MATHEMATICAL BIOSCIENCES
AND ENGINEERING
Volume **8**, Number **2**, April **2011**

doi:10.3934/mbe.2011.8.445

pp. **445–461**

REGULATION OF MODULAR CYCLIN AND CDK FEEDBACK LOOPS BY AN E2F TRANSCRIPTION OSCILLATOR IN THE MAMMALIAN CELL CYCLE

Orit Lavi

Department of Mathematics, Bar Ilan University
Ramat Gan 52900, Israel

Doron Ginsberg

Life Science Faculty, Bar Ilan University
Ramat Gan 52900, Israel

Yoram Louzoun

Department of Mathematics, Bar Ilan University
Ramat Gan 52900, Israel

Abstract. The cell cycle is regulated by a large number of enzymes and transcription factors. We have developed a modular description of the cell cycle, based on a set of interleaved modular feedback loops, each leading to a cyclic behavior. The slowest loop is the E2F transcription and ubiquitination, which determines the cycling frequency of the entire cell cycle. Faster feedback loops describe the dynamics of each Cyclin by itself. Our model shows that the cell cycle progression as well as the checkpoints of the cell cycle can be understood through the interactions between the main E2F feedback loop and the driven Cyclin feedback loops. Multiple models were proposed for the cell cycle dynamics; each with differing basic mechanisms. We here propose a new generic formalism. In contrast with existing models, the proposed formalism allows a straightforward analysis and understanding of the dynamics, neglecting the details of each interaction. This model is not sensitive to small changes in the parameters used and it reproduces the observed behavior of the transcription factor E2F and different Cyclins in continuous or regulated cycling conditions. The modular description of the cell cycle resolves the gap between cyclic models, solely based on protein-protein reactions and transcription reactions based models. Beyond the explanation of existing observations, this model suggests the existence of unknown interactions, such as the need for a functional interaction between Cyclin B and retinoblastoma protein (Rb) de-phosphorylation.

1. **Introduction.** Eukaryotic intracellular dynamics are mediated by many different molecular components (e.g. proteins, metabolites, RNA, etc.). Each such component operates at different rates and often in different locations and conditions. The classical attitude of cellular mechanism analysis is the detection of all the molecular components involved in the process and construction of a "wiring diagram" showing who affects whom [36]. The dynamics resulting from such a diagram can be intuitively understood as long as the diagram does not contain interleaved

2000 *Mathematics Subject Classification.* Primary: 58F15, 58F17; Secondary: 53C35.
Key words and phrases. Biomathematics, Stability, ODE, robustness, forced oscilators.

feedback loops. Once multiple feedback loops are involved, a more quantitative description is required. In such cases, the dynamics are often studied using Ordinary Differential Equations (ODE)[1]. An ODE-based methodology has been successful for simple cases containing a limited number of components and a limited number of feedback loops [40, 79]. In more complex situations, such as the cell cycle studied here, a more abstract description may be required, maintaining the mathematical description of the different interactions but relaxing the constraint for a precise numerical quantitative value for the concentration of each molecular component. We propose such a method to study the cell cycle regulation and show that direct and precise molecular conclusions can be derived from it. A large number of different models were previously built, each explaining some of the cell cycle properties [7]. Most such models contain a large number of parameters (in some cases more than a hundred). Some models are highly detailed while others are minimalist schemes [70, 57]. The existence of so many different models highlights the limits of classical modeling focused on the time course of different molecules. We here propose an alternative, putting the focus on the interplay between feedback loops and not the proteins inside each feedback loop. This attitude provides a mechanistic understanding of the cell cycle that is not sensitive to the details of the molecular interactions. The cell cycle contains interleaved feedback loops. It affects and must synchronize a large number of intracellular elements. It requires the buildup of new molecules within the cell and the associated increase in its mass and volume. At the same time it requires the cells rapid division into two almost equally sized daughter cells. Finally, all chromosomes must be doubled [13]. This complex mechanism is orchestrated by several proteins. Knock-out experiments were performed on most of these proteins. In almost all cases, some sort of cell division was observed. The cell cycle has four main stages: In G1, cell growth occurs through RNA transcription and protein translation; in S, DNA synthesis and chromosome duplication take place; in G2, the cell continues to produce new proteins and grows in size; finally, in M, chromosomes segregate and cell division ends the cycle. The concentrations or the number of molecules and therefore the cell mass is inherent to the cell cycle dynamics and is not solely dictated by an external mechanism [8]. Four families of Cyclins (D, E, A and B) combined with four types of CDKs (CDK4/6, CDK2 and CDK1) and the CKIs, Cyclin/CDKs Inhibitor families (the CIP/KIP family e.g. p21 and p27; and the INK4 family e.g. p16 and p19) are strongly associated with the mammalian cell cycle and its regulation. The cyclin/CDK complexes operate in an orderly manner and distinct cyclin/CDK complexes are active in different stages of the cell cycle. Specifically cyclins D/CDK4,6 function in G1; cyclins E/CDK2 functions in late G1 and early S; cyclin A/CDK2 in S phase; cyclin A/CDK1 in S and G2 and lastly cyclin B/CDK1 at the M phase. The cell cycle is also regulated by the CDC25 phosphatases, members of the ubiquitin system (mainly SCF and APC) and transcription factors (E2F, Myc and p53). Other proteins from the pocket protein family (pRB, p107 and p130), a repressive cofactor to the E2F family, are also known to affect the cell cycle [77, 53, 1, 26]. Most of these proteins have been shown to be replaceable in the cell cycle dynamic. KO experiments have shown that Cyclin

[1]The following abbreviations are used in the text Ko-Knock-Out,CKI Cyklin Kinase Inhibitor, CDK Cyclin Dependent Kinase,ODE ordinary differential equation, TF Transcription Factor, CDC25- Cell Division Cycle 25, CAK- Cdk-activating kinases ,SCF- Skpl-cullin-F-box-protein complex,APC- Anaphase-Promoting Complex/Cyclosome, CB- Cyclin Box, EB E2F Box,Skp2- S-phase kinase-associated protein 2, Rb-RetinoBlastoma, TFBS- Transcription factor binding site.

D [65, 66], Cyclin E1 and Cyclin E2 [65, 18, 62, 71], CDK2 [71, 4], Cyclin A1, Cyclin B2 [6], CDK4/6 [43], p27 [49, 37], p21 [72, 9, 31],CDC25B-C [63], Skp2 [37], and Rb [30] can be removed without preventing continuous cell division. Some components, however, such as CDK1 [64], Cyclin A2 [48] and Cyclin B1 [6] are essential for the cell cycle and cannot be replaced. Furthermore, combined deletion of E2F 1, 2 and 3 prevents cell cycle progression, at least in some settings [83]. These experiments reveal that there are several proteins/activities that can be categorized as a must in the simplest cyclic dynamic, while other proteins are important, yet not essential for the minimal periodic behavior. We model here only the Cyclins, CDKs, CKIs, transcription factors and E3 Ubiquitin ligases. Many other proteins are involved in the cell cycle, but those are either downstream the signaling cascade or only affecting the cell cycle in special situations (e.g. stress) and are ignored in the current analysis. The proposed model is modular and includes all the above mentioned components and checkpoints of the cell cycle. The model is based on a slow negative feedback loop between a transcription factor and an ubiquitin ligase. This feedback loop represents the balance between protein, transcription and degradation. By itself, such a feedback loop would lead to a stable steady state, but if a faster positive feedback loop is coupled to it, a cyclic behavior is obtained. We have previously shown that a minimal model, composed of a positive feedback loop containing a transcription factor (TF), Cyclins and CDKs, accompanied by a negative feedback loop of the same TF with other Cyclins and E3 ubiquitin ligases can induce robust oscillations [38]. Similar dynamic structures (coupled feedback loops with TF and Ubiquitin ligases) play an important role in diverse cellular processes, including cell cycle regulation, signal transduction and the circadian rhythm [27, 88, 3]. We here further develop the analysis of models as a combination of feedback loops to the interplay between multiple loops. Specifically, we propose a description based on feedback loops containing a TF (e.g., E2F1) interacting with a set of Cyclins (in the current context, Cyclin D, E, A and B) and the appropriate CDKs. This model is further regulated by E3 ubiquitin ligases. A model with such variables can be studied in detail as a complex system. Here, we will adopt a different attitude and build a model composed of a set of similar modules. We will assume an internal module containing a similar dynamic structure for the protein-protein interaction of each Cyclin with its CDK, CDC25, E3 Ubiquitin ligases, and CKI (with possible different parameters for each such module). These modules themselves manifest cyclic behavior as often shown and modeled [20, 60, 16, 76] and assume an external transcription factor. The general cell cycle is obtained from the interaction of E2F with these modules and with Ubiquitin ligases. We show that such a description produces the known cyclic behavior of the cell cycle and its stop-points. The general model can be described as a TF interacting with multiple similar modules, each containing only protein-protein interactions (Cyclin boxes). These Cyclin protein-protein interactions have been studied extensively. In most studies, the Cyclin box (CB) contains mainly Cyclins, CDKs, CKIs, different forms of CDC25, and E3 Ubiquitin ligases. The box can be approximately described through the following well-accepted reactions: An active complex of Cyclin and CDK is created by a series of phosphorylations and de-phosphorylations by CAK, wee1 and CDC25 [28, 68, 67]. The degradation of Cyclins, CDKs and CKIs occurs primarily through the proteasome following their ubiquitination. Two known E3 Ubiquitin ligase families affect cell cycle progression, the SCF and the APC. These families control the degradation of Cyclins and other cell cycle elements [56, 5, 50, 46, 19, 80, 35]. CDK inhibitor

