Modelling the eukaryotic cell cycle

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ABSTRACT

The cell division cycle is a biological mechanism that ensures the production of new cells by mitosis. This process is cyclic, begins with a growth factor (GF) and progresses by the sequential activation of cyclin and cyclin-dependent kinases (cdk). In this thesis, we propose a new model for the eukaryotic cell cycle.

The model gives a more complete description than previous reported models. It considers that all cyclin-cdk complexes participating in the cell cycle can describe the global mechanism in a minimal way. We simulate the time evolution concentration of the complexes cyclinD-cdk4-6, cyclinE-cdk2, cyclinA-cdk1-2, cyclinB-cdk1, transcription factor E2F and of the GF in a network of enzymatic reactions. Some of these enzymatic reactions have positive feedback loops.

Positive feedback loops provided periodic solutions for both cyclinE-cdk2 and cyclinB-cdk1 complexes. CyclinD-cdk4-6 and cyclinA-cdk1-2 don't have positive feedback loops and don't oscillate, unless by external oscillation of GF which can induce a global cyclin-cdk oscillation.

A Cell cycle checkpoint arises by the phosphatase cdc25. This protein participates in positive feedback loops and induces the system to cross a Hopf bifurcation, leading to stable oscillations of cyclinE-cdk2 and cyclinB-cdk1, corresponding to the G1/S and G2/M transitions, respectively.

Globally, the differential equation model obtained with the *mass-action law*, sets the growth factor as the master regulator of the cell cycle and connects important extracellular and intracellular components for the generation of the eukaryotic cell cycle.

Index Terms - Cell cycle, Growth Factor (GF), cyclins, cyclin-dependent kinase (Cdk), systems biology.

1. INTRODUCTION

In 1982, Tim Hunt and Paul Nurse identified a new class of proteins they called cyclins, partially solving the question of "who are the agents" responsible for cell cycle progression. The cyclins associate with a specific cdk (cyclin dependent kinase) and originate different complexes, each one responsible for a different cell cycle stage.

Describing and understanding the mechanisms of cyclin-cdk complexes is important to describe the modern eukaryotic-human cell cycle but also to establish an evolutionary bridge between different eukaryotic organisms. Understanding the cell cycle dynamics may become a powerful tool in cancer therapeutics and other cell diseases.

The cell division cycle

The cell cycle is divided in two phases: the interphase and mitosis (M). The interphase is subdivided in 4 phases: G0, G1, S and G2. The mitosis is also subdivided in 4 phases: Prophase, Metaphase, Anaphase and Telophase.

1.1. G0

In G0, the cell is in a quiescent state (non-dividing state) and must receive an external stimulus from a growth factor (GF) in order to leave G0 and initiate a new cell cycle.

1.2. Early G1 and the restriction point (R)

In the early G1, the GF activates membrane receptors that send signals to intracellular protein kinases and phosphatases through the RAS/RAF/MEK/ERK pathway, [1]. The ERK activates

the Myc and AP-1 transcription factors that promote the expression of important genes such as the genes for cyclinD and for proteins/enzymes required for progression in G1, [2].

Growth factor signaling pathway is fundamental for the production of cyclinD and experimental results show that ectopic expression of cyclinD makes the GF dispensable to initiate the cell cycle confirming its primary role in activating cyclinD, [3].

Once the cell enters in the G1 phase it must receive continuous supply of nutrients and produce specific proteins otherwise it returns to G0. In mid-G1, in the restriction point (R), the cell confirms if these conditions are verified.

To pass the R-point, the complex cyclinD-cdk4-6 has to oppose the inhibitory effect of the retinoblastoma protein pRb. CyclinD-cdk-4-6 phosphorylates and partly dissociates the pRb from the E2F and allows this partial-free version of the E2F to act as a transcription factor for the gene of cyclinE, [3].

1.3. Late G1

After the restriction point, the pRb is further inhibited by the cyclinE-cdk2. This inhibition reflects a positive feedback loop. As more cyclinE-cdk2 is formed more pRb is inhibited and consequently more cyclinE is produced by the E2F, increasing significantly the concentration of cyclinE-cdk2. At the same time, as more cyclinE is produced, more cdc25A is activated and, since cdc25A also contributes to cyclinE-cdk2 activation, we have a second positive feedback loop enhancing cyclinE-cdk2 concentration.

CyclinE-cdk2 is important to initiate the S-phase: it phosphorylates substrates related to DNA elongation and replication and substrates that increase the cell size. Cells with an excess of cyclinE concentration have a quicker progression through G1 and smaller cells reaching the S-phase, [4].

In figure 1, we show how cyclinDcdk4-6 and cyclinE-cdk2 concentration changes along the G1 phase.
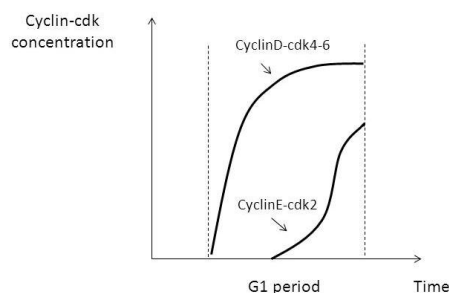

Figure 1: Qualitative concentration of cyclinDcdk4-6 and cyclinE-cdk2 during the G1 phase.

1.4. S–phase (synthesis phase)

The S-phase is marked by the replication of the DNA. CyclinA is expressed in the mid-S-phase by the E2F and forms a complex with cdk1 or cdk2. CyclinA-cdk1-2 induces DNA replication and ensures that DNA replication only happens once per cell cycle.

In the S-phase cyclinA-cdk1-2 concentration increases and cyclinD-cdk4-6 and cyclinE-cdk2 concentration decreases.

CyclinD-cdk4-6 represses DNA replication and its concentration must decay in the S-phase. CyclinD is phosphorylated in the Threonine-286 which makes it vulnerable to ubiquitination and degradation by the proteasome p26, [5].

CyclinE-cdk2 is also degraded in the S-phase by the glycogen synthase kinase 3b (GSK3b) that phosphorylates the residues Thr-380 and Ser-384. This also induces the ubiquitylation and degradation of the complex by the proteasome p26, [6].

The concentration of cyclinE-cdk2, cyclinA-cdk1-2 and cyclinD-cdk4-6 in the S-phase follows the curves indicated in figure 2.
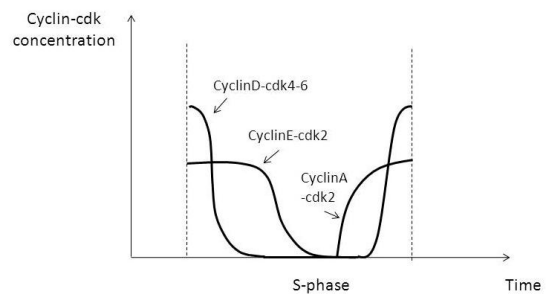

Figure 2: Qualitative concentration of cyclinD, cyclinE and cyclinA complexes during the S-phase.

1.5. G2

The S/G2 transition is promoted by the cyclinA-cdk1-2. The G2 is characterized by a rapid cell growth and synthesis of proteins necessary for mitosis.

In this stage, the relevant cyclins-cdk complexes are cyclinA-cdk1, cyclinA-cdk2 and cyclinB-cdk1 (MPF). CyclinA-cdk1-2 is sufficient to drive G2 phase cells into mitosis but abnormal features may occur. Correct passage into mitosis requires the presence of both cyclinA-cdk1-2 and MPF, [7].

The protein kinase Wee1 is known to have a negative effect on the MPF, [8]. The activated MPF has the residues tyrosine 15 and Threonine 14 dephosphorylated and the residue Threonine 161 phosphorylated. The Wee1 phosphorylates the Tyr-15 and Thr-14

residues and inactivates the MPF. The protein cdc25C induces the dephosphorylation of the Tyr-15 and Thr-14 residues and contributes to MPF activation, [1].

