

Relation Between the Cell Volume and the Cell Cycle Dynamics in Mammalian cell

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Abstract. The main goal of this work is to add and analyze an equation that represents the volume in a dynamical model of the mammalian cell cycle proposed by Gérard and Goldbeter (2011) [1]. The cell division occurs when the *cyclinB/Cdk1* complex is totally degraded (Tyson and Novak, 2011)[2] and it reaches a minimum value. At this point, the cell is divided into two newborn daughter cells and each one will contain the half of the cytoplasmic content of the mother cell. The equations of our base model are only valid if the cell volume, where the reactions occur, is constant. Whether the cell volume is not constant, that is, the rate of change of its volume with respect to time is explicitly taken into account in the mathematical model, then the equations of the original model are no longer valid. Therefore, every equations were modified from the mass conservation principle for considering a volume that changes with time. Through this approach, the cell volume affects all model variables. Two different dynamic simulation methods were accomplished: deterministic and stochastic. In the stochastic simulation, the volume affects every model's parameters which have molar unit, whereas in the deterministic one, it is incorporated into the differential equations. In deterministic simulation, the biochemical species may be in concentration units, while in stochastic simulation such species must be converted to number of molecules which are directly proportional to the cell volume. In an effort to understand the influence of the new equation a stability analysis was performed. This elucidates how the growth factor impacts the stability of the model's limit cycles. In conclusion, a more precise model, in comparison to the base model, was created for the cell cycle as it now takes into consideration the cell volume variation

1. Introduction

There is an increasing interest in modeling the cell cycle, specially the mammalian cell cycle. This may be explained as some diseases, like the cancer, or even the aging process are directly related to the cell cycle. Thus, understanding the cell cycle and how it is affected by those problems is essential for proposing new treatments. On that account, several models for the cell cycle were proposed. There are, for instance, models from a simple yeast cell to a complex human cell. There are mainly two methods of simulation, the ordinary deterministic and the stochastic. The deterministic method simulates the average concentration, which is in a large system precise and simple. On the other hand, a stochastic method simulates each reaction individually, following the probability of each reaction occurs. This is computationally more expensive, but can capture the stochastic noise that in small systems, like a cell, may lead to a different result in relation to the average.



This work proposes an improvement for the model of the mammalian cell cycle proposed by [1, 3], to explicitly take in account the cell volume variation. This new model is then analyzed in order to verify how the volume variation affects the dynamics of the differential equations. Finally, the model is simulated using a deterministic and a stochastic method.

2. Proposed Model

This work develops an extension for the model proposed by [1]. The original model is composed by a set of differential equations (1-6). However, those equations do not contain any information related to the cell volume. In an effort to improve the model's completeness, information about how the cell volume changes along the cell cycle will be added.

$$\frac{dM_d}{dt} = v_{sd} \cdot \left(\frac{GF}{K_{gf} + GF} \right) - V_{dd} \cdot \left(\frac{M_d}{K_{dd} + M_d} \right) \quad (1) \quad \frac{dM_a}{dt} = v_{sa} \cdot E2F - V_{da} \cdot Cdc20 \cdot \left(\frac{M_a}{K_{da} + M_a} \right) \quad (4)$$

$$\frac{dE2F}{dt} = V_{1e2f} \cdot \left(\frac{E2F_{tot} - E2F}{K_{1e2f} + E2F_{tot} - E2F} \right) \cdot (M_d + M_e) \quad (2) \quad \frac{dM_b}{dt} = v_{sb} \cdot M_a - V_{db} \cdot Cdc20 \cdot \left(\frac{M_b}{K_{db} + M_b} \right) \quad (5)$$

$$- V_{2e2f} \cdot \left(\frac{E2F}{K_{2e2f} + E2F} \right) \cdot M_a \quad (2) \quad \frac{dCdc20}{dt} = V_{1cdc20} \cdot M_b \cdot \left(\frac{Cdc20_{tot} - Cdc20}{K_{1cdc20} + Cdc20_{tot} - Cdc20} \right)$$

$$\frac{dM_e}{dt} = v_{se} \cdot E2F - V_{de} \cdot M_a \cdot \left(\frac{M_e}{K_{de} + M_e} \right) \quad (3) \quad - V_{2cdc20} \cdot \left(\frac{Cdc20}{K_{2cdc20} + Cdc20} \right) \quad (6)$$

As we are working with concentrations ($\frac{\mu M}{L}$), the Equations (1-6) are only valid when the cell volume is constant. So, when the cell volume is not constant, these equations are no longer valid. However, these equations were developed from the mass conservation principle, therefore, it is the mass that is conserved not the concentration. Let N_e be the equivalent of M_e in moles $[M]$ and V the cell volume $[L]$. Now, the Equation (3) can be rewrite as in Equation (7), having $M_e \cdot V = N_e$.