(CKI) also acts as a negative regulator of active Cyclin/CDK complexes. The Cyclin/CDK/CKI complex is degraded/dissociated following phosphorylation by the active Cyclin/CDK complex [45]. The degradation/dissociation of this complex frees the bound Cyclin/CDK, thereby activating it and forming another positive feedback loop [60]. It is here assumed that the protein synthesis rates and total levels of CDK are not a limiting factor for the activity of the cyclin/CDK complex [67]. We will use these very basic interactions to form the CB. Most elements included in the CB fluctuate. Gene expression experiments have shown the cyclic behavior of Cyclin, CDK and CKI [82, 69, 33, 34] with proteins and mRNA transcripts varying on a scale of 4-5 orders of magnitude (e.g. [69, 85]). Most, albeit not all, of the mammalian E3 ubiquitin ligase elements have been proved to fluctuate during cell cycle. This behavior results from a combination of synthesis, degradation and phosphorylations and is true for both APC [56, 58, 81, 15, 86], and F-box proteins [10, 87, 90, 39].Some mammalian E3 ubiquitin ligase have been not been proved to cycle yet (e.g., fbxw7). We assume here their periodic expression, similarly to other ligases (e.g., Skp2). Many different realizations of the Cyclin Box can mathematically lead to cyclic behavior; different mathematical descriptions can generate different dynamics. Moreover, simplification or expedition of the same box can also change the system dynamic [38, 20, 60, 16, 76, 75, 29]. In the results presented here, we make further simplifications for the sake of the presentation simplicity. These simplifications do not affect the results (supplementary files: Figure 2). We assume that each CB does not interact with other CBs. This is true for most Cyclins, with some minor exceptions, such as the mutual effect of Cyclin A box and Cyclin B [22], the presence of elements which may be common to multiple boxes (e.g., p21 and p27), and the common effect of ubiquitin ligases on multiple boxes (e.g., SCF and APC) [56, 78, 2]. These missing interactions can create time correlations and limit the flexibility of the full system [42]. An important advantage of this box model is the possibility of replacing one box with another or exchanging components between boxes to understand recent experimental results. It has been shown, for example, that Cyclin E can replace Cyclin D after the replacement of their promoters [17].

2. **Results.** The cell cycle dynamics can be simplified and studied dynamically by identifying a two-level hierarchy of slow and fast time scales. The first level represents the slow dynamics based mainly on gene transcription and protein destruction [38]. The second level is a more detailed molecular model, taking into account both slow and fast dynamics. This second level is constructed to explain not only the cyclic behavior but also the different stopping points of the cell cycle (e.g., G1/S, G2/M, senescence). It is a modular expansion of each feedback in the first level into a distinct module. In the present case the interactions composing the first level abstract model are transcription factors (e.g. E2F) accumulation and degradation. We label this sub-network as the E2F box (EB). The more detailed model is an expansion of the accumulation and destruction of E2F to include the dynamics of a set of CBs, each representing the interaction of E2F with one of the Cyclins (A, B and E) and the protein-protein interaction of these Cyclins. Note that the slow rates of the EB and the fast dynamics of the CB have dynamic consequences. While the CBs can cycle by themselves, the main E2F cycle (representing the full cell cycle length) is dictated by the slower dynamics of the E2F cycle. The CBs (with the fast dynamics) synchronize with the E2F box (with the slow dynamics) and induce combined dynamics describing the entire cell cycle. The CB has a much

faster dynamic than the EB, since there are only protein-protein interactions in the CBs, while the EB contains gene transcription and protein degradation. We first study the faster CB dynamics and then describe how they can all be merged in the context of the EB (Figure 1).

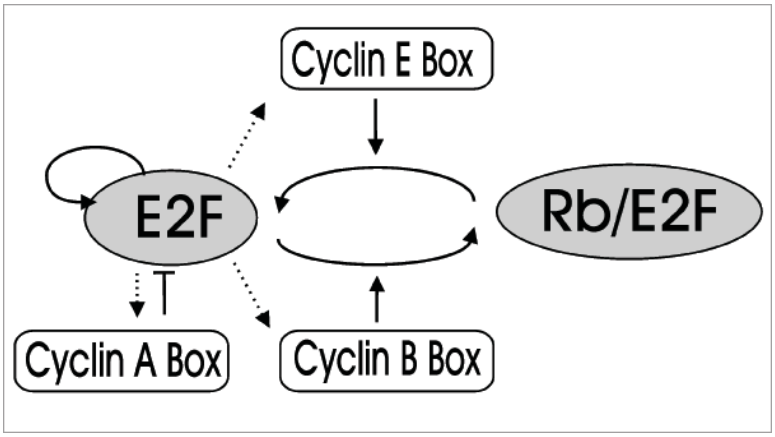

FIGURE 1. Combination of Cyclin Boxes modules. Three CB modules are used in the current analysis. The first CB to be activated by E2F is the Cyclin E CB, which closes a positive feedback loop with E2F through the decomposition of the Rb/E2F complex. The second CB to be activated is Cyclin A, which reduces the total E2F concentration by sending E2F to ubiquitination. The Cyclin A CB closes a negative feedback loop with E2F. The last CB to be activated is the Cyclin B CB, which also inhibits E2F through the enhancement of the E2F/Rb complex formation rate. Note that the last assumption has no direct experimental backing. We further assume a self activation process of E2F leading to an increase in E2F that is independent of the CBs. We did not model explicitly Cyclin D in the current analysis.

