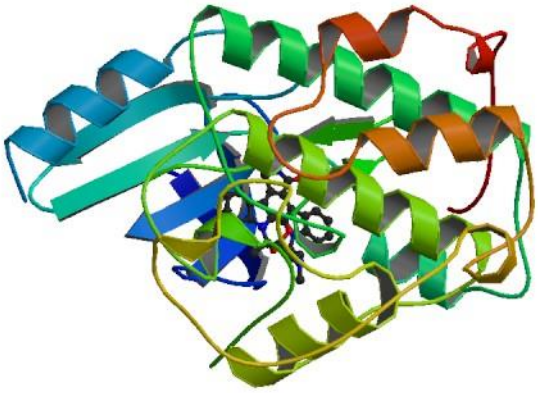
A Minimal Cascade Model for the Mitotic Oscillator Involving CyclinB and Cdc2 Kinase



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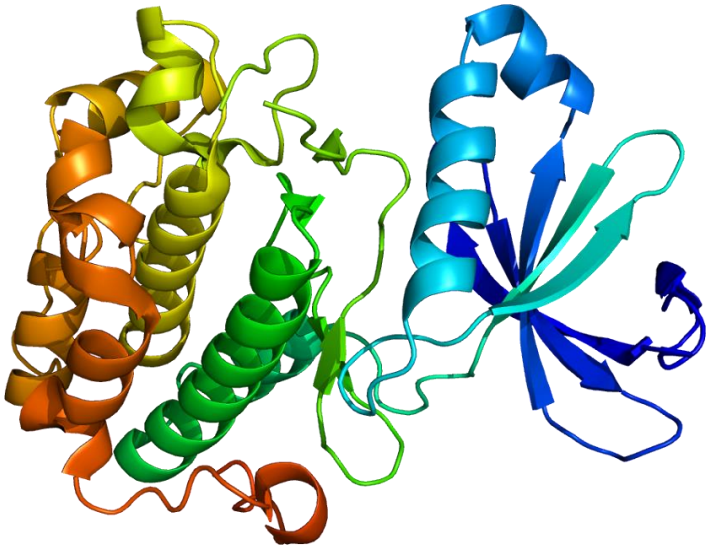


Introduction

Cell division and duplication permit cells to pass on their genetic information to their descendants. In the process of cell division, there are four main phases, including G1, S, G2, and M phase. These four phases play a crucial role in living system. Each phase will check the cells for possible mutations. Three checkpoints in G1, G2, and M phases are responsible for the correction of replication. If there is mutation or damage in the DNA, cell duplication/division stops and damages will be fixed in these checkpoints. In the G2 checkpoint, cell cycle is governed by two proteins, namely CyclinB and Cdc2. Both of the proteins act as guards to decide if the cell could enter the M phase. Therefore, the regulations of both two proteins are of great importance during cell division. In this report we focus on the regulation of CyclinB and CyclinB dependent kinase (Cdc2) (Vermeulen, Van Bockstaele et al. 2003).