It is thought that if the cell does not acquire a sufficient size for mitosis the Wee1 remains active and maintains the MPF inactivated. Once the cell reaches a sufficient size, coherent with the mitosis requirement, the Wee1 is inactivated and the MPF is activated, allowing progression to mitosis, [9].

There are two positive feedback loops in G2 phase. The first one is between the MPF and Wee1. The Wee1 inactivates the Wee1 but the active MPF phosphorylates and inactivates the Wee1, silencing it and promoting more MPF activation. The second positive feedback loop includes the phosphatase cdc25C and the MPF. Cdc25C is activated by the MPF and the cdc25C also activates MPF, [10].

CyclinD concentration increases before the beginning of G2. CyclinD is especially important to stimulate the cell to have another mitotic cycle once this cycle finishes. The GF must be present in the extracellular medium in late G2, because cyclinD is produced by the GF, otherwise the cell will enter in a quiescence state after this mitosis, [11].

The evolution of MPF, cyclinA-cdk1-2 and cyclinD-cdk4-6 concentration during G2 follows, approximately, the curves represented in figure3.
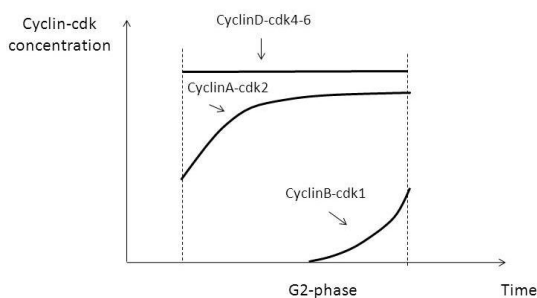

Figure 3: Qualitative concentration of cyclinD, cyclinA and cyclinB complexes during G2 phase.

1.6. Mitotic phase

CyclinA-cdk1-2 is important until the end of the prophase and the MPF is important for prophase and metaphase, participating in the chromosome condensation, spindle arrangement and nuclear membrane breakdown, [7].

CyclinA and CyclinB are degraded by the APC, [12]. Both APC and cdc20 are phosphorylated by the MPF and form the complex APC-cdc20. The APC-cdc20 attaches a polyubiquitin chain (tail of ubiquitin molecules) causing MPF and cyclinA-cdk1-2 degradation via the proteasome p26. This describes a negative feedback loop for the MPF: the MPF activates the APC but the APC forces the MPF to be degraded by the p26, [12].

The APC-cdc20 is responsible for sister chromatids separation and anaphase initiation.

The evolution of cyclinA-cdk1-2, cyclinD-cdk4-6 and MPF concentration in the M-phase is represented in figure 4.
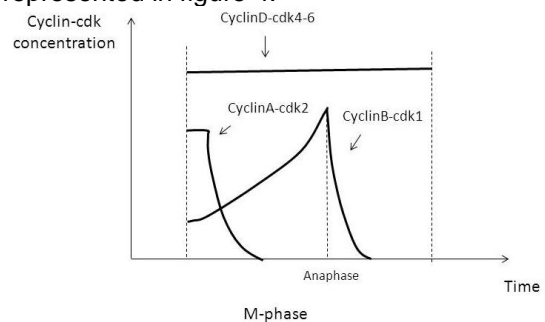

Figure 4: Qualitative concentration of cyclin D, cyclin A and cyclin B complexes during the M-phase.

1.7. Mitotic exit

In the final steps of the cell cycle the substrates that were phosphorylated by cyclin-cdk complexes are dephosphorylated by APC-cdh14 and the cell returns to G0.

2. MINIMAL CELL CYCLE MODEL FOR A SOMATIC EUKARYOTIC CELL

2.1. Mass-action law

The *mass-action law* establishes the differential equations describing the time-evolution of the concentration of the elements that make up a chemical reaction.

For a general reaction with substances, A_j, j=1,2,….m, we consider n possible chemical reactions, i=1,2….n,

$$v_{i1}A_1 + \cdots + v_{im}A_m \xrightarrow{k_i} \mu_{i1}A_1 + \cdots + \mu_{im}A_m$$
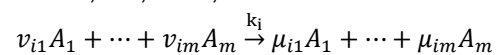
where v_{ij} and μ_{ij} are integer stoichiometric coefficients, and k_i is the velocity of the reactions.

The density of the chemical species is considered to be uniformly distributed in the

reaction medium and so the concentration for each specie A_j changes in time according to,

$$\frac{dA_j}{dt} = \sum_{i=1}^{n} k_i\left(u_{ij} - v_{ij}\right)A_1^{v_{i1}} \dots A_m^{v_{im}} \qquad (1)$$

and this equation can be written in the matrix form,

$$\frac{d}{dt}\begin{pmatrix} A_1 \\ \vdots \\ A_m \end{pmatrix} = \begin{pmatrix} u_{11} - v_{11} & \cdots & u_{n1} - v_{n1} \\ \vdots & \ddots & \vdots \\ u_{1m} - v_{1m} & \cdots & u_{nm} - v_{nm} \end{pmatrix}\begin{pmatrix} k_1 A_1^{V_{11}} & \cdots & A_n^{V_{im}} \\ \vdots & \ddots & \vdots \\ k_n A_1^{V_{n1}} & \cdots & A_m^{V_{nm}} \end{pmatrix}(2)$$

Denoting by r the rank of the matrix in (2), there are only r independent equations and consequently, (m-r) conservation laws, [13].

2.2. Enzymatic chain of reactions with feedback and feedforward loops

In order to apply the mass-action law, we first need to define the type of reactions we are simulating. Kinetic reactions are represented by the general collisional diagram

$$A + B \xrightarrow{k_1} C$$
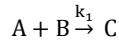
where A, B and C indicate atoms or molecules participating in the reaction. Enzymatic reactions are described differently,

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \xrightarrow{k_2} E + P \qquad (3)$$
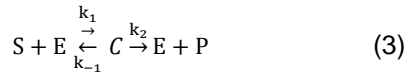
where E is an enzyme, C an intermediate complex and P the product of the reaction. In this work, the mechanism in (4), a Michaelis-Menten chain reaction, is abbreviated and written as,

$$S - - \xrightarrow{E} P \qquad (4)$$

It is common to assume a steady state approximation, where C and E are taken as constants, [15]. An enzymatic reaction can be associated to a mechanism of feedback or feedforward activation or inhibitory loop. In figure 5, we show a possible mechanism with a feedback activation loop,
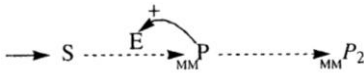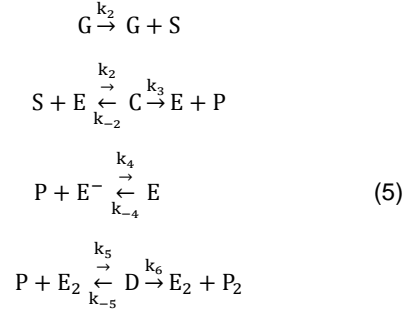

Figure 5: Minimal chain of enzymatic reactions that produce oscillatory solutions. This mechanism consists of two enzymatic reactions coupled to a positive feedback loop.

The global kinetic reactions for the mechanism in figure 5 are,

$$G \xrightarrow{k_2} G + S$$

$$S + E \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C \xrightarrow{k_3} E + P$$

$$P + E^- \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} E \qquad (5)$$

$$P + E_2 \underset{k_{-5}}{\overset{k_5}{\rightleftarrows}} D \xrightarrow{k_6} E_2 + P_2$$

The first step in (5) is the production of the protein S by transcription of the gene G. The second and fourth reactions are Michaelis-Menten enzymatic reactions. The third reaction is the positive feedback loop that in figure 5 is signed with a "**+**" arrow.