2.1. **CB model.** Based on the CB molecular interactions discussed in the introduction, we formulate a simple modular box containing only four elements (Cyclin, CDK, CKI and Ubi) which can be qualitatively described by four ODEs (Eq.1). In order to obtain an abstract model of the Cyclin dynamics, we simplify the interactions of the known network. We assume a constant external creation for all elements as well as a constant self-destruction for x_2 and x_3 (Eq.1). We do not explicitly model a natural decay for x_1, since it does not affect the feedback loops determining the model dynamics. This is equivalent to assuming a slow degradation rate compared with the ubiquitinization rate. The inclusion of such natural decay terms does not affect the results. We further assume a mutual positive feedback between Cyclin (x_1) and CDC25 (x_2) and a mutual negative regulation of Cyclin (x_1) and CKI (x_4), based on the rapid formation of a complex and its slow destruction. Note that the destruction of the Cyclin/CDK/CKI complex requires multiple phosphorylation steps of CDC25 and CKI and can thus be safely assumed to be slow [47, 51]. The last interaction included in the CB is the direct destruction of

Cyclin (x_1) by an ubiquitination-mediated degradation (x_3) [56, 5, 46]. Different ubiquitination components also destroy the other elements of the current system, but only the active form of the ubiquitin destroying the Cyclin is known to cycle and has therefore been included in the current model. This sub-network is similar among different Cyclins and can thus be defined as a modular unit. We present here the analysis of this unit. The model is obviously a simplification of the CB; it can be replaced by any other similar model, such as the ones proposed by [60] or [16] without significantly affecting the results.

$$\begin{aligned} x'_1 &= P_1 - P_2x_1x_3 + P_3x_1x_2 - P_{12}x_1x_4 \\ x'_2 &= P_9 + P_4x_1 - P_5x_2 \\ x'_3 &= P_8 + P_6x_1 - P_7x_3 \\ x'_4 &= P_{10} - P_{11}x_1x_4 - P_{13}x_4 \\ x_1 &= \text{cyclin}, x_2 = \text{cdc25}, x_3 = \text{ubiquitin}, x_4 = \text{cki} \end{aligned} \tag{1}$$

The CB dynamics can be a limit cycle, a stable fixed point or an unstable one, each covering wide regions in parameter space. At the biological level, this can represent unlimited cell division or arrest of the cell cycle. A typical periodic behavior can be seen in the numeric solution of Eq.1 (Figure 2-A). For most parameters, the dynamics of the CB is robust to large parameter changes over a wide range of parameters (e.g. Figure 2-B and in supplementary files: Table 1 and Figure 1)). Note that Intracellular molecule numbers can be computed in two ways. One can either compute the total protein copy number in the cell, which is approximately divided by a factor of 2 when the cell divide, or one can treat the number of proteins per unit volume in the cell. In such a case, this number is slowly diluted when the cell grows, but it is not affected by the cell division. The second approach seems more appropriate for mass-action formalism, since the mass-action rate constants represent the interaction probability within a given volume. In the equations used here a constant decay was assumed for most parameters. This decay is assumed to include the dilution due to cell size growth. Robustness to parameter variation is essential in cell cycle modeling. The same type of cell cycle mechanism operates in all eukaryotes (e.g., fission yeast [53], budding yeast [24, 11, 25], and the mammalians [36]). The differences in the cell cycle components between different species obviously affect their interaction rate constants. Moreover, even in different cell types of the same organism or in the same cell type under different conditions (e.g., cell size, temperature, and pH), the interaction rates between proteins can change widely. These changes often do not affect the cells capacity to divide [43, 74]. Furthermore, the same cycle can occur at different rates (division time) [12, 61, 52]. Thus if a generic mechanism exists for the cell cycle, it should be insensitive to drastic parameter changes.

2.2. **Stopping conditions of a single CB: The functionality of CKI and CDC25.** The eukaryotic cell cycle has a few possible arrest points (stable states) quiescence, senescence and three main checkpoints the G1, S and G2 checkpoints [32]. Quiescent cells are induced to reenter the cell cycle by mitogenic stimulation. We have assumed the existence of such an external signal and therefore focus only on the two checkpoints of dividing cells. The key regulator of the G1 stage is the Cyclin D box. The G1/S transition and S phase progression are regulated by the Cyclin E and Cyclin A boxes, and the G2/M transition is regulated by the Cyclin A and Cyclin B boxes. For the sake of modularity, we examined the regulation

capacity of an isolated CB. We studied the stopping conditions set by CKI and CDC25 and the way in which the CB dynamics vary after concentration changes in active CKI or active CDC25 or both. This analysis can mimic the behavior of normal or mutant cells. We also checked the effect of the length of CKI over-expression time on the probability of resuming cyclic behavior. In all these cases the model changed its stability from a limit cycle (representing cell division) to another state (cell death, cell cycle arrest or senescence) (Figure 2-C, supplementary files: Table 1). The control of the CB (e.g., by p53) can be introduced into our model as either an increased production of CKI (P_3) or an inhibition of CDC25 (P_{12}) or both. The control mechanism can be simulated through short or long simultaneous activations of the control unit: up-regulation of CKI protein level (P_3 in Eq.1) and down-regulation of CDC25 protein level (P_{12} in Eq.1) (P_3=1/100, P_{12}=100) (Eq.1). In the cases of a short activation of the control sub-unit, the cell cycle is temporarily stopped by either CKI or CDC25 or both and then resumed. In the case of long activations, over-expression of CKI may induce an irreversible switch (supplementary files: Table 1 and Figure 2-C). These results correspond to the observed effect of CKI and CDC25. One can observe the negative feedback of the ubiquitin E3 ligase on the Cyclin. In the absence of this feedback their dynamics will always lead to a fixed point. The negative feedback loop is balanced by a positive feedback loop. The increased CKI or reduced CDC25 expressions upset the balance between the positive and negative feedback loops and lead to a fixed point.

2.3. **Combination of cyclin boxes to produce full model of cell cycle.** Each CB is actually part of a larger cycle regulated by a TF (in the present case, E2F 1-3). We maintained the modular model by assuming a set of CBs interacting with an EB. Since each CB has a faster dynamic than the EB, we assume that the slow EB drives the fast dynamic of the different CBs (Figure 3-A). Before simulating the entire system, we checked the effect of an external cyclic forcing on a single CB. This cyclic forcing represents the effect of the E2F modulation on the CB. We replaced the constant activation term P_1 by a cyclic function $P_1(t)$. A simple positive forcing is a set of positive pulses is: $P_1(t) = A_1 \sum_{i=1}^{n} e^{-A_2(t-a_i)^2}$ (Eq.2 and Figure 3)

$$x'_1 = P_1 \cdot A_1 \sum_{i=1}^{n} e^{-A_2(t-a_i)^2} - P_2 x_1 x_3 + P_3 x_1 x_2 - P_{12} x_1 x_4 \tag{2}$$

$$x'_2 = P_9 + P_4 x_1 - P_5 x_2$$

$$x'_3 = P_8 + P_6 x_1 - P_7 x_3$$

$$x'_4 = P_{10} - P_{11} x_1 x_4 - P_{13} x_4$$

x_1= cyclin,x_2= cdc25,x_3= ubiquitin,x_4= cki

The forced CB again has a cyclic behavior, but the cycle is now adapted to the external forcing, if the forcing is strong enough. This behavior is similar to a forced oscillator. Interestingly, many previously proposed models used the CB as the sole source of oscillations. The combined model shows that while the CB indeed oscillates independently, in reality it may oscillate at an induced frequency and not at its natural frequency. The different CBs share the same transcription factor, E2F; however, their accumulation and activation are not at the same stage of the cycle. One way to explain the time lag between the CBs is by the strength of