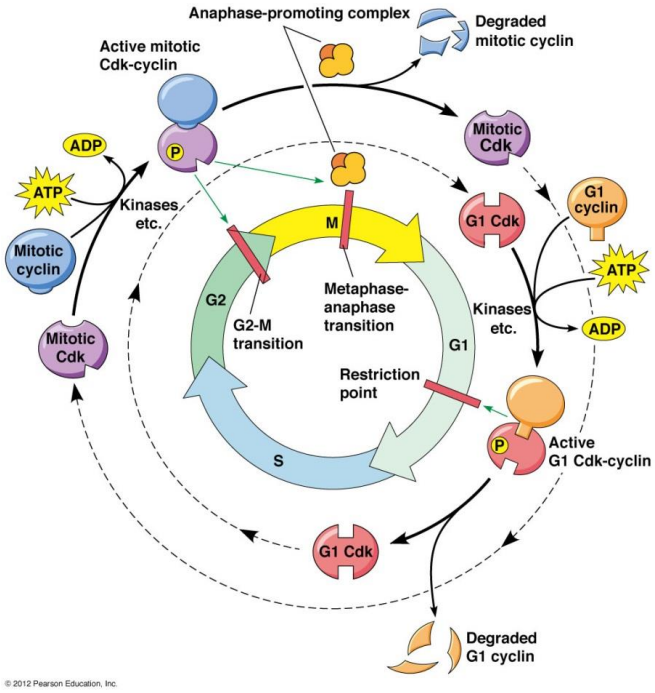
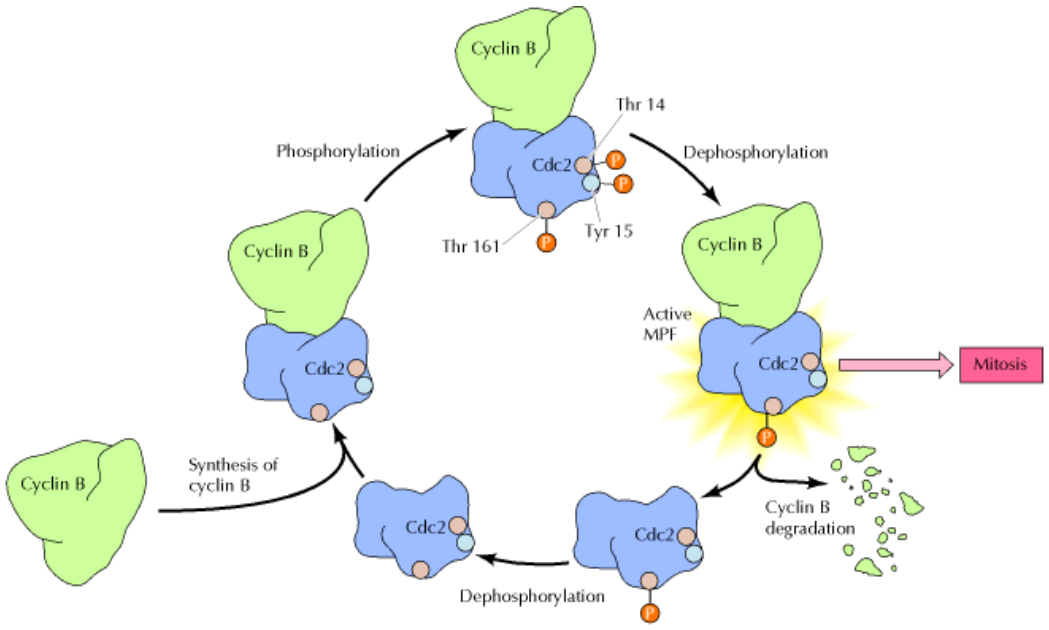


Figure1. Cell cycle is divided into four phase, G1, S, G2, and M phases. In the G1 phase, cells grow, and take in nutrients needed for mitosis. Upon entering S phase cells start to replicate their DNA. During the G2 phase, cells prepare proteins needed for mitosis. Finally in the M phase, cells divide into two daughter cells. There are three checkpoints in the cell cycle to ensure DNA replication is correct.

In each checkpoint, sensor mechanism detects DNA damages. If DNA damages are detected, the sensor proteins may induce signal mechanism to stall the cell until DNA damages were repaired. If a cell fails to be repaired, it will be destructed by effector mechanism, called Apoptosis. There are three checkpoints in cell cycle. Each checkpoint assesses DNA damage by the same sensor-signal-effector mechanism.

In the G2 checkpoint, CyclinB and Cdc2 are the main proteins regulating cell cycle. As the CyclinB-Cdc2 complex, also known as MPF, is activated, cell cycle proceeds into Mitosis. This activation process is due to the phosphorylation of Cdc2. This process starts from the accumulation of CyclinB, which would later bind with Cdc2 to form MPF and induce a Kinase named Wee1 and CAK to phosphorylate at Thr14, Tyr15, and Thr161 on Cdc2. However Thr14, and Tyr15 is the inhibitory site of Cdc2. In order to complete MPF activation, Thr14, and Tyr15 would need to be dephosphorylated by Cdc25. When only Thr161 is phosphorylated, Mitosis will begin (Ubersax, Woodbury et al. 2003). After the cell enters Mitosis, the activated Cdc2 would trigger a protease to degrade CyclinB. After MPF dissociation, Cdc2 is dephosphorylated, and return to the inactivated form, preventing infinite replication.