Applying the mass-action law to (5), we obtain the differential equations describing the concentration of each agent in the mechanism (5),

$$C'(t) = k_{-2}\left(-C(t)\right) - k_3 C(t) + k_2 E(t)S(t)$$
$$D'(t) = k_{-5}\left(-D(t)\right) - k_6 D(t) + k_6 E_2(t)P(t)$$
$$E^{-'}(t) = k_{-4}E(t) - k_4 E^-(t)P(t)$$
$$P'(t) = k_3 C(t) + k_{-5}D(t) + k_{-4}E(t) - k_4 E^-(t)P(t) - k_5 E_2(t)P(t)$$
$$S'(t) = k_{-2}C(t) + k_1 G(t) - k_2 E(t)S(t)$$

$$\qquad (6)$$

with the conservation laws,

$$G(t) = G_0$$

$$D(t) + E_2(t) = E_2(0) \qquad (7)$$

$$C(t) + E(t) + E^-(t) = E(0)$$

where G_0 is the concentration of the gene G and $E(0)$ and $E_2(0)$ are the initial concentration of the enzymes E and E_2, respectively.

Solving the conservation-laws (7) with the steady-state conditions in (8),

$$C'(t) = 0, D'(t) = 0 \text{ and } E^{-'} = 0 \qquad (8)$$

in order to the variables E, E_2, C, D and E^-, we obtain,

$$\begin{cases} E_2(t) = \frac{E_2(0)(k_{-5}+k_6)}{k_5 P(t)+k_{-5}+k_6} \\ \\ E(t) = \frac{E(0)(k_{-2}+k_3)k_4 P(t)}{k_4 P(t)(k_2 S(t)+k_{-2}+k_3)+k_{-4}(k_{-2}+k_3)} \\ C(t) = \frac{E(0)k_2 k_4 P(t)S(t)}{k_4 P(t)(k_2 S(t)+k_{-2}+k_3)+k_{-4}(k_{-2}+k_3)} \\ D(t) = \frac{E(0)k_5 P(t)}{k_5 P(t)+k_{-5}+k_6} \\ E^-(t) = \frac{E(0)k_{-4}(k_{-2}+k_3)}{k_4 P(t)(k_2 S(t)+k_{-2}+k_3)+k_{-4}(k_{-2}+k_3)} \end{cases} \qquad (9)$$

Substitution of (9) into (8) simplifies equations in (8) into a system of two equations that

describe the time–evolution concentration of S(t) and P(t), where $\nu = k_1 G_0$

$$\begin{cases} S'(t) = \nu - \dfrac{E(0)k_3 P(t)S(t)}{P(t)\left(S(t)+\frac{k_{-2}+k_3}{k_2}\right)+\frac{k_{-4}}{k_4}} \\ P'(t) = \dfrac{E(0)k_3 P(t)S(t)}{P(t)\left(S(t)+\frac{k_{-2}+k_3}{k_2}\right)+\frac{k_{-4}}{k_4}} - \dfrac{E_2(0)k_6 P(t)}{P(t)+\frac{k_{-5}+k_6}{k_5}} \end{cases} \quad (10)$$
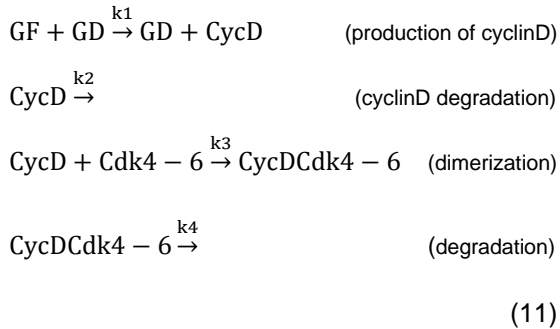
3. RESULTS

3.1. A new model for the eukaryotic cell cycle

During the last decades many minimalistic models have been published but, in general, the models are just too simple and can't explain the skeletal reactions of the cell cycle.

In this work we developed a new model for the eukaryotic cell cycle based on the mechanisms in which cyclin-cdk complexes participate. We constructed the model by translating the biochemical processes into skeletal kinetic reactions and we characterized these reactions by differential equations that describe the time-evolution concentration of these agents.

3.1.1. CyclinD

According to section 1, we defined the next biochemical mechanisms for cyclinD:

$$GF + GD \xrightarrow{k1} GD + CycD \qquad \text{(production of cyclinD)}$$

$$CycD \xrightarrow{k2} \qquad \text{(cyclinD degradation)}$$

$$CycD + Cdk4-6 \xrightarrow{k3} CycDCdk4-6 \qquad \text{(dimerization)}$$

$$CycDCdk4-6 \xrightarrow{k4} \qquad \text{(degradation)}$$

$$(11)$$

The first reaction represents CycD production by the action of GF. The gene works as a template and is not consumed in the reaction, [15]. The other reactions are simple kinetic mechanisms.

The mechanisms (11) were introduced into the *Mathematica* programming language and simulated with the software named Kinetics, [16]. The software returns the differential equations describing the temporal evolution of each chemical specimen, applying the mass-action law and Michaelis-Menten formulas described in sections 2.1 and 2.2.

test1 = {GF + GD -> GD + CycD, CycD ->ϕ_1 , CycD + Cdk46 -> CycDCdk46, CycDCdk46 ->ϕ_2 };
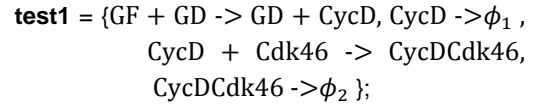
ϕ_1 and ϕ_2 represent the degraded version of the respective proteins that we will not follow. The differential equations for this mechanism are,

```
Cdk46'[t] == -Cdk46[t] CycD[t] k₃
CycD'[t] == GD[t] GF[t] k₁ - CycD[t] k₂ - Cdk46[t] CycD[t] k₃
CycDCdk46'[t] == Cdk46[t] CycD[t] k₃ - CycDCdk46[t] k₄
GD'[t] == 0
GF'[t] == -GD[t] GF[t] k₁
ϕ1'[t] == CycD[t] k₂
ϕ2'[t]|== CycDCdk46[t] k₄
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$$(12)$$

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Conservation Laws:
 Cdk46[t] + CycDCdk46[t] + ϕ2[t]
 -Cdk46[t] + CycD[t] + GF[t] + ϕ1[t]
 GD[t]
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$$(13)$$

The only conservation law considered was $GD[t] = GD0$. Since both cdk4 and cdk6 are not limiting agents they were simulated as constants values. CycD and CycDCdk46 are associated with differential equations (14),

$$\begin{cases} CycD'[t] = \alpha1 * GF[t] - \alpha2 * CycD[t] \\ CycDCdk46'[t] = \alpha3 * CycD[t] - \alpha4 * CycDCdk46[t] \end{cases}$$

$$(14)$$

Considering that the cell cycle occurs when GF is present and vanishes when GF is absent, we chose the usual unit step Heaviside function to define the GF[t],

$$GF[t] = \sum_{i=0}^{N} H(t - Ti) - H(t - (Ti - S))$$

$$(15)$$

During the period T, GF[t] is 1 for t in the interval [0,S] and zero in the interval [S,T]. The parameter N determines the number of cycles in the GF signal. Substituting (15) in (14) we get the periodic solution represented in figure 6.

The concentrations of cyclinD and of the correspondent complex describe periodic oscillations under the effect of a periodic behavior for GF[t].
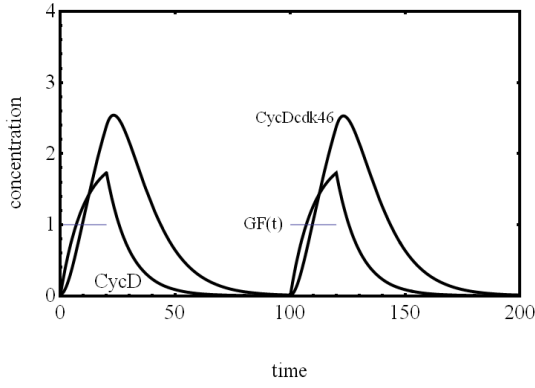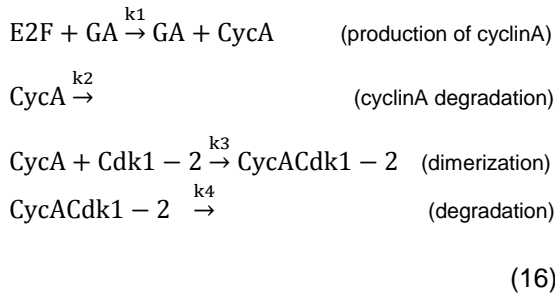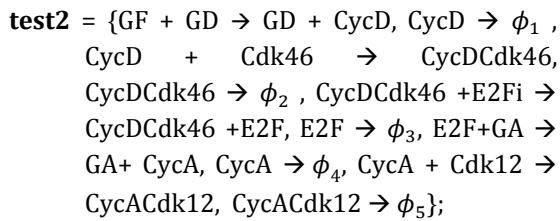
Figure 6: Time evolution of the concentration of GF, cyclinD and cyclinD-cdk46, considering that the growth factor as the periodic function (15). The parameter values of the simulation are: $\alpha 1 = 0.2, \alpha 2 = 0.1, \alpha 3 = 0.2, \alpha 4 = 0.1, N = 1, T = 100$ and $S = 20$.

3.1.2. CyclinA + cyclinD

We defined the following set of biochemical mechanisms for cyclinA, very similar to the ones described for cyclinD.

$$E2F + GA \xrightarrow{k1} GA + CycA \qquad \text{(production of cyclinA)}$$

$$CycA \xrightarrow{k2} \qquad \text{(cyclinA degradation)}$$

$$CycA + Cdk1 - 2 \xrightarrow{k3} CycACdk1 - 2 \quad \text{(dimerization)}$$

$$CycACdk1 - 2 \xrightarrow{k4} \qquad \text{(degradation)}$$

(16)

CyclinA is expressed by the E2F and there are not positive feedback loops in its intrinsic mechanisms. Since the mechanisms in (16) are very close from the ones shown in (11) and we would get the same results, so we decided to extend the analysis and include cyclinD and cyclinA in the same simulation:

test2 = {GF + GD → GD + CycD, CycD → ϕ_1 , CycD + Cdk46 → CycDCdk46, CycDCdk46 → ϕ_2 , CycDCdk46 +E2Fi → CycDCdk46 +E2F, E2F → ϕ_3, E2F+GA → GA+ CycA, CycA → ϕ_4, CycA + Cdk12 → CycACdk12, CycACdk12 → ϕ_5};

The differential equations associated with test2 are,