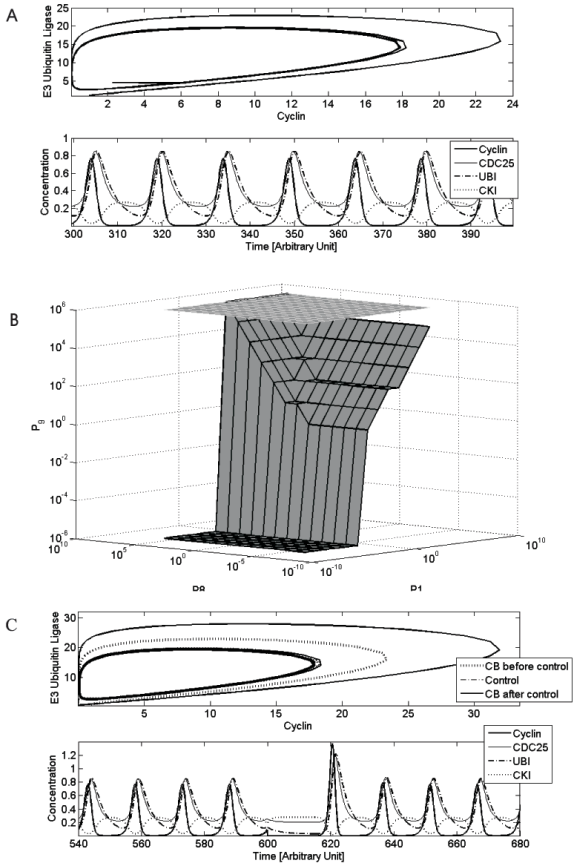

FIGURE 2. A (upper two drawings): Numeric solution for ODE. Limit cycle behavior produced by the four-variable model described in Eq.1. A similar behavior is obtained for a large number of parameter sets. The upper drawing represents the phase space and the lower drawing represents the components concentrations as a function of time. B (central drawing): Parameters space for P_1, P_8, P_9. In the plane of Cyclin synthesis rate (P_1), CDC25 synthesis rate constant (P_9) and CKI synthesis rate constant (P_8), stably kinase activity is the area between the two surfaces. Robust oscillating kinase activity (limit cycle) is the area above the upper surface, while the other activities are below the lower surface. C (lower two drawings): Simultaneous control activation of CDC25 and CKI This plot can be divided into three sections: the first is the initiation of normal cell cycle until a crisis occurs. The second section starts with the activation of a control sub-unit by stopping the oscillations to a constant level of concentrations. When (or if) the damage is fixed, the cell cycle continues with the initial amplitudes. The parameter set of Figure 2-A are used with the activation of control sub-unit by P_3=1/100, P_{12}=100.

their regulation by E2F. We examined the coupling of the CBs and the external forcing (Eq.2) as a function of the E2F induced transcription rate (P_1). At very low transcription level, the CB oscillates at its own period. When the transcription level is above a minimal level, the CB couples to the external forcing and oscillates much more slowly (Figure 3-B). Note that in this simplified case the external forcing period is fixed and not affected by the internal oscillations of the CB.

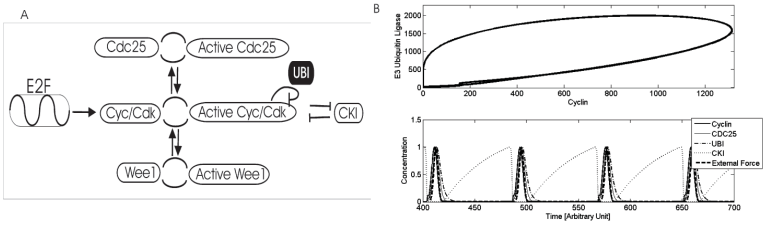

FIGURE 3. Cyclic external input to Single CB. A (left drawing): Schematic plot of CB with cyclic external input to Cyclin. This input can mimic E2F activity through the cell cycle. The external input can be applied to other proteins in CB with the same qualitative results. B (right drawing): Numeric solution for ODE. Limit cycle behavior produced by the four-variable model with cyclic external input described in Eq.2. Phase space (upper drawing) and the variation of concentrations over time (lower drawing) are plotted.

2.4. **Box combination location is important.** The CBs can be merged to produce a detailed modular model of the cell cycle (Eq.3 and Figure 1). There are three CBs: the Cyclin E box (x_1= cyclinE,x_2= cdc25,x_3= ubiquitin,x_4= cki), the Cyclin A box (x_5= cyclinA,x_6= cdc25,x_7= ubiquitin,x_8= cki) and Cyclin B box (x_9= cyclinB, x_{10}= cdc25, x_{11}= ubiquitin, x_{12}= cki). Using the stopping condition of each CB, a combined model can produce all cell cycle stopping points. In order to allow for a detailed analysis of the model properties, we simplified the interactions to be fully modular, i.e. we assume that each CB operates independently and that the only interaction between CBs is through the mutual effects of the CB and E2F (x_{13}) on each other. Although theoretical models backed by experimental data show that a cycle can occur when the CBs are connected (e.g. the effect of Cyclin A over Cyclin B through intermediate cdh1 [42]), here we discuss the simplified situation, without such connections, and show that cyclic behavior can be obtained in a simplified model. The modularity also implies that all CBs have different components. For example, while CDK2 is common to the Cyclin A and Cyclin E boxes, in the present case we only treat the Cyclin/CDK complex as a component. We explicitly assume that the CKI operating on Cyclin E is different from the one operating on Cyclin A. This can happen if different phosphorylations are required for the interaction of CKI with different Cyclins or if multiple CKI operate. This has not been tested experimentally and it thus remains an assumption. Still, we have shown numerically that assuming a common CKI does not affect the results of the model (Eq.4 and Figure 2 both in the Supplementary files:) For CKI and CDC25, this assumption is of minimal importance, since their active concentrations

increase/decrease rapidly and are thus probably not affected by their active concentration in earlier CBs. This assumption can have significant consequences in the context of ubiquitin E3 ligases, as will be discussed below. We further assumed that the internal structure of each CB is identical except for the rate constants, which can vary among CBs. The CBs are joined through the E2F box. We propose a minimal E2F box model producing robust oscillations containing at least one positive and one negative feedback loop [38]. The most natural negative feedback loops are either the loss of activity of E2F mediated by Cyclin A ($-P_{15}x_{13}x_5$) (blocking DNA binding) [84] and by Skp2 (E2F degradation) [44, 23], or a potential involvement of Cyclin B in the E2F/Rb complex formation ($P_{16}x_{13}x_9$) [59, 73, 41]. The simplest positive feedback loop is the decomposition of the E2F/Rb complex (x_{14}) mediated by Cyclin E ($P_{14}x_1x_{14}$). Thus in the short run, Cyclin E raises the active E2F concentration, while in the long run Cyclin A and perhaps also Cyclin B decreases the concentration of active E2F, but only Cyclin A induces an irreversible drop in the concentration of active E2F. The CBs were placed within these feedback loops to produce a general model of the cell cycle (Eq.3): The equations for the Cyclin box are:

$$\begin{aligned} x'_i &= k_j P_1 x_{13} - P_2 x_i x_{i+2} + P_3 x_i x_{i+1} - P_{12} x_i x_{i+3} \\ x'_{i+1} &= P_9 + P_4 x_i x_{i+1} - P_5 x_{i+1} \\ x'_{i+2} &= P_8 + P_6 x_i x_{i+2} - P_7 x_{i+2} \\ x'_{i+3} &= P_{10} - P_{11} x_i x_{i+3} \end{aligned} \tag{3}$$

$i = 1, j = 1$ for Cyclin E box, $i = 5, j = 2$ for Cyclin A box, $i = 9$, $j = 3$ for Cyclin B box. The equations for the E2F box are:

$$\begin{aligned} x'_{13} &= P_{13}x_{13} + P_{14}x_1x_{14} - P_{15}x_{13}x_5 - P_{16}x_{13}x_9 \\ x'_{14} &= P_{16}x_{13}x_9 - P_{14}x_1x_{14} \end{aligned} \tag{4}$$

All boxes are combined through their interaction with E2F (x_{13}). The combination of the CB and the EB indeed produce a cyclic behavior, with each Cyclin rising at the appropriate time. Moreover, for a large range of parameters the ratio between the minimal and maximal amplitudes of the Cyclins is of the order of 1.e3-1.e5, which fits the experimental RNA transcript levels. The phases of each Cyclin's activation are set by the strength of its TFBS (Figure 4-A). One can now wonder if the robustness of a single CB induces a similar robustness on the global model. Robustness can have two meanings. In order for the model to represent reality it must cycle and all Cyclins must appear in the proper order. In order to check for the robustness of the model, we defined a basic model, where the affinity of Cyclins E2F TFBS is reduced by a factor of 10 between Cyclin E and Cyclin A and a factor of 10 between Cyclin A and Cyclin B. For this system, we explicitly estimated the fixed points of the system, and computed for each fixed point the Jacobian and its eigenvalues. We then numerically substituted 10,000 different sets of parameters into the eigenvalues. The initial parameter sets were chosen from a log-normal distribution centered on (-1), with 4 orders of variability. Only parameter sets producing a cyclic behavior were then chosen, and their geometric average is computed. This average was used as the center of the distribution for a new group of 10,000 parameter sets. The same process was then iteratively repeated. In large parts of the tested phase space (43%) the cyclic behavior and the order of Cyclins was maintained.