Firuge2. CyclinB and Cdc2 complex (MPF) activates through phosphorylation. CyclinB would accumulate in cells, and increase Cdc2 activity by binding together.

After forming MPF, Cdc2 will be activated by phosphorylating at Thr161, and cells will enter Mitosis. After Mitosis, CyclinB would be degraded by proteasome. Therefore, MPF are back to its inactivate state. This step can be thought of as a negative regulation preventing infinite mitosis.

Problem Statement

In this project we focused on the self-oscillating, self-governing nature of MPF and its role in cell division. This involves two stages of activation, including the Phosphorylation of MPF, and degradation of CyclinB. We chose these two parts to simplify the whole activation. In our project, the experiment model of choice is Yeast (*S. cerevisiae*). The reasons for are (1) short duplication time (2) Easier to observe for cell division. (3) Relatively low-cost

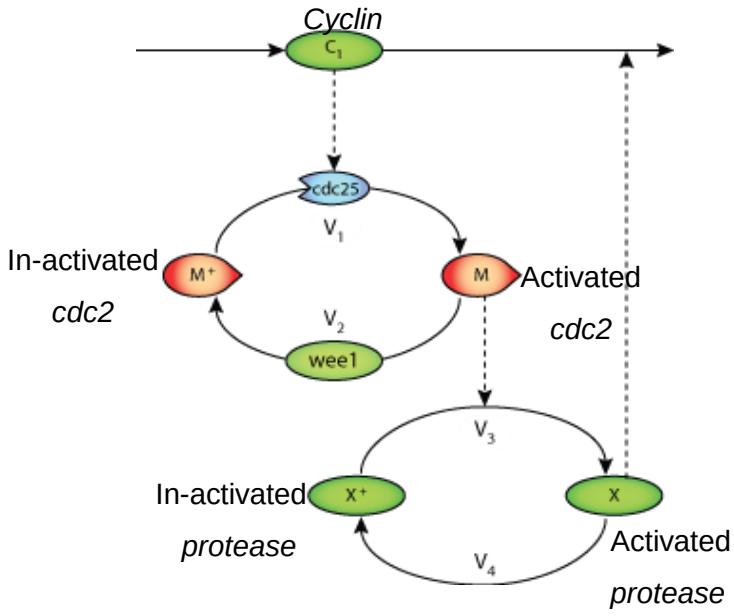


Figure3. Model of report focused on three parts. One is Cdc2 activation by CyclinB, and another one is protease activation by Cdc2, and the third one is CyclinB degradation by protease.

Model Description

Below is a pathway of post-translational modifications made to the MPF complex with regards to the mitosis cycle. The Cdc2 catalytic subunit is inactive as a monomer. Association with a CyclinB regulatory subunit is required for activity.

A simplified model for this mitotic oscillator was proposed by Goldbeter in 1991, and is the model of choice in our presentation.