```
Cdk12'[t] == -Cdk12[t] CycA[t] k9
Cdk46'[t] == -Cdk46[t] CycD[t] k3
CycA'[t] == E2F[t] GA[t] k7 - CycA[t] k8 - Cdk12[t] CycA[t] k9
CycACdk12'[t] == Cdk12[t] CycA[t] k9 - CycACdk12[t] k10
CycD'[t] == GD[t] GF[t] k1 - CycD[t] k2 - Cdk46[t] CycD[t] k3
CycDCdk46'[t] == Cdk46[t] CycD[t] k3 - CycDCdk46[t] k4
E2F'[t] == CycDCdk46[t] E2Fi[t] k5 - E2F[t] k6 - E2F[t] GA[t] k7
E2Fi'[t] == -CycDCdk46[t] E2Fi[t] k5
GA'[t] == 0
GD'[t] == 0
GF'[t] == -GD[t] GF[t] k1
φ1'[t] == CycD[t] k2
φ2'[t] == CycDCdk46[t] k4
φ3'[t] == E2F[t] k6
φ4'[t] == CycA[t] k8
φ5'[t] == CycACdk12[t] k10
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(17)

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Conservation Laws:

Cdk12[t] + CycACdk12[t] + φ5[t]
-Cdk12[t] + CycA[t] + E2F[t] + E2Fi[t] + φ3[t] + φ4[t]
Cdk46[t] + CycDCdk46[t] + φ2[t]
-Cdk46[t] + CycD[t] + GF[t] + φ1[t]
GD[t]
GA[t]
```

(18)

Only the conservation laws $GD[t] \rightarrow GD0, GE[t] \rightarrow GE0$ were considered (once again we are not interested in describing the degraded proteins). The differential equation model for this system is,

$$\begin{cases} CycD'[t] = \alpha 1 GF[t] - \alpha 2 CycD[t] \\ CycDCdk46'[t] = \alpha 3 CycD[t] - \alpha 4 CycDCdk46[t] \\ CycA'[t] = \alpha 5 * E2F[t] - \alpha 6 CycA[t] \\ CycACdk2'[t] = \alpha 7 * CycA[t] - \alpha 8 * CycACdk2[t] \\ E2F'[t] = CycDCdk46[t] * \alpha 9 - \alpha 10 E2F[t] \end{cases}$$

(19)

Analysis of system (19) gives the possible periodic solution in figure 7.

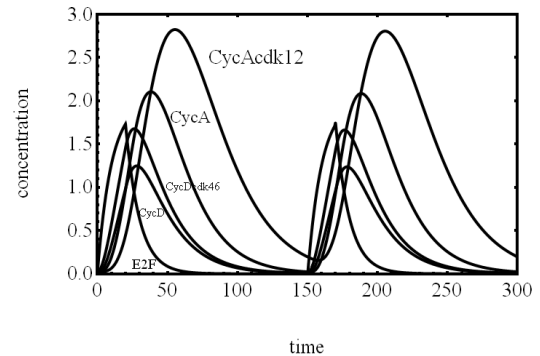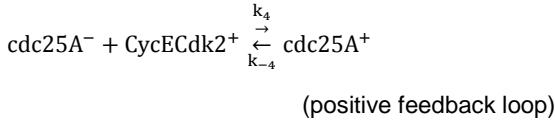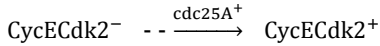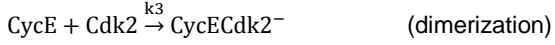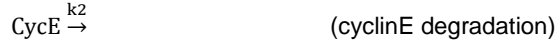**Figure 7**: Time-evolution concentration for GF, cyclin D, cyclin D-cdk46, E2F, cyclin A and cyclin A-cdk2 considering the growth factor as the periodic function (15). The parameter values chosen were: α1=0.2, α2=0.1, α3=0.09, α4=0.05, α5=0.2, α6=0.1, α7=0.09, α8=0.05, α9=0.45, α10=0.6, T=100, S=20 and N=1.

3.1.3. CyclinB and CyclinE

CyclinB and cyclinE mechanisms are quite similar. The production of cyclinE is associated with the mechanisms,

$$E2F + GE \xrightarrow{k1} GE + CycE \qquad \text{(production of cyclinE)}$$

$$CycE \xrightarrow{k2} \qquad \text{(cyclinE degradation)}$$

$$CycE + Cdk2 \xrightarrow{k3} CycECdk2^- \qquad \text{(dimerization)}$$

$$CycECdk2^- \quad -\,\xrightarrow{cdc25A^+}\quad CycECdk2^+$$

$$cdc25A^- + CycECdk2^+ \underset{k_{-4}}{\overset{k_4}{\underset{\leftarrow}{\rightarrow}}} cdc25A^+$$

$$\text{(positive feedback loop)}$$

$$CycECdk2^+ \quad -\,\xrightarrow{p26}\quad \text{(degradation)}$$

$$(20)$$

test3= {E2F + GE → GE + CycE, CycE → ϕ_1 , CycE + Cdk2 → CycECdk2⁻, CycECdk2⁻+cdc25A⁺ ↔ complex1, complex1→ CycECdk2⁺+cdc25A⁺, CycECdk2⁺ + p26 ↔ complex2, complex2 → p26 + ϕ_2, cdc25A⁻ + CycECdk2⁺ ↔ cdc25A⁺}

The package Kinetics returned the next differential equations for the mechanisms in test3.