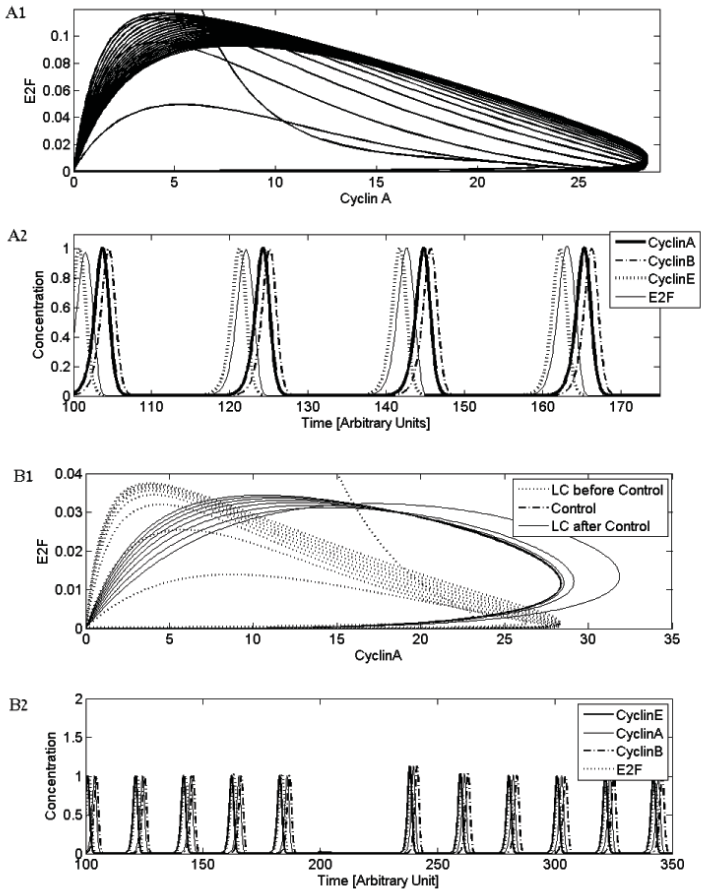

FIGURE 4. The complete modular model. A (upper drawing): The numeric solution of the modular system described by Eq.3. The parameter sets used are: k=[10^2, 10^1, 10^0], P=[0.0356, 0.4416, 1.3013, 0.203, 0.5467, 0.6108, 0.2844, 0.1952, 0.7739, 0.2782, 0.4302, 0.1691, 0.02291, 5.6201e-04, 1.4627e-04, 0.24555, 1 0.01]. P_{12} of the Cyclin B box is smaller than the other two CBs since it represents lower transcription rate. B (lower drawing): Simultaneous control activation of CDC25 and CKI to the modular system. Similar to Figure 2-C, this plot can be divided also into three sections: limit cycle, stable and limit cycle states. The same parameter sets of Figure 4-A are used with the activation of control sub-unit by P_3=1/100, P_{12}=5.

2.5. **Stopping conditions.** Another property maintained from the CB to the full model is the effect of CKI and CDC25 on the stopping conditions. The stopping conditions of the integrated system were examined when changing CKI and CDC25 level/activity either together (Figure 4-B) or separately (data not shown); and indeed, changing the level/activity of even one of the CKIs or CDC25s can lead to

a cell cycle arrest. Another element studied was whether CKI and CDC25 affect all CBs simultaneously or only a single CB at a time. Hypothetically, CKI and CDC25 can equally lead to cell cycle arrest. Therefore, each CB can be controlled by CKI over-expression (P_{12} is increased by a factor of 30), CDC25 under-expression (P_3 is reduced by a factor of 30), or both. Indeed, raising CKI and reducing CDC25 in all CBs produces a stable steady state. When the control effect is removed, the limit cycle resumes. The same response is induced by CDC25 without CKI or by activation by CKI alone. We then studied the effect of controlling the entire system through a single CB (data not shown). Controlling only the Cyclin A box can stop the cell cycle, while affecting the Cyclin E box does not stop or even change the stability of the entire system. When the Cyclin B box was affected, the limit cycle values were changed but, it did not become a stable point. To the best of our knowledge, these results have not been checked experimentally. Our model is also consistent with the return to perfectly normal cell cycle after an arrest of a few full cycles (Figure 4-B).

2.6. **Order of cyclin expression.** The synchronization between the different CBs with E2F and the entire cell cycle can be understood using our model. E2F controls the timing of Cyclins expression through their transcription rate. The parameters of each CB ($P_1 - P_{12},\ P_{17}$) can be different (data not shown) or equal (Figure 4) and still maintain the proper order between the CBs in the majority of simulated cases. The order is maintained, since we ensure a difference between the transcription rates of each CB. We only checked the order of expression and not the precise kinetics of each Cyclin and CDK. In order to reproduce this kinetics, we need to change the parameters of each CB. These parameters cannot be critical since the replacement of one cyclin by the other does not affect drastically the cell cycle [17].

3. **Discussion.** The cell cycle modeling literature contains a wide variety of cell cycle models. Different models contain very different driving mechanisms, but two general frameworks are usually used. Some models are based on the protein-protein interactions of Cyclins and CDKs, while others are based on the regulation of TF, such as E2F. Both frameworks have experimental validations. The cell cycle is arrested in the absence of E2F1-3, in some settings [83], showing the importance of TFs in cell cycle regulation. Experimental data supports the proposal that periodic transcription is an emergent property of a transcription factor network that can function as a cell-cycle oscillator independently of, and in tandem with, the CDK oscillator [54]. On the other hand, Cyclin-based models seem to have a large number of precise predictions. We show that these attitudes are not contradictory but rather complementary. While the Cyclin-CDK interactions can indeed produce cyclic behavior, this cyclic behavior may be constrained by a slower E2F cycle. Thus, the cell cycle may actually be driven by a set of interleaved feedback loops. We have developed a modular model of the cell cycle in which each module represents the dynamics of a single Cyclin and all modules are integrated into a general cyclic dynamics, much slower than the dynamics of each individual module. The internal dynamics of each module is actually not crucial for the large cycle. We have modified the Qu. model [60] for the Cyclin dynamics and produced a CB containing a schematic description of the basic protein-protein interactions involved in Cyclin regulation. We then integrated the dynamics of Cyclins E, A and B and of E2F into a coherent scheme. By itself, each CB produced the observed stopping points following CDC25 protein level down-regulation or CKI protein level up-regulation.

