decreasing the level of Cdh1 or by increasing the level of the phosphatase Cdc25. Similarly, the model shows that, from the black dot in (b), cell proliferation is impeded by decreasing ECM or by increasing the level of CI. In (a), parameter values are as in Figure S4C in Ref 25, while in (b), parameter values are as in Figure 7B in Ref 31. The diagrams are adapted from Refs 25 and 31.

steady states corresponding to cell cycle arrest, the transition to cell proliferation may be induced by an increase in Cdc25 activity (vertical red arrow) or by a decrease in Cdh1 activity (horizontal red arrow). Both tumorigenic effects have been documented experimentally.[48,49] The transition to cell proliferation can in fact be induced similarly by a sufficient increase (decrease) in any of the biochemical parameters measuring factors that promote (impede) progression in the cell cycle. Thus, increasing the activity of E2F or decreasing that of pRB can trigger cell proliferation, which holds with the observation that loss of pRB is often associated with cancer.[50]

Turning to the control of the cell cycle by extrinsic factors characterizing the cellular environment, we see in Figure 6(b) how a suprathreshold increase in CI, which rises with cell density, or a sufficient decrease in stiffness of the ECM can induce the switch from cell proliferation to cell cycle arrest (red arrows). Similar diagrams for the transition between cell cycle arrest and cell proliferation can thus be established for regulatory factors intrinsic or extrinsic to the Cdk network.

Healthy cells need both the presence of growth factors in the serum and proper anchorage of the cell to the ECM to enter into a proliferative state. This situation defines an AND gate between GF and ECM for entry into the cell cycle; in contrast, transformed cells and cancer cells can exhibit serum- and/or anchorage-independent growth.[51] The extended model for the Cdk network allows us to consider both cases. Let us first deal with the case of healthy cells (Figure 7(a)–(c)). In the presence of GF and cell anchorage to ECM, cells enter into a proliferative mode characterized by sustained oscillations of the various cyclin/Cdk complexes (Figure 7(a)). The cells evolve to a stable, quiescent steady state either when the growth factor is absent (Figure 7(b)) or when cell anchorage is missing or the stiffness of the ECM is too low (Figure 7(c)). In contrast, when the balance is strongly tilted toward cell proliferation—e.g., by hyperactivation of FAK in Figure 7(d), overexpression of AP1 in Figure 7(e), or overexpression of E2F in Figure 7(f)—cells proliferate in the absence of GF (Figure 7(d)), or when cell anchorage is missing or the stiffness of the ECM is too low (Figure 7(e)), or when growth factor signaling and cell anchorage are both missing or too weak (Figure 7(f)).

The model therefore indicates that overactivation of FAK in the absence of GF (Figure 7(d)), or overexpression of AP1 in the absence of anchorage to ECM (Figure 7(e)) can lead to serum- or anchorage-independent growth, respectively. This result supports experimental observations showing the role of FAK and AP1 in serum- and anchorage-independent cell growth.[52] In particular, a recent study showed that overexpression of AP1 upregulates cyclin D, leading to anchorage-independent cell growth.[53] The model further indicates that the overexpression of oncogenes such as the transcription factor E2F can elicit anchorage- and serum-independent growth (Figure 7(f)), which is a characteristic of cancer cells.[54,55]