```
Cdk2'[t] == -Cdk2[t] CycE[t] k₃
complex1'[t] ==
 -complex1[t] k₋₄ - complex1[t] k₅ + k₄ CycECdk2⁻[t] cdc25A⁺[t]
complex2'[t] ==
 -complex2[t] k₋₆ - complex2[t] k₇ + p26[t] k₆ CycECdk2⁺[t]
CycE'[t] == E2F[t] GE[t] k₁ - CycE[t] k₂ - Cdk2[t] CycE[t] k₃
E2F'[t] == -E2F[t] GE[t] k₁
GE'[t] == 0
p26'[t] ==
 complex2[t] k₋₆ + complex2[t] k₇ - p26[t] k₆ CycECdk2⁺[t]
φ1'[t] == CycE[t] k₂
φ2'[t] == complex2[t] k₇
(cdc25A⁻)'[t] == k₋₈ cdc25A⁺[t] - k₈ cdc25A⁻[t] CycECdk2⁺[t]
(CycECdk2⁻)'[t] == complex1[t] k₋₄ +
  Cdk2[t] CycE[t] k₃ - k₄ CycECdk2⁻[t] cdc25A⁺[t]
(cdc25A⁺)'[t] ==
 complex1[t] k₋₄ + complex1[t] k₅ - k₋₈ cdc25A⁺[t] -
  k₄ CycECdk2⁻[t] cdc25A⁺[t] + k₈ cdc25A⁻[t] CycECdk2⁺[t]
(CycECdk2⁺)'[t] ==
 complex2[t] k₋₆ + complex1[t] k₅ + k₋₈ cdc25A⁺[t] -
  p26[t] k₆ CycECdk2⁺[t] - k₈ cdc25A⁻[t] CycECdk2⁺[t]
```

$$(21)$$

```
Conservation Laws:

 Cdk2[t] + complex1[t] + complex2[t] +
  φ2[t] - cdc25A⁻[t] + CycECdk2⁻[t] + CycECdk2⁺[t]
 complex1[t] + cdc25A⁻[t] + cdc25A⁺[t]
 -Cdk2[t] + CycE[t] + E2F[t] + φ1[t]
 complex2[t] + p26[t]
 GE[t]
```

$$(22)$$

The conservation laws (22) (without the equations for ϕ_1 and ϕ_2) and the steady state conditions,

$$\text{complex1}'(t) = 0, \text{complex2}'(t) = 0$$
$$\text{cdc25A}^{-\,'}(t) = 0 \text{ and } \text{cdc25A}^{+\,'}(t) = 0$$

can be solved in order to complex1, complex2, cdc25A⁻, p26 and cdc25A⁺, to obtain the next differential equations,

```
GE[t] → GE0
```

$$p26[t] \to \frac{p260\,(k_{-6} + k_7)}{k_{-6} + k_7 + k_6\,CycECdk2^+[t]}$$

$$complex2[t] \to \frac{p260\,k_6\,CycECdk2^+[t]}{k_{-6} + k_7 + k_6\,CycECdk2^+[t]}$$

$$cdc25A^-[t] \to$$
$$\frac{k_{-8}\,(k_{-4} + k_5)\,cdc25A0^+}{k_{-8}\,(k_{-4} + k_5) + k_8\,(k_{-4} + k_5 + k_4\,CycECdk2^-[t])\,CycECdk2^+[t]}$$

$$complex1[t] \to$$
$$\frac{k_4\,k_8\,cdc25A0^+\,CycECdk2^-[t]\,CycECdk2^+[t]}{k_{-8}\,(k_{-4} + k_5) + k_8\,(k_{-4} + k_5 + k_4\,CycECdk2^-[t])\,CycECdk2^+[t]}$$

$$cdc25A^+[t] \to$$
$$\frac{(k_{-4} + k_5)\,k_8\,cdc25A0^+\,CycECdk2^+[t]}{k_{-8}\,(k_{-4} + k_5) + k_8\,(k_{-4} + k_5 + k_4\,CycECdk2^-[t])\,CycECdk2^+[t]}$$

$$(23)$$

By introducing (23) into (21) the system is simplified into,

$$\begin{cases} CycE'[t] = \alpha1 - \alpha2 * CycE[t] \\[2mm] CycECdk2^{-\,'}[t] = \alpha3 * CycE[t] - \dfrac{\beta1 * CycECdk2^-[t] * CycECdk2^+[t]}{\alpha4 + \alpha5 * CycECdk2^+[t] + CycECdk2^+[t] * CycECdk2^-[t]} \\[2mm] CycECdk2^{+\,'}[t] = \dfrac{\beta1 * CycECdk2^-[t] * CycECdk2^+[t]}{\alpha4 + \alpha5 * CycECdk2^+[t] + CycECdk2^+[t] * CycECdk2^-[t]} - \dfrac{\beta2 * CycECdk2^+[t]}{\alpha6 + CycECdk2^+[t]} \end{cases}$$

$$(24)$$

This system of differential equations has one fixed point with coordinates,

$$\begin{cases} CycE^* = \dfrac{\alpha1}{\alpha2} \\[4mm] CycECdk2^{-*} = \dfrac{\frac{\alpha1\alpha3\alpha5\alpha6}{\alpha2} + \alpha4\left(\beta2 - \frac{\alpha1\alpha3}{\alpha2}\right)}{\alpha6 * \left(\beta1 - \frac{\alpha1\alpha3}{\alpha2}\right)} \\[4mm] CycECdk2^{+*} = \dfrac{\alpha6 * \alpha1 * \alpha3}{\alpha2\left(\beta2 - \frac{\alpha1\alpha3}{\alpha2}\right)} \end{cases}$$

$$(25)$$

The fixed point is positive if $\beta1 > \frac{\alpha1\alpha3}{\alpha2}$ and $\beta2 > \frac{\alpha1\alpha3}{\alpha2}$. Looking at the determinant and trace of the Jacobian matrix, calculated at

fixed point (25) we see that the determinant is always positive and the trace is only positive when expression (26) is verified,

$$\alpha 4(\beta 1 - \beta 2)\left(\beta 2 - \frac{\alpha 1 \alpha 3}{\alpha 2}\right)^2 >$$

$$\alpha 4^2 \beta 2 \left(\beta 1 - \frac{\alpha 1 \alpha 3}{\alpha 2}\right)^2 + \alpha 5 \alpha 6 \beta 1 \left(\beta 2 - \frac{\alpha 1 \alpha 3}{\alpha 2}\right)^2$$

$$(26)$$

which, to be true, implies $\beta 1 > \beta 2$. In figure 8, we show the region of the parameter space $(\beta 1, \beta 2)$ where condition (26) is true and where the equation (24) assumes stable limit cycle solutions.
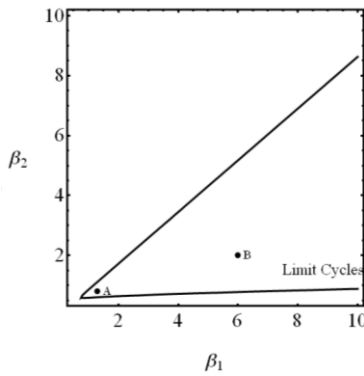

Figure 8: Inside the "triangular–like" region the model has sustainable limit cycle solutions for cyclinE and for cyclinE-cdk2 complex. In the interior, both trace and determinant of the Jacobian matrix are positive. The boundary curve was obtained by equaling the trace of the Jacobian matrix to zero. The point A has coordinates β1=1.3 and β2=0.8 and point B β1=6 and β2=2.0. The parameter values were fixed and chosen as: $\alpha_1 = 0.5$, $\alpha_2 = 1.1$, $\alpha_3 = 1.0$, $\alpha_4 = 1.0$, $\alpha_5 = 1.0$, $\alpha_6 = 0.12$.

Points A and B are inside the triangular-like region in figure 8 correspond to periodic behavior as we show in figure 9.
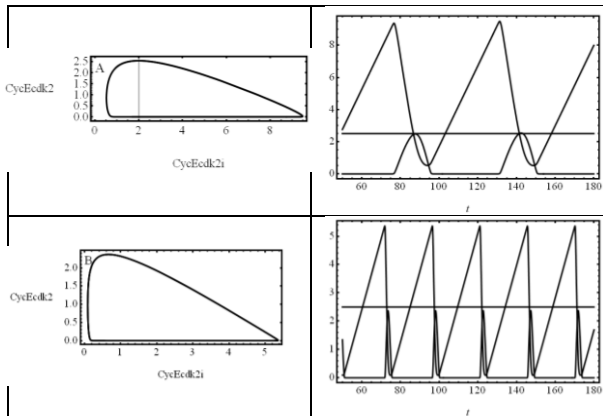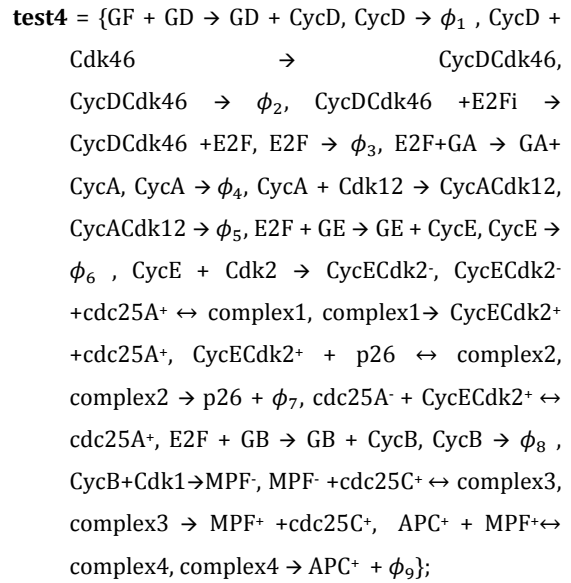

Figure 9: Limit cycle solutions acquired for points A and B identified in figure 8.

Even without the growth factor stimulation there are periodic solutions. The presence of positive feedback loops is crucial for this behavior.

3.1.4. Temporal evolution for all cyclin-cdk integrated in the same simulation.

If we now join all the previous mechanisms from 3.1.1 to 3.1.3, we get the global reactions for all the cyclins and cdks that participate in the cell cycle.

test4 = {GF + GD → GD + CycD, CycD → ϕ_1 , CycD + Cdk46 → CycDCdk46, CycDCdk46 → ϕ_2, CycDCdk46 +E2Fi → CycDCdk46 +E2F, E2F → ϕ_3, E2F+GA → GA+ CycA, CycA → ϕ_4, CycA + Cdk12 → CycACdk12, CycACdk12 → ϕ_5, E2F + GE → GE + CycE, CycE → ϕ_6 , CycE + Cdk2 → CycECdk2$^-$, CycECdk2$^-$ +cdc25A$^+$ ↔ complex1, complex1→ CycECdk2$^+$ +cdc25A$^+$, CycECdk2$^+$ + p26 ↔ complex2, complex2 → p26 + ϕ_7, cdc25A$^-$ + CycECdk2$^+$ ↔ cdc25A$^+$, E2F + GB → GB + CycB, CycB → ϕ_8 , CycB+Cdk1→MPF$^-$, MPF$^-$ +cdc25C$^+$ ↔ complex3, complex3 → MPF$^+$ +cdc25C$^+$, APC$^+$ + MPF$^+$↔ complex4, complex4 → APC$^+$ + ϕ_9};

Simulating the reactions in test4, we got the next differential equations for this system,