Combining the CBs produced a model with 14 variables and 18 parameters. In order to simplify the model, we assumed that all the parameters in each CB were identical, with the exception of the E2F-induced Cyclin creation. This assumption does not limit the validity of our model, since the model was validated to be robust to parameter variation. We further assumed no interaction between the different CBs and/or their components. The removal of this assumption was also tested not to affect the qualitative results of the model. These limitations are essential from a methodological point of view, since by using a larger number of parameters or interactions, one can obtain any desired dynamics and thus it becomes impossible to make any concrete statements about the system studied. The proposed model contains fast and slow mechanisms. The slow mechanism is a ubiquitination based negative feedback loop over E2F and a transcription-based positive feedback loop (or multiple loops) over E2F. A faster set of mechanisms are phosphorylation, complex formation/dissociation-based Cyclin, CDK, CKI and CDC25 protein-protein interactions. The two slow processes (transcription and ubiquitination) correspond to the minimal positive and negative feedback loops required for a predator prey-like dynamic. The fast Cyclin-CDK interaction can lead to an independent cycle of each CB and can also explain the cell cycle control checkpoints. The CBs were built in order to mimic the molecular interaction of their components. The resulting dynamics agree with a large array of experimental observations, including the effect of CDC25 and CKI combinations on the cell cycle checkpoints [55, 21]. The proposed combination of positive and negative feedback loops yields a cyclic behavior that does not depend on the details of each feedback loop or its components [38]. Thus the slow dynamics of the model are hardly affected by the fine details of the fast dynamics, or by the simplifying assumption used to produce them. A particularly important element of the model is a negative feedback between TF and ubiquitin ligases. In the case of the cell cycle, the TFs are the E2F family members and the known E3 ubiquitin ligase is Skp2 [89]. However, the deletion of Skp2 was proved not to stop the cell cycle [14]. We must thus conclude that there is at least one more E3 ubiquitin ligase leading directly or indirectly to E2F 1-3 destruction. The general framework presented here provides both a mathematical and a qualitative way of understanding the effects of gene knock-outs. Instead of analyzing the activity of a single gene, we analyze the effect of its removal on the different feedback loops. If a gene is a part of a non-redundant feedback loop, its removal would alter the dynamics. If, on the other hand, a gene participates in a feedback loop that can be replaced by a different loop with the same dynamics, its removal would not stop the cell cycle. Thus, removing Cyclin E1,2 or Cyclin B2 is not expected to arrest the cell cycle machinery, but the removal of Cyclin A2 is expected to stop the cell cycle, as is indeed observed (e.g., Cyclin E1 and Cyclin E2 [65, 18, 62, 71], CDK2 [71, 4], Cyclin A1, Cyclin B2 [6]). Note that redundancy alone is not a sufficient condition for the safe removal of a gene. If two loops are redundant but one has much lower rate constants than the other, it may not be possible to safely remove the strong loop. For example, although the negative feedback loop of Cyclin B on E2F is paralleled by that of Cyclin A, and its positive feedback loop is paralleled by that of Cyclin E, if the effect of Cyclin A or Cyclin E is too weak it would not be possible to remove Cyclin B and still obtain a cell cycle. This distinction may be the origin of the difference between Cyclin B1 and B2, as well as between Cyclin A1 and A2. Using the proposed logic we can conclude from the fact that Cyclin A2 and Cyclin B1 were experimentally proved to be essential for the cell cycle, that they are likely

to be negative regulators of E2F. Beyond the explanatory power of this model and the prediction of the expected effect of removing genes based on their participation in feedback loops, the model makes very specific predictions about expected interactions that were not measured. The main prediction is the existence of a functional interaction between Cyclin B and retinoblastoma protein (Rb) de-phosphorylation. This interaction is essential in order to close the E2F feedback loop. Supplementary files: http://peptibase.cs.biu.ac.il/frames/Supplementary_files.pdf.

REFERENCES

[1] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, "Molecular Biology of the Cell," fifth edition, Garland Science, a member of the Taylor and Francis Group, 29 West 35th Street, New York, NY, 2007.

[2] T. Bashir, N. V. Dorrello, V. Amador, D. Guardavaccaro and M. Pagano, *Control of the scf(skp2-cks1) ubiquitin ligase by the apc/c(cdh1) ubiquitin ligase*, Nature, **428** (2004), 190–193.

[3] D. Bech-Otschir, M. Seeger and W. Dubiel, *The cop9 signalosome: At the interface between signal transduction and ubiquitin-dependent proteolysis*, J. Cell Sci., **115** (2002), 467–73.

[4] C. Berthet, E. Aleem, V. Coppola, L. Tessarollo and P. Kaldis, *Cdk2 knockout mice are viable*, Curr. Biol., **13** (2003), 1775–1785.

[5] M. Bilodeau, H. Talarmin, G. Ilyin, C. Rescan, D. Glaise, S. Cariou, P. Loyer, C. Guguen-Guillouzo and G. Baffet, *Skp2 induction and phosphorylation is associated with the late g1 phase of proliferating rat hepatocytes*, FEBS Lett, **452** (1999), 247–253.

[6] M. Brandeis, I. Rosewell, M. Carrington, T. Crompton, M. A. Jacobs, J. Kirk, J. Gannon and T. Hunt, *Cyclin b2-null mice develop normally and are fertile whereas cyclin b1-null mice die in utero*, Proc. Natl. Acad. Sci. U.S.A., **95** (1998), 4344–4349.

[7] K. C. Chen, L. Calzone, A. Csikasz-Nagy, F. R. Cross, B. Novak and J. J. Tyson, *Integrative analysis of cell cycle control in budding yeast*, Mol. Biol. Cell, **15** (2004), 3841–3862.

[8] L. Chen, R. Wang, T. J. Kobayashi and K. Aihara, *Dynamics of gene regulatory networks with cell division cycle*, Phys. Rev. E Stat. Nonlin. Soft Matter Phys., **70** (2004), 011909.

[9] M. Cheng, P. Olivier, J. A. Diehl, M. Fero, M. F. Roussel, J. M. Roberts and C. J. Sherr, *The p21(cip1) and p27(kip1) cdk 'inhibitors' are essential activators of cyclin d-dependent kinases in murine fibroblasts*, Embo. J., **18** (1999), 1571–1583.

[10] S. Chu, J. DeRisi, M. Eisen, J. Mulholland, D. Botstein, P. O. Brown and I. Herskowitz, *The transcriptional program of sporulation in budding yeast*, Science, **282** (1998), 699–705.

[11] J. Culotti and L. H. Hartwell, *Genetic control of the cell division cycle in yeast. 3. seven genes controlling nuclear division*, Exp. Cell Res., **67** (1971), 389–401.

[12] S. J. D'Souza, A. Vespa, S. Murkherjee, A. Maher, A. Pajak and L. Dagnino, *E2f-1 is essential for normal epidermal wound repair*, J. Biol. Chem., **277** (2002), 10626–10632.

[13] H. L. Ford and A. B. Pardee, *Cancer and the cell cycle*, J. Cell Biochem., Suppl. **32-33** (1999), 166–172.

[14] A. Fotovati, K. Nakayama and K. I. Nakayama, *Impaired germ cell development due to compromised cell cycle progression in skp2-deficient mice*, Cell Div., **1** (2006), 4.

[15] J. M. Galan and M. Peter, *Ubiquitin-dependent degradation of multiple f-box proteins by an autocatalytic mechanism*, Proc. Natl. Acad. Sci. U.S.A., **96** (1999), 9124–9129.

[16] T. S. Gardner, M. Dolnik and J. J. Collins, *A theory for controlling cell cycle dynamics using a reversibly binding inhibitor*, Proc. Natl. Acad. Sci. U.S.A., **95** (1998), 14190–14195.

[17] Y. Geng, W. Whoriskey, M. Y. Park, R. T. Bronson, R. H. Medema, T. Li, R. A. Weinberg and P. Sicinski, *Rescue of cyclin d1 deficiency by knockin cyclin e*, Cell, **97** (1999), 767–777.

[18] Y. Geng, Q. Yu, E. Sicinska, M. Das, J. E. Schneider, S. Bhattacharya, W. M. Rideout, R. T. Bronson, H. Gardner and P. Sicinski, *Cyclin e ablation in the mouse*, Cell, **114** (2003), 431–443.

[19] M. Glotzer, A. W. Murray and M. W. Kirschner, *Cyclin is degraded by the ubiquitin pathway*, Nature, **349** (1991), 132–138.

[20] A. Goldbeter, *A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase*, Proc. Natl. Acad. Sci. U.S.A., **88** (1991), 9107–9111.