As the cell volume is not constant, the product rule must be applied on the left side of the Equation (7). The equation is then divided by V and the original term $\frac{dM_e}{dt}$ is isolated on the left side, resulting in the Equation (8).

It is reasonable to suppose that a cell growth is proportional to its volume, following an exponential growth during an active cell cycle [4]. Thus, the cell volume may be represented by the Equation (9).

Using this expression (Eq. 9) for $\frac{dV}{dt}$ in the equation (8) results in a simpler equation (10). This equation now takes into account the cell volume variation, and the term $-\mu M_e$ is called dilution factor due the cell growth. The same procedure can be applied to each equation of the original model, resulting in a new set of differential equation composed by the transformed equations in addition to the volume differential equation (9). The cell division is done when the *cyclinB/Cdk1* complex is totally degraded, like the work presented by [2]. At this moment the volume V is cut by half. In the stochastic version, the amount of molecules of each chemical is also reduced to its half.

$$\frac{d(VM_e)}{dt} = V \left[v_{se} \cdot E2F - V_{de} \cdot M_a \cdot \left(\frac{M_e}{K_{de} + M_e} \right) \right] \quad (7) \quad \frac{dV}{dt} = \mu V \quad (9)$$

$$\frac{dM_e}{dt} = v_{se} \cdot E2F - V_{de} \cdot M_a \cdot \left(\frac{M_e}{K_{de} + M_e} \right) - \frac{M_e}{V} \frac{dV}{dt} \quad (8) \quad \frac{dM_e}{dt} = v_{se} \cdot E2F - V_{de} \cdot M_a \cdot \left(\frac{M_e}{K_{de} + M_e} \right) - \mu M_e \quad (10)$$

2.1. Stochastic

As cells are small, the stochasticity may impact the behavior of the system [5]. Thence, a stochastic version of this model is also proposed. To do so, each variable and parameter having a concentration unit ($\frac{\mu M}{L}$) must be converted to number of molecules. This number is directly proportional to the cell volume. This conversion is done by multiplying the value in concentration unit by a factor of $\Omega = V_c \cdot N_A \cdot 10^{-6}$, where V_c is the cell volume in liters (L), N_A is the Avogadro constant and the 10^{-6} factor is to convert the parameters unit from $\frac{\mu M}{L}$ to $\frac{M}{L}$ [6].

In order to model the system as a stochastic process, each differential equation has to be separated in two [4]. The first one represents the activation a new molecule, while the second represents the deactivation of a molecule. The stochastic model is represented in the Table (1). Additionally, we implement the direct method, proposed by [6], for simulating the stochastic model.

Table 1: Stochastic model.

Reaction Id	Reaction	Propensity of the reaction
1	$\rightarrow \text{Md}$	$v_{sd} \cdot \Omega \cdot \left(\frac{GF}{K_{gf} \cdot \Omega + GF} \right)$
2	$\text{Md} \rightarrow$	$V_{dd} \cdot \Omega \cdot \left(\frac{Md}{K_{dd} \cdot \Omega + Md} \right)$
3	$\rightarrow \text{E2F}$	$V_{1e2f} \cdot \left(\frac{E2F_{tot} \cdot \Omega - E2F}{K_{1e2f} \cdot \Omega + E2F_{tot} \cdot \Omega - E2F} \right) \cdot (Md + Me)$
4	$\text{E2F} \rightarrow$	$V_{2e2f} \cdot \left(\frac{E2F}{K_{2e2f} \cdot \Omega + E2F} \right) \cdot Ma$
5	$\rightarrow \text{Me}$	$v_{se} \cdot E2F$
6	$\text{Me} \rightarrow$	$V_{de} \cdot Ma \cdot \left(\frac{Me}{K_{de} \cdot \Omega + Me} \right)$
7	$\rightarrow \text{Ma}$	$v_{sa} \cdot E2F$
8	$\text{Ma} \rightarrow$	$V_{da} \cdot Cdc20 \cdot \left(\frac{Ma}{K_{da} \cdot \Omega + Ma} \right)$
9	$\rightarrow \text{Mb}$	$v_{sb} \cdot Ma$
10	$\text{Mb} \rightarrow$	$V_{db} \cdot Cdc20 \cdot \left(\frac{Mb}{K_{db} \cdot \Omega + Mb} \right)$
11	$\rightarrow \text{Cdc20}$	$V_{1cdc20} \cdot Mb \cdot \left(\frac{Cdc20_{tot} \cdot \Omega - Cdc20}{K_{1cdc20} \cdot \Omega + Cdc20_{tot} \cdot \Omega - Cdc20} \right)$
12	$\text{Cdc20} \rightarrow$	$V_{2cdc20} \cdot \Omega \cdot \left(\frac{Cdc20}{K_{2cdc20} \cdot \Omega + Cdc20} \right)$

This model is easily adapted to a model with a non constant volume. As we are working with number of molecules, the equations are already valid for a variable volume. However, the conversion factor Ω will no longer be constant. Even though this seems a small change, it is not, because Ω affects the value of several parameters.