```
cdk1′[t] == -cdk1[t] CycB[t] k₂₁
Cdk12′[t] == -Cdk12[t] CycA[t] k₉
Cdk2′[t] == -Cdk2[t] CycE[t] k₁₃
Cdk46′[t] == -Cdk46[t] CycD[t] k₃
complex1′[t] == -complex1[t] k₋₁₄ -
  complex1[t] k₁₅ + k₁₄ CycECdk2⁻[t] cdc25A⁺[t]
complex2′[t] ==
 -complex2[t] k₋₁₆ - complex2[t] k₁₇ + p26[t] k₁₆ CycECdk2⁺[t]
complex3′[t] ==
 -complex3[t] k₋₂₂ - complex3[t] k₂₃ + k₂₂ MPF⁻[t] cdc25C⁺[t]
complex4′[t] ==
 -complex4[t] k₋₂₅ - complex4[t] k₂₆ + k₂₅ APC⁺[t] MPF⁺[t]
CycA′[t] == E2F[t] GA[t] k₇ - CycA[t] k₈ - Cdk12[t] CycA[t] k₉
CycACdk12′[t] == Cdk12[t] CycA[t] k₉ - CycACdk12[t] k₁₀
CycB′[t] == E2F[t] GB[t] k₁₉ - CycB[t] k₂₀ - cdk1[t] CycB[t] k₂₁
CycD′[t] == GD[t] GF[t] k₁ - CycD[t] k₂ - Cdk46[t] CycD[t] k₃
CycDCdk46′[t] == Cdk46[t] CycD[t] k₃ - CycDCdk46[t] k₄
CycE′[t] == E2F[t] GE[t] k₁₁ - CycE[t] k₁₂ - Cdk2[t] CycE[t] k₁₃
E2F′[t] == CycDCdk46[t] E2Fi[t] k₅ - E2F[t] k₆ -
```

$$\quad E2F[t]\ GA[t]\ k_7 - E2F[t]\ GE[t]\ k_{11} - E2F[t]\ GB[t]\ k_{19}$$
$$E2Fi'[t] == -CycDCdk46[t]\ E2Fi[t]\ k_5$$
$$GA'[t] == 0$$
$$GB'[t] == 0$$
$$GD'[t] == 0$$
$$GE'[t] == 0$$
$$GF'[t] == -GD[t]\ GF[t]\ k_1$$
$$p26'[t] ==$$
$$\quad complex2[t]\ k_{-16} + complex2[t]\ k_{17} - p26[t]\ k_{16}\ CycECdk2^+[t]$$
$$\phi1'[t] == CycD[t]\ k_2$$
$$\phi2'[t] == CycDCdk46[t]\ k_4$$
$$\phi3'[t] == E2F[t]\ k_6$$
$$\phi4'[t] == CycA[t]\ k_8$$
$$\phi5'[t] == CycACdk12[t]\ k_{10}$$
$$\phi6'[t] == CycE[t]\ k_{12}$$
$$\phi7'[t] == complex2[t]\ k_{17}$$
$$\phi8'[t] == CycB[t]\ k_{20}$$
$$\phi9'[t] == complex4[t]\ k_{26}$$
$$(cdc25A^-)'[t] == k_{-18}\ cdc25A^+[t] - k_{18}\ cdc25A^-[t]\ CycECdk2^+[t]$$
$$(cdc25C^-)'[t] == k_{-24}\ cdc25C^+[t] - k_{24}\ cdc25C^-[t]\ MPF^+[t]$$

$$(CycECdk2^-)'[t] == complex1[t]\ k_{-14} +$$
$$\quad Cdk2[t]\ CycE[t]\ k_{13} - k_{14}\ CycECdk2^-[t]\ cdc25A^+[t]$$
$$(MPF^-)'[t] ==$$
$$complex3[t]\ k_{-22} + cdk1[t]\ CycB[t]\ k_{21} - k_{22}\ MPF^-[t]\ cdc25C^+[t]$$
$$(APC^+)'[t] ==$$
$$complex4[t]\ k_{-25} + complex4[t]\ k_{26} - k_{25}\ APC^+[t]\ MPF^+[t]$$
$$(cdc25A^+)'[t] ==$$
$$complex1[t]\ k_{-14} + complex1[t]\ k_{15} - k_{-18}\ cdc25A^+[t] -$$
$$\quad k_{14}\ CycECdk2^-[t]\ cdc25A^+[t] + k_{18}\ cdc25A^-[t]\ CycECdk2^+[t]$$
$$(cdc25C^+)'[t] ==$$
$$complex3[t]\ k_{-22} + complex3[t]\ k_{23} - k_{-24}\ cdc25C^+[t] -$$
$$\quad k_{22}\ MPF^-[t]\ cdc25C^+[t] + k_{24}\ cdc25C^-[t]\ MPF^+[t]$$
$$(CycECdk2^+)'[t] ==$$
$$complex2[t]\ k_{-16} + complex1[t]\ k_{15} + k_{-18}\ cdc25A^+[t] -$$
$$\quad p26[t]\ k_{16}\ CycECdk2^+[t] - k_{18}\ cdc25A^-[t]\ CycECdk2^+[t]$$

$$(MPF^+)'[t] == complex4[t]\ k_{-25} + complex3[t]\ k_{23} +$$
$$\quad k_{-24}\ cdc25C^+[t] - k_{24}\ cdc25C^-[t]\ MPF^+[t] - k_{25}\ APC^+[t]\ MPF^+[t]$$

$$(27)$$

Conservation Laws:

$$cdk1[t] + complex3[t] + complex4[t] +$$
$$\quad \phi9[t] - cdc25C^-[t] + MPF^-[t] + MPF^+[t]$$
$$Cdk2[t] + complex1[t] + complex2[t] +$$
$$\quad \phi7[t] - cdc25A^-[t] + CycECdk2^-[t] + CycECdk2^+[t]$$
$$complex3[t] + cdc25C^-[t] + cdc25C^+[t]$$
$$complex1[t] + cdc25A^-[t] + cdc25A^+[t]$$
$$complex4[t] + APC^+[t]$$
$$-cdk1[t] - Cdk12[t] - Cdk2[t] + CycA[t] + CycB[t] +$$
$$\quad CycE[t] + E2F[t] + E2Fi[t] + \phi3[t] + \phi4[t] + \phi6[t] + \phi8[t]$$
$$Cdk12[t] + CycACdk12[t] + \phi5[t]$$
$$Cdk46[t] + CycDCdk46[t] + \phi2[t]$$
$$-Cdk46[t] + CycD[t] + GF[t] + \phi1[t]$$
$$complex2[t] + p26[t]$$
$$GE[t]$$
$$GD[t]$$
$$GB[t]$$
$$GA[t]$$

$$(28)$$

The conservation laws (28) and the steady state assumptions,

$$complex1'[t] = 0, complex2'[t] = 0, complex3'[t] = 0$$
$$complex4'[t] = 0 \ , \qquad p26'[t] = 0 \ , cdc25A^{-\prime}[t] = 0 \quad \text{and}$$
$$cdc25C^{-\prime}[t] = 0$$

allow the following set of solutions,

$$GD[t] \to GD0$$

$$GA[t] \to GA0$$

$$GE[t] \to GE0$$

$$GB[t] \to GB0$$

$$cdc25A^-[t] \to (k_{-18}\ (k_{-14} + k_{15})\ cdc25A0^-)\ /\ (k_{-18}\ (k_{-14} + k_{15}) +$$
$$\quad k_{18}\ (k_{-14} + k_{15} + k_{14}\ CycECdk2^-[t]))\ CycECdk2^+[t])$$

$$cdc25C^-[t] \to$$
$$\frac{k_{-24}\ (k_{-22} + k_{23})\ cdc25C0^-}{k_{-24}\ (k_{-22} + k_{23}) + k_{24}\ (k_{-22} + k_{23} + k_{22}\ MPF^-[t])\ MPF^+[t]}$$

$$APC^+[t] \to \frac{(k_{-25} + k_{26})\ APC0^-}{k_{-25} + k_{26} + k_{25}\ MPF^+[t]}$$

$$cdc25A^+[t] \to$$
$$((k_{-14} + k_{15})\ k_{18}\ cdc25A0^-\ CycECdk2^+[t])\ /\ (k_{-18}\ (k_{-14} + k_{15}) +$$