[21] C. H. Golias, A. Charalabopoulos and K. Charalabopoulos, *Cell proliferation and cell cycle control: A mini review*, Int. J. Clin. Pract., **58** (2004), 1134–1141.

[22] D. Gong, J. R. Pomerening, J. W. Myers, C. Gustavsson, J. T. Jones, A. T. Hahn, T. Meyer and J. Ferrell, *Cyclin a2 regulates nuclear-envelope breakdown and the nuclear accumulation of cyclin b1*, Curr. Biol., **17** (2007), 85–91.
[23] J. W. Harper and S. J. Elledge, *Skipping into the e2f1-destruction pathway*, Nat. Cell Biol., **1** (1999), E5–7.
[24] L. H. Hartwell, *Genetic control of the cell division cycle in yeast. ii. genes controlling dna replication and its initiation*, J. Mol. Biol., **59** (1971), 183–194.
[25] L. H. Hartwell, J. Culotti and B. Reid, *Genetic control of the cell-division cycle in yeast. i. detection of mutants*, Proc. Natl. Acad. Sci. U.S.A., **66** (1970), 352–359.
[26] A. Hershko, *The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle*, Cell Death Differ., **12** (2005), 1191–1197.
[27] A. Hershko and A. Ciechanover, *The ubiquitin system*, Annual Rev. Biochem., **67** (1998), 425-4-79.
[28] I. Hoffmann, G. Draetta and E. Karsenti, *Activation of the phosphatase activity of human cdc25a by a cdk2-cyclin e dependent phosphorylation at the g1/s transition*, Embo. J., **13** (1994), 4302–4310.
[29] K. Iwamoto, Y. Tashima, H. Hamada, Y. Eguchi and M. Okamoto, *Mathematical modeling and sensitivity analysis of g1/s phase in the cell cycle including the dna-damage signal transduction pathway*, Biosystems, **94** (2008), 109–117.
[30] T. Jacks, A. Fazeli, E. M. Schmitt, R. T. Bronson, M. A. Goodell and R. A. Weinberg, *Effects of an rb mutation in the mouse*, Nature, **359** (1992), 295–300.
[31] S. Jirawatnotai, D. S. Moons, C. O. Stocco, R. Franks, D. B. Hales, G. Gibori and H. Kiyokawa, *The cyclin-dependent kinase inhibitors p27kip1 and p21cip1 cooperate to restrict proliferative life span in differentiating ovarian cells*, J. Biol. Chem., **278** (2003), 17021–17027.
[32] M. B. Kastan and J. Bartek, *Cell-cycle checkpoints and cancer*, Nature, **432** (2004), 316–323.
[33] D. Knapp, L. Bhoite, D. J. Stillman and K. Nasmyth, *The transcription factor swi5 regulates expression of the cyclin kinase inhibitor p40sic1*, Mol. Cell Biol., **16** (1996), 5701–5707.
[34] C. Koch and K. Nasmyth, *Cell cycle regulated transcription in yeast*, Curr. Opin. Cell Biol., **6** (1994), 451–459.
[35] D. M. Koepp, J. W. Harper and S. J. Elledge, *How the cyclin became a cyclin: Regulated proteolysis in the cell cycle*, Cell, **97** (1999), 431–434.
[36] K. W. Kohn, *Molecular interaction map of the mammalian cell cycle control and dna repair systems*, Mol. Biol. Cell, **10** (1999), 2703–2734.
[37] U. Kossatz, N. Dietrich, L. Zender, J. Buer, M. P. Manns and N. P. Malek, *Skp2-dependent degradation of p27kip1 is essential for cell cycle progression*, Genes Dev., **18** (2004), 2602–2607.
[38] O. Lavi and Y. Louzoun, *What cycles the cell? - robust autonomous cell cycle models*, Math Med Biol, **26** (2009), 337–359.
[39] J. Lisztwan, A. Marti, H. Sutterluty, M. Gstaiger, C. Wirbelauer and W. Krek, *Association of human cul-1 and ubiquitin-conjugating enzyme cdc34 with the f-box protein p45(skp2): Evidence for evolutionary conservation in the subunit composition of the cdc34-scf pathway*, EMBO J., **17** (1998), 368–383.
[40] A. J. Lotka, "Elements of Physical Biology," Williams and Wilkins, Baltimore, 1925.
[41] J. W. Ludlow, C. L. Glendening, D. M. Livingston and J. A. DeCarprio, *Specific enzymatic dephosphorylation of the retinoblastoma protein*, Mol. Cell Biol., **13** (1993), 367–372.
[42] C. Lukas, C. S. Sorensen, E. Kramer, E. Santoni-Rugiu, C. Lindeneg, J. M. Peters, J. Bartek and J. Lukas, *Accumulation of cyclin b1 requires e2f and cyclin-a-dependent rearrangement of the anaphase-promoting complex*, Nature, **401** (1999), 815–818.
[43] M. Malumbres, R. Sotillo, D. Santamaria, J. Galan, A. Cerezo, S. Ortega, P. Dubus and M. Barbacid, *Mammalian cells cycle without the d-type cyclin-dependent kinases cdk4 and cdk6*, Cell, **118** (2004), 493–504.
[44] A. Marti, C. Wirbelauer, M. Scheffner and W. Krek, *Interaction between ubiquitin-protein ligase scfskp2 and e2f-1 underlies the regulation of e2f-1 degradation*, Nat. Cell Biol., **1** (1999), 14–19.
[45] A. Montagnoli, F. Fiore, E. Eytan, A. C. Carrano, G. F. Draetta, A. Hershko and M. Pagano, *Ubiquitination of p27 is regulated by cdk-dependent phosphorylation and trimeric complex formation*, Genes Dev., **13** (1999), 1181–1189.