3. Results

This section will present an analysis of the the proposed method, along with the results found when the model is both stochastically and deterministically simulated. In an effort to stimulate the reproducibility, the values used for each parameter of the model can be found in [7].

3.1. Stability analysis

In order to present a stable oscillatory behavior, an ODE system must have at least one limit cycle [8]. The original model, proposed in [1], has a limit cycle. Thus, the model proposed here must preserve this limit cycle. As our model introduces an extra parameter, an analysis of the impact of this new parameter is presented.

The first thing one must do to perform a stability analysis is to find the stable points. These points can be found by solving a nonlinear system which is obtained when each derivate is made equal to zero[9]. Although the system obtained has several solutions, when the solutions are limited to the positive Real numbers there are only two solutions P_1 and P_2 (Tab. 2). However, the second point P_2 is also not valid because the $E2F$ value exceeds the maximum value allowed of 3.0 defined by the parameter $E2F_{tot}$. So, the following analysis will only focus in the valid point P_1 .

Table 2: Equilibrium points

	$Cdc20$	$E2f$	Ma	Mb	Me	Md
P_1	0.1738902240	0.2448834860	0.2901560559	0.3365279086	0.08954976057	0.165495
P_2	1.786431803	3.010457122	2.715508604	1.834578112	0.1783832744	0.165495

In an effort to further analyze the equilibrium point P_1 , a linearized version of the system was used in its the neighborhood. In this simplified version the eigenvalues were computed for inferring the stability of the point. These values are found in [7]. As there are complex eigenvalues, we can conclude that the system presents an oscillatory behavior in the neighborhood of the point P_1 [9].

For validating our model, we must verify the impact of the parameter μ over the equilibrium point. To perform this analysis, the `matcont`[10] extension of the well-known MATLAB software was used. The result shows that the system behavior is preserved until the critical point $\mu = 0.11$ (Fig. 1). At this point, a Hopf bifurcation occurs, this means that a limit cycle is created or vanished at this point [11]. Hopefully, this values is much bigger than the actual value computed for being used $\mu = 0.0385$.

The Figures (Fig. 2) and (Fig. 3) show the phase plane $M_a \times M_b$ for several values of μ . However, the first one shows distinct cycles on the plane, while the second is a 3D graph composed by phase plane $M_a \times M_b$ and a height given by μ . These graphs evince that as μ approaches 0.11, the limit cycle shrinks until it reaches the critical point 0.11 where it vanishes. In fact, this evinces that the parameter μ influences the stability of the limit cycle, but it also evinces that it only changes the system's behavior when its value is much greater than the value we estimate (0.0385 against 0.11).

3.2. Deterministic Simulation

The Figure 4 shows the results of simulating the original model proposed by [1], while the Figure 5 shows the results of our new model. Even though our model presents lower concentrations, it is clear that they have a similar behavior. These lower concentrations are expected as we introduce a factor of dilution. One may also notice that the length of the cycle is slighted shorter, to be precise the cycle reduces from 23,573 to 21,816 hours, in our model. However, this is not sufficient to invalidate our model.

3.3. Stochastic Simulation

In an effort to produce reliable results, the results are presented in terms of concentrations. This allows a direct comparison between the stochastic version and the deterministic version. In order to obtain the concentration C , the following operation was done $C = \frac{N_m}{\Omega}$, where N_m is the number of molecules.

The graph illustrated in Figure 6 shows the results for our new model. The volume here varies between $0.334pL$ to $0.7pL$ ($0.334pL$ is the average volume of human granulocytes [12]), following the Differential Equation 7. This result is close to deterministic version, indicating that our stochastic model is also correct.

4. Conclusion and Future Works

In this work a new mathematical model for the mammalian cell cycle was developed. This new model takes into account not only the concentrations of some key elements, but also explicitly models the cell volume variation. Finally, this model also has a key moment where the cell division explicitly occurs, cutting by a half the cell volume.

Another important contribution of this work is the analysis of the impact of the new parameter μ over the stability of the model. This analysis concludes that only high values of μ affects the

model's stability. As values of this magnitude are not viable from a physiological point of view, we may conclude that parameter impact, over the stability, is very small.

As a future work, we can include the influence of a chemotherapeutic on the cell cycle model. Among several chemotherapeutic, an interesting options is the cisplatin family, which inhibits the DNA synthesis. Therefore, it can be included in the model affecting the phase S.