$$\quad k_{18}\ (k_{-14} + k_{15} + k_{14}\ CycECdk2^-[t]))\ CycECdk2^+[t])$$

$$complex1[t] \to (k_{14}\ k_{18}\ cdc25A0^-\ CycECdk2^-[t]\ CycECdk2^+[t])\ /$$
$$\quad (k_{-18}\ (k_{-14} + k_{15}) +$$
$$\quad k_{18}\ (k_{-14} + k_{15} + k_{14}\ CycECdk2^-[t]))\ CycECdk2^+[t])$$

$$complex4[t] \to \frac{k_{25}\ APC0^-\ MPF^+[t]}{k_{-25} + k_{26} + k_{25}\ MPF^+[t]}$$

$$cdc25C^+[t] \to$$
$$\frac{(k_{-22} + k_{23})\ k_{24}\ cdc25C0^-\ MPF^+[t]}{k_{-24}\ (k_{-22} + k_{23}) + k_{24}\ (k_{-22} + k_{23} + k_{22}\ MPF^-[t])\ MPF^+[t]}$$

$$complex3[t] \to$$
$$\frac{k_{22}\ k_{24}\ cdc25C0^-\ MPF^-[t]\ MPF^+[t]}{k_{-24}\ (k_{-22} + k_{23}) + k_{24}\ (k_{-22} + k_{23} + k_{22}\ MPF^-[t])\ MPF^+[t]}$$

$$complex2[t] \to \frac{p260\ k_{16}\ CycECdk2^+[t]}{k_{-16} + k_{17} + k_{16}\ CycECdk2^+[t]}$$

$$p26[t] \to \frac{p260\ (k_{-16} + k_{17})}{k_{-16} + k_{17} + k_{16}\ CycECdk2^+[t]}$$

$$(29)$$

to be substituted into the relevant cyclin and cdk equations in (27),

$$CycD'[t] == GF[t]\ \alpha_1 - CycD[t]\ \alpha_2$$

$$CycDCdk46'[t] == CycD[t]\ \alpha_3 - CycDCdk46[t]\ \alpha_4$$

$$CycE'[t] == E2F[t]\ \alpha_5 - CycE[t]\ \alpha_6$$

$$(CycECdk2^+)'[t] ==$$
$$-\frac{\alpha_7\ CycECdk2^+[t]}{\alpha_8 + CycECdk2^+[t]} + \frac{\alpha_9\ CycECdk2^-[t]\ CycECdk2^+[t]}{\alpha_{10} + (\alpha_{11} + CycECdk2^-[t])\ CycECdk2^+[t]}$$

$$(CycECdk2^-)'[t] ==$$
$$CycE[t]\ \alpha_{12} - \frac{\alpha_9\ CycECdk2^-[t]\ CycECdk2^+[t]}{\alpha_{10} + (\alpha_{11} + CycECdk2^-[t])\ CycECdk2^+[t]}$$

$$CycA'[t] == E2F[t]\ \alpha_{13} - CycA[t]\ \alpha_{14}$$

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$$\text{CycACdk12}'[t] == \text{CycA}[t]\,\alpha_{15} - \text{CycACdk12}[t]\,\alpha_{16}$$

$$\text{E2F}'[t] == \text{CycDCdk46}[t]\,\alpha_{17} - \text{E2F}[t]\,\alpha_{18}$$

$$\text{CycB}'[t] == \text{E2F}[t]\,\alpha_{19} - \text{CycB}[t]\,\alpha_{20}$$

$$(\text{MPF}^-)'[t] == \text{CycB}[t]\,\alpha_{21} - \frac{\alpha_{22}\,\text{MPF}^-[t]\,\text{MPF}^+[t]}{\alpha_{23} + (\alpha_{24} + \text{MPF}^-[t])\,\text{MPF}^+[t]}$$

$$(\text{MPF}^+)'[t] == -\frac{\alpha_{25}\,\text{MPF}^+[t]}{\alpha_{26} + \text{MPF}^+[t]} + \frac{\alpha_{22}\,\text{MPF}^-[t]\,\text{MPF}^+[t]}{\alpha_{23} + (\alpha_{24} + \text{MPF}^-[t])\,\text{MPF}^+[t]}$$

(30)

The equations in (30) were simulated with the parameters defined in table 1.

Table 1: Parameters for the system of differential equations (30).

Parameters	Values chosen for the simulation
$\alpha_1 = \text{GD0} * k_1$	0.1
$\alpha_2 = k_2 + \text{Cdk46}[t] * k_3$	0.05
$\alpha_3 = k_3 * \text{Cdk46}[t]$	0.09
$\alpha_4 = k_4$	0.05
$\alpha_5 = \text{GE0} * k_{11}$	0.2
$\alpha_6 = k_{12} + \text{cdk2}[t] * k_{13}$	0.1
$\alpha_7 = \text{p260}k_{17}$	0.04
$\alpha_8 = (k_{-16} + k_{17})/k_{16}$	0.05
$\alpha_9 = k_{15}\text{cdc25A0}^-$	1.2
$\alpha_{10} = k_{-18} * (k_{-14} + k_{15}) \\ /k_{14}k_{18}$	0.3
$\alpha_{11} = (k_{-14} + k_{15})/k_{14}$	0.3
$\alpha_{12} = \text{Cdk2}[t] * k_{13}$	0.01
$\alpha_{13} = \text{GA0} * k_7$	0.2
$\alpha_{14} = k_8 + \text{Cdk12}[t] * k_9$	0.1
$\alpha_{15} = \text{Cdk12}[t] * k_9$	0.03
$\alpha_{16} = k_{10}$	0.05
$\alpha_{17} = \text{E2F} * k_5$	0.2
$\alpha_{18} \\ = k_5 + \text{GA0}k_7 + \text{GE0}k_{11} \\ - \text{GB0}k_{19}$	0.1
$\alpha_{19} = \text{GB0}k_{19}$	0.2
$\alpha_{20} = k_{20} + k_{21}\text{Cdk1}[t]$	0.1
$\alpha_{21} = k_{21}\text{Cdk1}[t]$	0.01
$\alpha_{22} = k_{23}\text{cdc25C0}^-$	1.2
$\alpha_{23} = k_{-24} * (k_{-22} + k_{23}) \\ /k_{22}k_{24}$	0.3
$\alpha_{24} = (k_{-22} + k_{23})/k_{22}$	0.3
$\alpha_{25} = k_{26}\text{APC0}^-$	0.03
$\alpha_{26} = (k_{-25} + k_{26})/k_{25}$	0.05

A possible oscillatory solution for cyclinD-cdk46, cyclinE-cdk2, cyclinA-cdk2 and MPF for the system (30) is plotted in figure 10.
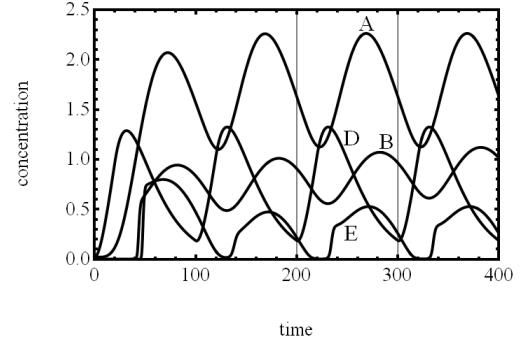

Figure 10: Oscillatory solutions for cyclinD-cdk46, cyclinE-cdk2, cyclinA-cdk2 and MPF of the system (30) obtained with the parameter values indicated in table 1. The GF[t] function was the same as previously defined in (15). The letters A, B, E, and D indicate the curves for cyclinAcdk12, cyclinBcdk1, cyclinEcdk2 and CycDcdk46, respectively.