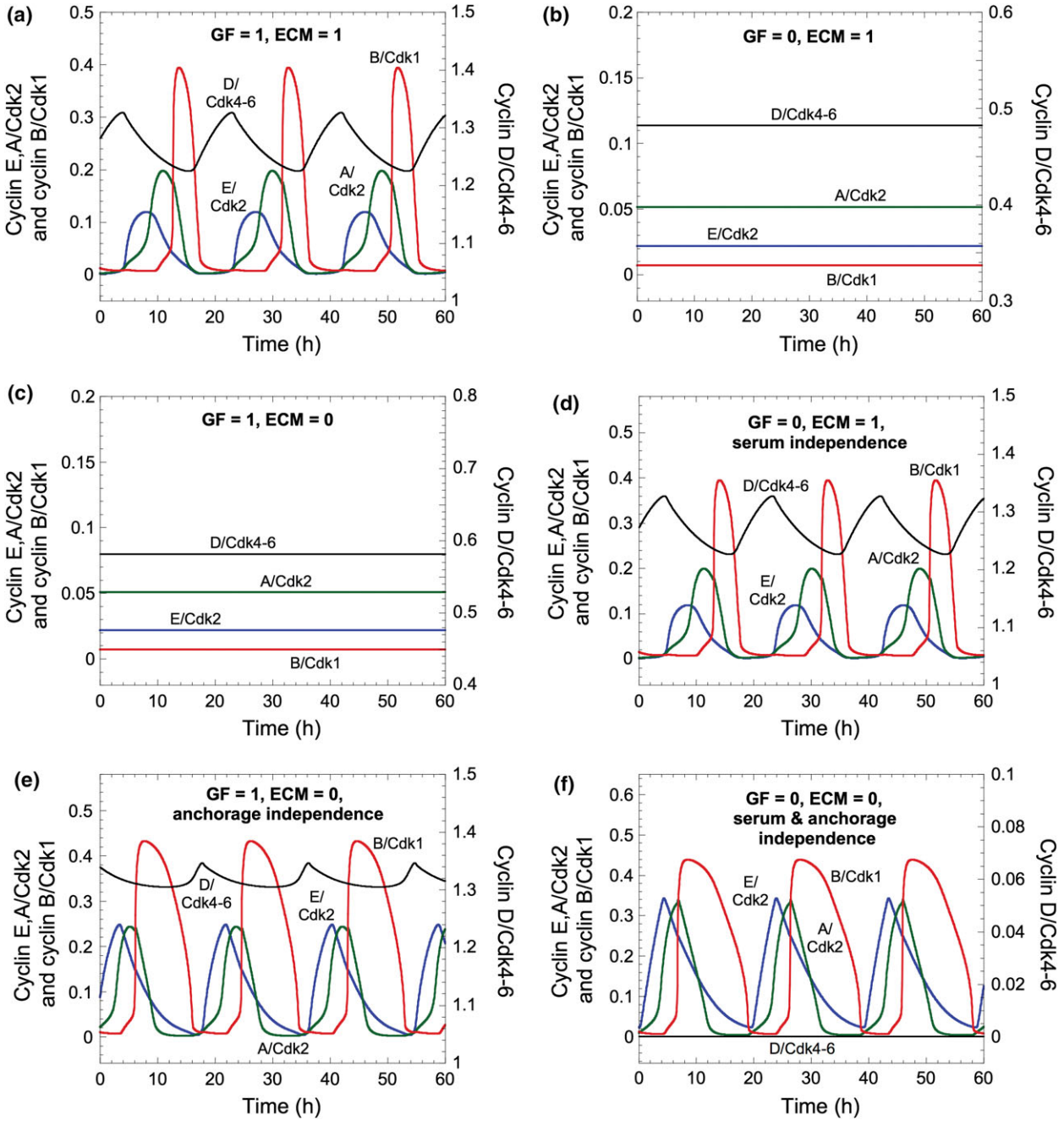

FIGURE 7 | Regulation of the cyclin-dependent kinase (Cdk) network by extracellular matrix (ECM) and growth factor (GF): serum- and anchorage-dependent or independent growth. The time evolution of cyclin D/Cdk4−6, cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1 is shown in the presence ((a), (c), (e)) or absence of soluble growth factors ((b), (d), (f)), and in the presence ((a), (b), (d)) or absence of ECM stiffness ((c), (e), (f)). (a) Healthy cell proliferation, characterized by the repetitive, sequential activation of the various cyclin/Cdk complexes, depending on GF and on the stiffness in ECM. From that condition (GF = ECM = 1), removing GF in (b) (GF = 0, ECM = 1) or reducing the stiffness of ECM in (c) (GF = 1, ECM = 0) elicits cell cycle arrest. (d) Increasing the rate of focal adhesion kinase (FAK) activation leads to cell proliferation even without GF (GF = 0, ECM = 1), resulting in serum-independent cell growth. Moreover, in (e) an increase in the rate of synthesis of AP1 allows cell proliferation without stiffness in ECM (GF = 1, ECM = 0), leading to anchorage-independent growth. Overexpression of the transcription factor E2F by increasing its rate of synthesis elicits cell proliferation in the absence of GF and without stiffness in ECM (GF = 0, ECM = 0), which situation defines serum- and anchorage-independent growth. The figure is adapted from Figure 4 in Ref 31.