Another future can be adapting the model for representing the cell cycle of a cancer cell. This may enhance the comprehension of which parts of the cell cycle are affected by each type cancer, guiding the development of efficient drugs.

5. Figures

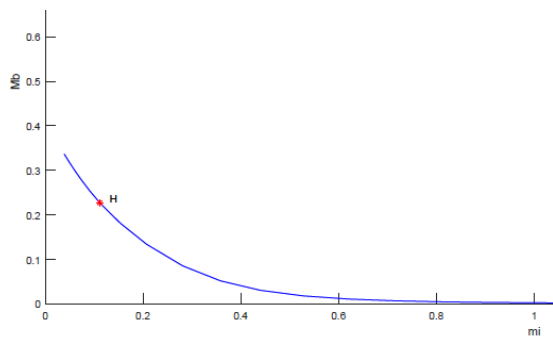


Figure 1: Diagram of bifurcation. Equilibrium value of Mb in relation the μ value. The point H represents the Hopf bifurcation ($\mu = 0.11$).

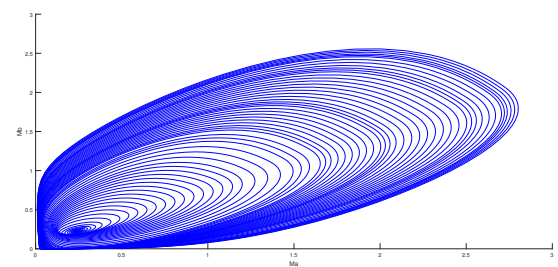


Figure 2: Phase plane $M_a \times M_b$. Each curve represents a distinct value of μ

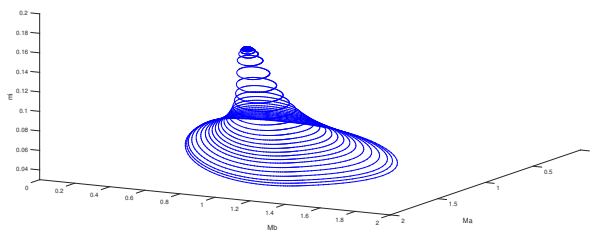


Figure 3: Graph of several phase planes $M_a \times M_b$ with μ being the height.

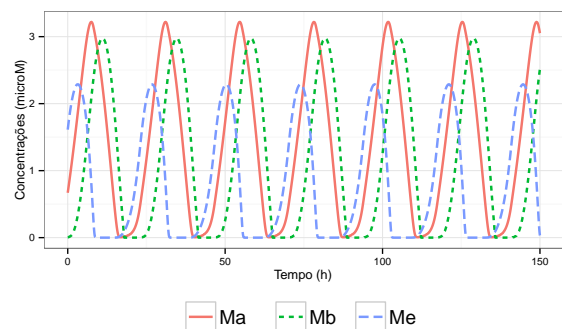


Figure 4: Complexes of *cyclinA/Cdk2* (M_a), *cyclinB/Cdk1* (M_b) and *cyclinE/Cdk2* (M_e). Deterministic simulation of the cell cycle with constant volume[1].

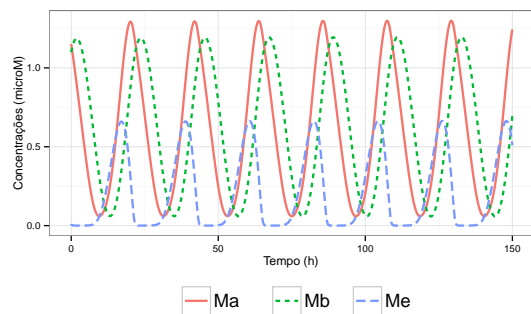


Figure 5: Complexes of *cyclinA/Cdk2* (M_a), *cyclinB/Cdk1* (M_b) and *cyclinE/Cdk2* (M_e). Deterministic simulation of the model proposed here.

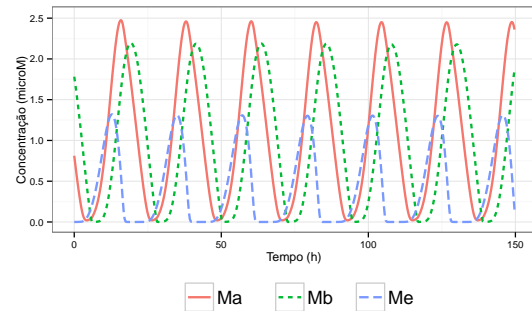


Figure 6: Complexes of *cyclinA/Cdk2*, *cyclinB/Cdk1* and *cyclinE/Cdk2*. Mean concentrations obtained by the average of 500 stochastic simulations of the model with variable volume.

6. Acknowledgements

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