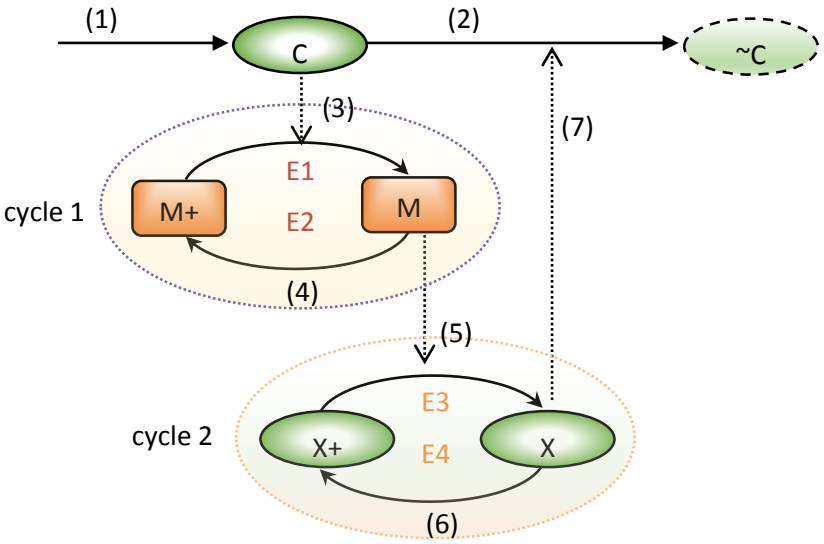


Figure4. Simplified model by Goldbeter

In this model, C1 corresponds to CyclinB. In cycle 1, M+ and M corresponds to the inactive and active form of Cdc2. The periodic fluctuation of active Cdc2 concentration is of great importance in cell's decision to enter mitosis. Cycle 2, not shown in figure 4, is a self-regulatory system of CyclinB protease which plays a major role in CyclinB degradation, enabling the system to "restart" automatically after a fixed period of time. The concentration of each element over time can be described by a system of non-linear differential equations as follows:

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_{\mathrm{d}} C$$

$$\frac{dM}{dt} = V_1 \frac{(1-M)}{K_1 + (1-M)} - V_2 \frac{M}{K_2 + M}$$

$$\frac{dX}{dt} = V_3 \frac{(1-X)}{K_3 + (1-X)} - V_4 \frac{\mathrm{X}}{\mathrm{K}_4 + \mathrm{X}}$$

$$V_1 = \frac{C}{K_c + C} V_{M1},\ V_3 = M\, V_{M3}$$

A close-up look at our model details its functions and some simplifications made:

(1) CyclinB is synthesized at a constant rate v_i.

(2) CyclinB undergoes non-specific self-degradation at a rate proportional to its concentration with a factor k_{d}.

(3) CyclinB attaches to Cdc2 to form CyclinB/Cdc2 inactive MPF complex. The docking process can be described by ligand-protein docking model (Langmuir equation, therefore the term $\frac{C}{K_c+C}$) in which both the concentration of the CyclinB and the number of remaining binding sites on Cdc2 are taken into account. Here, K_c denotes the dissociation constant for CyclinB/Cdc2 complex. The MPF complex is activated through Thr14 and Tyr15 dephosphorylation. We assume this process autocatalytic, conforming to the Michaelis–Menten enzyme kinetics model. Note that since enzyme E1 binds to inactive MPF (concentration: $(1-M)$) during its transformation to active MPF, the form is written as this: $V_1 \frac{(1-M)}{K_1+(1-M)}$, where $V_1 = \frac{C}{K_c+C} V_{M1}$ and V_{M1} being the maximum transformation rate of enzyme E1. Here V_1 is the *effective* maximum reaction rate of enzyme E1, the fraction $\frac{C}{K_c+C}$

can also reflect the proportion of bound CyclinB/Cdc2 complex to all CyclinB since only bound CyclinB/ Cdc2 can catalyze activation of MPF.

- *This step is actually greatly simplified. As shown in figure 2, phosphorylation on Thr161 is required for tight association with CyclinB and activation of the kinase Cdc25. However, we assume spontaneous phosphorylation of Thr161 when CyclinB attaches to Cdc2. Another difference lies in the phosphorylation of inhibitory sites Tyr15 and Thr14. In figure 2, Thr14 and Tyr15 are phosphorylated following CyclinB/Cdc2 attachment under the catalysis of Wee1 and CAK. However, since this reaction is rather fast compared with the docking of CyclinB/Cdc2, it is neglected in the simulation.*

(4) The term $-V_2 \frac{M}{K_2+M}$ reflects the inverse reaction of MPF activation, which is also assumed self-catalytic. Note that here active MPF (concentration$M$) becomes the substrate to which enzyme E2 binds.