The transitions to a stable steady state or to sustained Cdk oscillations occur in physiological conditions, during development or differentiation. Thus, cell cycle arrest often represents a first step toward differentiation. As indicated above, the reverse transition leading to spurious cell proliferation is often observed in pathological conditions. Which way the transition goes depends on the global balance between the multifarious, antagonistic factors that impact the balance between cell cycle arrest and cell proliferation.

DYNAMICS OF THE CELL CYCLE: THE NEED FOR A SYSTEMIC VIEW

The insights gained from a computational approach to the Cdk network that drives the mammalian cell cycle bear on the nature of the transition between cell cycle arrest and cell proliferation. The analysis of computational models of intermediate complexity (containing between 5 and 50 variables) for the Cdk network shows that the design regulatory principles on which it is built confer to this network the capability of temporal self-organization in the form of sustained Cdk oscillations. These oscillations ensure that each of the four cyclin/Cdk modules of the network is activated transiently in a repetitive, ordered manner that brings about the passage through the successive phases of the cell cycle. The key design feature of the network is that each module activates the following module(s) and inhibits the previous module(s) of the Cdk network. This regulatory design is achieved through tight intertwined regulations between modules, which take various forms such as control of cyclin synthesis and degradation, regulation by protein inhibitors of Cdk activity, and modulation of Cdk activity through phosphorylation–dephosphorylation.

The question arises as to how the mammalian cell cycle differs from cell cycles studied in yeast or the frog embryo. In the early cell cycles of amphibian embryos, oscillations in the activity of Cdk1 (initially referred to as the kinase Cdc2) result from the regulation of the fourth module of the Cdk network shown in Figure 1, and relies on intertwined positive and negative feedback loops. The other modules of the Cdk network do not seem to play any key role in these early embryonic cycles, which occur rapidly, with a period of the order of 30 min, owing to the absence of checkpoint mechanisms.[5] As recalled in section *Models for the Embryonic and Yeast Cell Cycles*, several models were proposed to account for the oscillatory dynamics of the embryonic cell cycle.[6,7,11,56]

In yeast, the situation appears to be more complex than in the early embryonic cell cycles, but less intricate than in the mammalian cell cycle, which involves a larger number of cyclin/Cdk complexes. In the detailed computational models proposed for the yeast cell cycle, cell mass plays the role of control parameter; its increase accompanies the all-or-none transitions between the successive phases of the cell cycle, which are analyzed by means of bifurcation diagrams displaying bistability.[12–14,17] Models were also proposed for the *Arabidopsis* cell cycle, using both a Boolean and a continuous description of regulatory interactions.[57]

As mentioned in section *Models for the Mammalian Cell Cycle*, several models were proposed for portions of the mammalian cell cycle. The model previously presented[25,26,31] and discussed here in detail, differs from these partial models in that it focuses on the dynamics of the full Cdk network and thereby provides a comprehensive picture of how Cdk oscillations drive the successive phases of the mammalian cell cycle. In contrast to the yeast cell cycle models, cell mass is not selected as control parameter because mammalian cell size does not seem to play a role as crucial as in yeast[58] for the dynamics of the cell cycle. Instead the various modes of dynamic behavior of the Cdk network are determined as a function of growth factor level and various other biochemical factors, internal or external to the Cdk network, such as E2F, pRB, Cdc25, Cdh1, stiffness of the ECM mediated by the kinase FAK, or CI mediated by the Hippo/YAP pathway. We focused here on the detailed model for the cell cycle in mammals because it allows us to discuss how a mathematical and computational approach helps clarifying the dynamics of the mammalian cell cycle in physiological and pathological conditions.