[46] D. O. Morgan, *Regulation of the apc and the exit from mitosis*, Nat. Cell Biol., **1** (1999), E47–53.
[47] M. C. Morris, A. Heitz, J. Mery, F. Heitz and G. Divita, *An essential phosphorylation-site domain of human cdc25c interacts with both 14-3-3 and cyclins*, J. Biol. Chem., **275** (2000), 28849–28857.
[48] M. Murphy, M. G. Stinnakre, C. Senamaud-Beaufort, N. J. Winston, C. Sweeney, M. Kubelka, M. Carrington, C. Brechot and J. Sobczak-Thepot, *Delayed early embryonic lethality following disruption of the murine cyclin a2 gene*, Nat. Genet., **15** (1997), 83–86.
[49] K. Nakayama, N. Ishida, M. Shirane, A. Inomata, T. Inoue, N. Shishido, I. Horii, D. Y. Loh and K. Nakayama, *Mice lacking p27(kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors*, Cell, **85** (1996), 707–720.
[50] K. I. Nakayama, S. Hatakeyama and K. Nakayama, *Regulation of the cell cycle at the g1-s transition by proteolysis of cyclin e and p27kip1*, Biochem. Biophys. Res. Commun., **282** (2001), 853–860.
[51] P. Nash, X. Tang, S. Orlicky, Q. Chen, F. B. Gertler, M. D. Mendenhall, F. Sicheri, T. Pawson and M. Tyers, *Multisite phosphorylation of a cdk inhibitor sets a threshold for the onset of DNA replication*, Nature, **414** (2001), 514–521.
[52] R. Norel and Z. Agur, *A model for the adjustment of the mitotic clock by cyclin and mpf levels*, Science, **251** (1991), 1076–1078.
[53] P. Nurse, *A long twentieth century of the cell cycle and beyond*, Cell, **100** (2000), 71–78.
[54] D. A. Orlando, C. Y. Lin, A. Bernard, J. Y. Wang, J. E. Socolar, E. S. Iversen, A. J. Hartemink and S. B. Haase, *Global control of cell-cycle transcription by coupled cdk and network oscillators*, Nature, **453** (2008), 944–947.
[55] M. Peter, *The regulation of cyclin-dependent kinase inhibitors (ckis)*, Prog. Cell Cycle Res., **3** (1997), 99–108.
[56] J. M. Peters, *Scf and apc: The yin and yang of cell cycle regulated proteolysis*, Curr. Opin. Cell Biol., **10** (1998), 759–768.
[57] B. Pfeuty and K. Kaneko, *Minimal requirements for robust cell size control in eukaryotic cells*, Phys. Biol., **4** (2007), 194–204.
[58] S. Prinz, E. S. Hwang, R. Visintin and A. Amon, *The regulation of cdc20 proteolysis reveals a role for apc components cdc23 and cdc27 during s-phase and early mitosis*, Curr. Biol., **8** (1998), 750–760.
[59] F. Puntoni and E. Villa-Moruzzi, *Phosphorylation of protein phosphatase-1 isoforms by cdc2-cyclin b in vitro*, Mol. Cell. Biochem., **171** (1997), 115–120.
[60] Z. Qu, W. R. MacLellan and J. N. Weiss, *Dynamics of the cell cycle: Checkpoints, sizers, and timers*, Biophys. J., **85** (2003), 3600–3611.
[61] T. Reis and B. A. Edgar, *Negative regulation of de2f1 by cyclin-dependent kinases controls cell cycle timing*, Cell, **117** (2004), 253–264.
[62] J. M. Roberts and C. J. Sherr, *Bared essentials of cdk2 and cyclin E*, Nat. Genet., **35** (2003), 9–10.
[63] J. Rudolph, *Targeting the neighbor's pool*, Mol. Pharmacol., **66** (2004), 780–782.
[64] D. Santamaria, C. Barriere, A. Cerqueira, S. Hunt, C. Tardy, K. Newton, J. F. Caceres, P. Dubus, M. Malumbres and M. Barbacid, *Cdk1 is sufficient to drive the mammalian cell cycle*, Nature, **448** (2007), 811–815.
[65] C. J. Sherr and J. M. Roberts, *Living with or without cyclins and cyclin-dependent kinases*, Genes Dev., **18** (2004), 2699–2711.
[66] P. Sicinski, J. L. Donaher, S. B. Parker, T. Li, A. Fazeli, H. Gardner, S. Z. Haslam, R. T. Bronson, S. J. Elledge and R. A. Weinberg, *Cyclin d1 provides a link between development and oncogenesis in the retina and breast*, Cell, **82** (1995), 621–630.
[67] M. J. Solomon, M. Glotzer, T. H. Lee, M. Philippe and M. W. Kirschner, *Cyclin activation of p34cdc2*, Cell, **63** (1990), 1013–1024.
[68] M. J. Solomon and P. Kaldis, *Regulation of cdks by phosphorylation*, Results Probl. Cell Differ., **22** (1998), 79–109.
[69] P. T. Spellman, G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders, M. B. Eisen, P. O. Brown, D. Botstein and B. Futcher, *Comprehensive identification of cell cycle-regulated genes of the yeast saccharomyces cerevisiae by microarray hybridization*, Mol. Biol. Cell, **9** (1998), 3273–3297.
[70] K. Sriram, G. Bernot and F. Kepes, *A minimal mathematical model combining several regulatory cycles from the budding yeast cell cycle*, IET Syst. Biol., **1** (2007), 326–341.

[71] T. T. Su and J. Stumpff, *Promiscuity rules? The dispensability of cyclin E and Cdk2*, Sci. STKE, **2004** (2004), pe11.
[72] M. Sugimoto, N. Martin, D. P. Wilks, K. Tamai, T. J. Huot, C. Pantoja, K. Okumura, M. Serrano and E. Hara, *Activation of cyclin d1-kinase in murine fibroblasts lacking both p21(Cip1) and p27(Kip1)*, Oncogene, **21** (2002), 8067–8074.
[73] S. Tamrakar, E. Rubin, and J. W. Ludlow, *Role of prb dephosphorylation in cell cycle regulation*, Front Biosci., **5** (2000), D121–137.
[74] O. Tetsu and F. McCormick, *Proliferation of cancer cells despite Cdk2 inhibition*, Cancer Cell, **3** (2003), 233–245.
[75] J. E. Toettcher, A. Loewer, G. J. Ostheimer, M. B. Yaffe, B. Tidor and G. Lahav, *Distinct mechanisms act in concert to mediate cell cycle arrest*, Proc. Natl. Acad. Sci. U.S.A., **106** (2009), 785–790.
[76] J. J. Tyson and B. Novak, *Regulation of the eukaryotic cell cycle: Molecular antagonism, hysteresis, and irreversible transitions*, J. Theor. Biol., **210** (2001), 249–263.
[77] S. van den Heuvel and N. J. Dyson, *Conserved functions of the prb and e2f families*, Nat. Rev. Mol. Cell Biol., **9** (2008), 713–724.
[78] H. C. Vodermaier, *Apc/c and scf: Controlling each other and the cell cycle*, Curr. Biol., **14** (2004), R787–796.
[79] V. Volterra, "Animal Ecology," Chapman R. N., McGraw-Hill, New York., 1931, 409–448.
[80] R. Wasch and F. R. Cross, *Apc-dependent proteolysis of the mitotic cyclin clb2 is essential for mitotic exit*, Nature, **418** (2002), 556–562.
[81] J. Weinstein, *Cell cycle-regulated expression, phosphorylation, and degradation of p55cdc. a mammalian homolog of cdc20/fizzy/slp1*, J. Biol. Chem., **272** (1997), 28501–28511.
[82] M. L. Whitfield, G. Sherlock, A. J. Saldanha, J. I. Murray, C. A. Ball, K. E. Alexander, J. C. Matese, C. M. Perou, M. M. Hurt, P. O. Brown and D. Botstein, *Identification of genes periodically expressed in the human cell cycle and their expression in tumors*, Mol. Biol. Cell, **13** (2002), 1977–2000.
[83] L. Wu, C. Timmers, B. Maiti, H. I. Saavedra, L. Sang, G. T. Chong, F. Nuckolls, P. Giangrande, F. A. Wright, S. J. Field, M. E. Greenberg, S. Orkin, J. R. Nevins, M. L. Robinson and G. Leone, *The E2f1-3 transcription factors are essential for cellular proliferation*, Nature, **414** (2001), 457–462.
[84] M. Xu, K. A. Sheppard, C. Y. Peng, A. S. Yee and H. Piwnica-Worms, *Cyclin a/Cdk2 binds directly to e2f-1 and inhibits the dna-binding activity of e2f-1/dp-1 by phosphorylation*, Mol. Cell Biol., **14** (1994), 8420–8431.
[85] K. Yang, M. Hitomi and D. W. Stacey, *Variations in cyclin d1 levels through the cell cycle determine the proliferative fate of a cell*, Cell Div., **1** (2006), 32.
[86] W. Zachariae and K. Nasmyth, *Whose end is destruction: Cell division and the anaphase-promoting complex*, Genes Dev., **13** (1999), 2039–2058.
[87] H. Zhang, R. Kobayashi, K. Galaktionov and D. Beach, *P19skp1 and p45skp2 are essential elements of the cyclin A-CDK2 S phase kinase*, Cell, **82** (1995), 915–925.
[88] J. Zhang, X. Dong, Y. Fujimoto and H. Okamura, *Molecular signals of mammalian circadian clock*, Kobe J. Med. Sci., **50** (2004), 101–109.
[89] L. Zhang and C. Wang, *F-box protein skp2: A novel transcriptional target of e2f*, Oncogene, **25** (2006), 2615–2627.
[90] P. Zhou and P. M. Howley, *Ubiquitination and degradation of the substrate recognition subunits of scf ubiquitin-protein ligases*, Mol. Cell, **2** (1998), 571–580.

Received April 14, 2010; Accepted October 30, 2010.

E-mail address: orit.lavi@nih.gov
E-mail address: ginsbed@mail.biu.ac.il
E-mail address: louzouy@math.biu.ac.il