(5) Cdc2 kinase activates a CyclinB protease, dubbed X, by reversible phosphorylation. The Michaelis–Menten enzyme kinetics model is again adopted, K_2 being the Michaelis–Menten constant of Cdc2 kinase. The process corresponds to $V_3 \frac{(1-X)}{K_3+(1-X)}$. The reaction rate depends on Cdc2 concentration since Cdc2 catalyzes synthesis of X, and this fact is evident in $V_3 = M\, V_{M3}$.

(6) As in (4), some active protease also undergoes reverse transformation.

(7) CyclinB protease degrades CyclinB following attachment to CyclinB. In a process similar to the docking of CyclinB to Cdc2 in (3), a ligand-protein kinetics model is deployed, with K_d being the Michaelis–Menten constant for CyclinB degradation.

Analysis

Goldbeter used following system of nonlinear differential equations as a model to describe interactions among chemical substance including CyclinB and Cdc2 kinase in Figure.

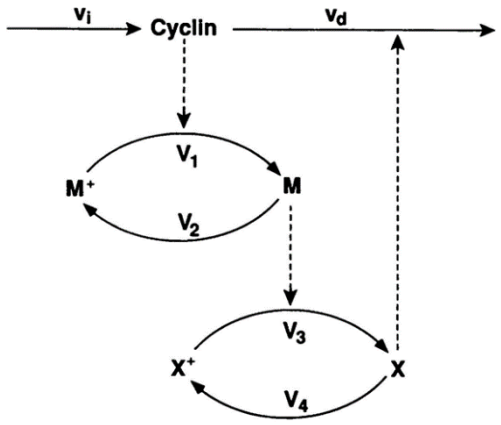


Figure 5. System dynamics of Goldbeter's model

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C$$
$$\frac{dM}{dt} = V_1 \frac{(1-M)}{K_1 + (1-M)} - V_2 \frac{M}{K_2 + M}$$
$$\frac{dX}{dt} = V_3 \frac{(1-X)}{K_3 + (1-X)} - V_4 \frac{X}{K_4 + X}$$

With

$$V_1 = \frac{C}{K_c + C} V_{M1}$$
$$V_3 = M V_{M3}.$$

Definition of constant variable is shown in Table 1.

From a cell's point of view, keeping concentration of substance within a fixed range is of vital importance. While some fluctuation of concentration is allowed, the value should not constantly excess certain threshold or be in short of supply for a long period of time. Therefore, a set of self-stabilized periodic solutions is expected.

Solutions to nonlinear system of differential equations of this kind cannot always be found easily. However, there are still several tricks that we could use to approximate solutions. For example, in the first differential equation, the third term on the right-hand side $-k_d C$ contributes much small than that of CyclinB degradation by protease X, and can be omitted; the fraction of the second term can be seen as a constant, providing that the value of C is greater than that of K_d for most of the time.

Table 1. Definition of variables in Goldbeter's model

v_i	CyclinB synthesis rate, set to $0.025\mu M\,\mathrm{min}^{-1}$
v_d	CyclinB degradation rate, set to $0.025\mu M\,\mathrm{min}^{-1}$
K_d	Michaelis constants for CyclinB degradation, set to $0.02\mu M$
k_d	apparent first-order rate constant related to nonspecific degradation of CyclinB
K_c	Michaelis constants for CyclinB activation of the phosphatase acting on the phosphorylated form of Cdc2 kinase, set to $0.5\mu M$
V_{M1}	the maximum rate of that enzyme reached at saturating CyclinB levels, set to $3\,\mathrm{min}^{-1}$
K_i	Michaelis-Menten constants of enzymes $E_i(i=1-4)$, all set to 0.005
V_2	Maximum reaction rate of converter enzymes E_2, set to $1.5\,\mathrm{min}^{-1}$
V_4	Maximum reaction rate of converter enzymes E_4, set to $0.5\,\mathrm{min}^{-1}$

Hence, we have

$$\frac{dC}{dt} = v_i - v_d X\,.$$

Similar tricks could be applies to the second and the third differential equations, and we could obtain

$$\frac{dM}{dt} = V_1 - V_2$$
$$\frac{dX}{dt} = MV_{M3} - V_4$$

Unfortunately, V_1 encapsulates a nonlinear term $V_{M1} \times C / (K_c + C)$, which cannot be simplified so easily since $K_c = 0.5$ and its value is neither large enough nor small enough in comparison to the value of C. A bypass would be setting $V_1 = CV_{M1}$, forcing it to be linear so that all these equations become linear. Admittedly, this is not a good approach, but we would like to see to what extends we could know about the system dynamics.