The period of oscillation for each cyclin-cdk complex, obtained from this simulation, is shown in table 2.

Table 2: Period of oscillation for cyclin-cdk complexes in figure 10.

Complex	Period of oscillation
cycDcdk46	100
cycAcdk12	100
cycEcdk2$^+$	100
MPF$^+$	99.91
GF	100

We can observe that the period of oscillation of all the complexes is 100 (or close to 100) which equals the period of the growth factor function defined in (15) that is also 100. This means that the growth factor establishes the periodicity of the cyclin-cdk complexes and consequently the periodicity of the cell cycle. We performed another simulation substituting N=0 in (15), which means that in this case the GF does not oscillate and only stimulates one mitotic cycle. The result of this simulation is represented in figure 11.
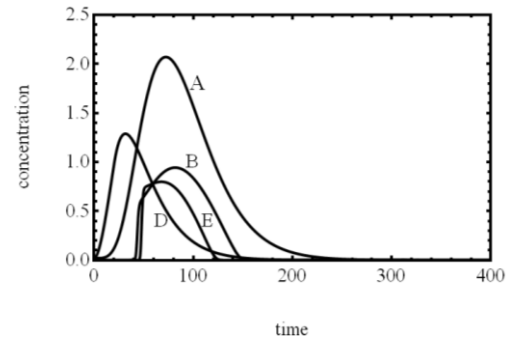

Figure 11: Time-evolution for the concentration of cyclin-cdk complexes in system (30), but for the specific case of a GF[t] with N=0 (only one impulse of GF, without periodic variation).

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From figure 11, we conclude that the non-periodicity of the GF eliminates the periodicity of the cell cycle, since the curves in figure 11 are no longer periodic. The presence of positive feedback loops for cyclinE and cyclinB and their correspondent complexes is not sufficient to induce periodic mitotic cycles when the GF[t] is not periodic.

In figure 12, we show a theoretical representation of the evolution of cyclin-cdk concentration along the cell cycle, obtained from experimental results.
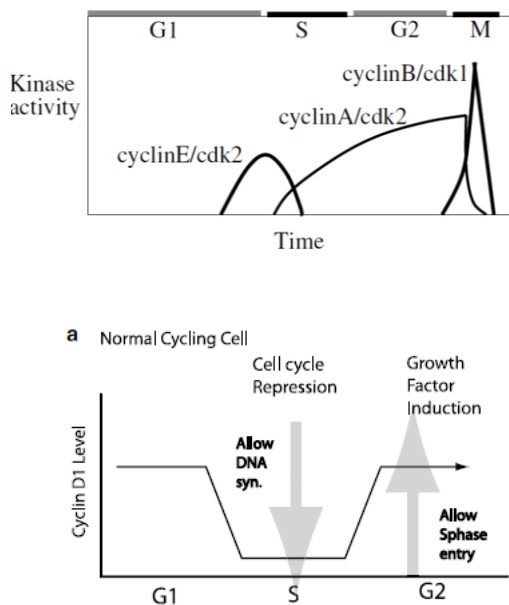

Figure 12: Temporal variation of cyclin-cdk activities during the mammalian cell cycle, obtained with experimental studies. Figure on the left extracted from [9] and on the right from [17].

The curves in figure 12 are more or less parabolic as the curves represented in figure 10 that result from our model. Accordingly to figure 12 (second image), for each cycle cyclinD should oscillate twice (have aproximately half the period, compared with cyclinE-cdk2, cyclinA-cdk12 and MPF) and that is not obtained from this model. CyclinD-cdk46 is degraded in the S-phase and is produced once again in the G2 period, but the exact mechanisms by which the GF re-increases the cyclinD concentration in G2 is not completely described and was not considered in this model.

4. CONCLUSION

In this thesis we have explored a model for the eukaryotic cell cycle. The model is based on the *mass-action law* and includes extracellular and intracellular agents, describing a cyclic time-variation for the concentration of cyclin-cdk complexes.
The model provides an important role for the positive feedback loops. These loops generate stable oscillations through the phosphatase cdc25 and allow the system to have periodic cyclin-cdk activation of cyclinE and cyclinB as long as the E2F is available in the cell. Since the E2F is activated by the action of cyclinD-cdk46 and this last complex depends on the growth-factor, positive feedback loops, are not capable of producing mitotic oscillations without the action of the growth-factor. The growth factor is the number-one agent to promote the cell cycle.

In embryonic cells, the MPF (cyclinB-cdk1) generates the mitotic oscillations of the first cell generations, in the absence of the growth factor. Perhaps the E2F is expressed in an alternative way or comes from maternal genetic material.

In figure 11, the fact that all cyclin-cdk complexes are oscillating together with the same period of oscillation highlights the idea that maybe there's a missing agent in the model. A repressor agent that could block cyclin gene transcription and establish a correct sequential activation of each cyclin-cdk complex at different times of the cell cycle is a possibility. As for the cyclinD, experimental results show that it oscillates twice for cell cycle but the other cyclins only oscillate once. Full theoretical information for cyclinD degradation and reuptake is not yet available and so it is an aspect that could not be represented by this model.

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