Among the dynamical properties that the model allows us to investigate are the existence of a restriction point in G1 beyond which the cell does not require the presence of growth factor to complete a cycle,[25] and the entrainment of the cell cycle by the circadian clock[30] through coupling mechanisms such as the circadian induction of the kinase Wee1, which plays the role of a Cdk inhibitor.[59] The model also accounts for the phenomenon of endoreplication when periodic peaks in cyclin E/Cdk2 and cyclin A/Cdk2 associated with DNA replication occur in the absence of significant peaks in cyclin B/Cdk1 needed for mitosis. The occurrence of such endocycles[60] results from the presence of multiple

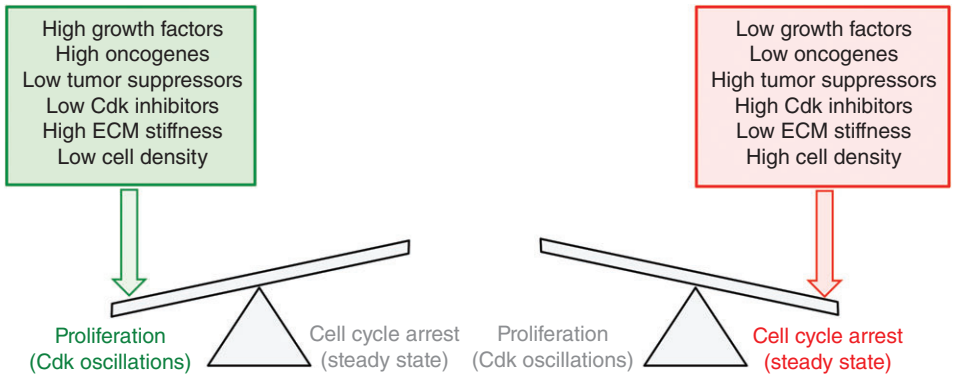

FIGURE 8 | The balance between cell cycle arrest and cell proliferation. The scheme integrates the multiple antagonistic factors that promote the occurrence of either sustained cyclin-dependent kinase (Cdk) oscillations, corresponding to cell proliferation, or a stable steady state of the Cdk network, corresponding to cell cycle arrest. These controlling factors are either intrinsic to the Cdk network—oncogenes, tumor suppressors, Cdk inhibitors—or extrinsic—growth factors, stiffness of the extracellular matrix, or cell density. Scheme adapted from Ref 31.

oscillatory circuits in the full Cdk network.[25] Each of these circuits is capable of producing oscillations on its own if uncoupled from the rest of the network. However, they are tightly coupled in physiological conditions so that they behave like a unique, global cell cycle oscillator. If they become uncoupled, the oscillatory potential of some of the circuits is revealed, as observed experimentally.[61,62] In the model this occurs, for example, at high levels of Cdh1, which, in agreement with experimental observations, give rise to endocycles.[63]

Finally, the computational approach throws light on the nature of the switch between cell cycle arrest and cell proliferation. The model suggests that this switch is associated with the passage through a bifurcation point separating a stable steady state of the Cdk network, corresponding to cell cycle arrest, and a regime of self-sustained oscillations in Cdk activity corresponding to cell proliferation. Where the Cdk network is located with respect to this bifurcation point at any given time, and thus whether the cell cycle is arrested or the cell is in proliferative mode depends on the global balance between two classes of antagonistic factors: those that promote and those that impede progression in the cell cycle (Figure 8). High levels of growth factors, high activity of oncogenes, low activity of tumor suppressors and Cdk inhibitors, increased stiffness of the ECM, and low cell density all tilt the balance toward cell proliferation. The reverse conditions tilt the balance toward cell cycle arrest. By providing a dynamical perspective on the behavior of the Cdk network, the computational approach contributes to the search[64] for an integrated, systemic view of cell cycle regulation in normal and pathological conditions. The passage through the bifurcation point may require more than one mutation, because a single move toward the Cdk oscillatory domain may not suffice to reach it and cross its boundary in a multidimensional parameter space. This view holds with the observation that several mutations are associated with cell transformation in many cancer types.[65,66]

What are the challenges ahead in using a computational approach to study the dynamics of the cell cycle in normal and pathological conditions? Beyond the general framework proposed here for the dynamics of the mammalian cell cycle, the computational model could be applied to analyze the dynamical properties of the cell cycle in specific cell types once quantitative data are collected that can be used in numerical simulations. Another challenge is to better understand the coupling of the cell cycle to the circadian clock, and its dynamical consequences.[30,67–69] Interestingly, the circadian clock appears to be disrupted in some tumor cells.[70–72] This could explain why cells in which the cell cycle becomes uncoupled from the circadian clock might proliferate more rapidly.[71]

ACKNOWLEDGMENT

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