Now the system of linear differential equations could be written in matrix form

$$\begin{bmatrix} C \\ M \\ X \end{bmatrix}' = \begin{bmatrix} 0 & 0 & -v_d \\ V_{M1} & 0 & 0 \\ 0 & V_{M3} & 0 \end{bmatrix} \begin{bmatrix} C \\ M \\ X \end{bmatrix} + \begin{bmatrix} v_i \\ -V_2 \\ -V_4 \end{bmatrix}.$$

The characteristic equation of the matrix is $\lambda^3 + v_d V_{M1} V_{M3} = 0$. Since all the variables are real and non-negative, the root of the characteristic equation should have one real value and two complex-conjugated ones, all having the same norm.

The only real root to the equation has negative value,

$$\lambda = -\sqrt[3]{v_d V_{M1} V_{M3}}$$

suggesting that the concentration of substance decays over time. This is mathematically possible, but if this were to happen in real cell, the substance would soon become short of supply. Hence, we can rule out this solution. Now it leaves us two complex-conjugated solutions, which imply periodic waveform with is period determined by the product of v_d, V_{M1}, and V_{M3}. This should not surprise us, for based on the

knowledge we already have, that the higher the synthesis rate of CyclinB is, the faster of our cycle in cell will be. Similar reasoning is also applicable to V_{M1} and V_{M3}.

The waveform and period of the oscillation is shown in Figure. Initial conditions are $C = 0.01\mu\mathrm{M}$, $M = X = 0.01$. Note since M and X are the value of relative concentration, they are dimensionless, whereas C is the absolute concentration measured in $\mu\mathrm{M}$. The values of these curves are obtained by using Euler's finite difference method of approximation.

From what we have observed from the result of numerical approximation, different initial conditions can cause different waveforms at the beginning of our simulation, and will eventually converge to the same waveform pattern. In Figure, we modify

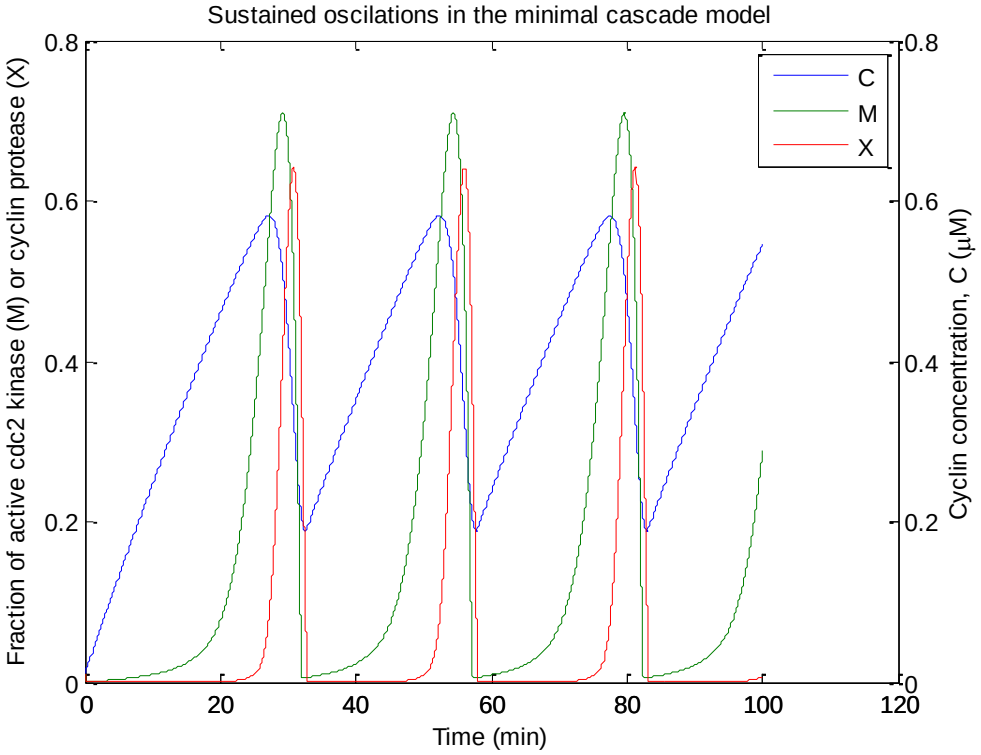


Figure 6. Sustained oscillations in the minimal cascade model involving CyclinB and Cdc2 kinase with Initial conditions $C = 0.01\mu\mathrm{M}$, $M = X = 0.01$.

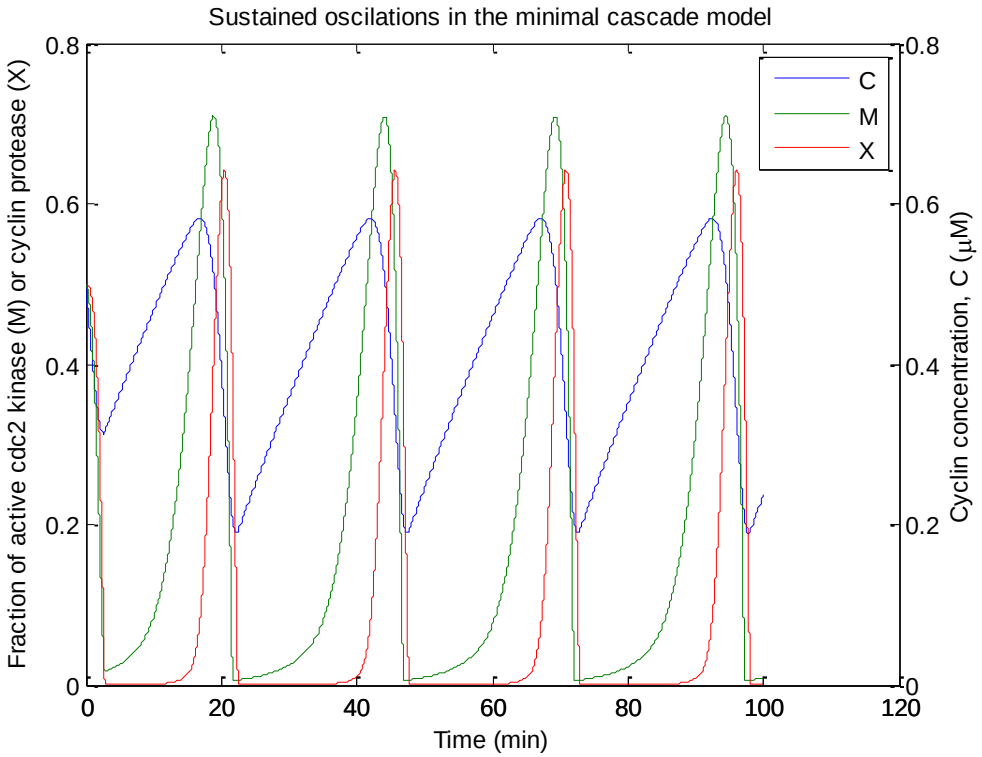


Figure 7. Sustained oscillations in the minimal cascade model involving CyclinB and Cdc2 kinase with Initial conditions $C = 0.50\mu\mathrm{M}$, $M = X = 0.5$.

the initial conditions to $C = 0.50\mu\mathrm{M}$ and $M = X = 0.5$. The result in Figure suggests that the system dynamics remains the same as that in Figure, meaning that our solutions are independent of initial conditions. The property suggests self-stabilization, i.e., that whenever there is a sudden change of concentration of a particular substance in cells, the effect result from this change should not last for long or result in unpredictable outcomes.

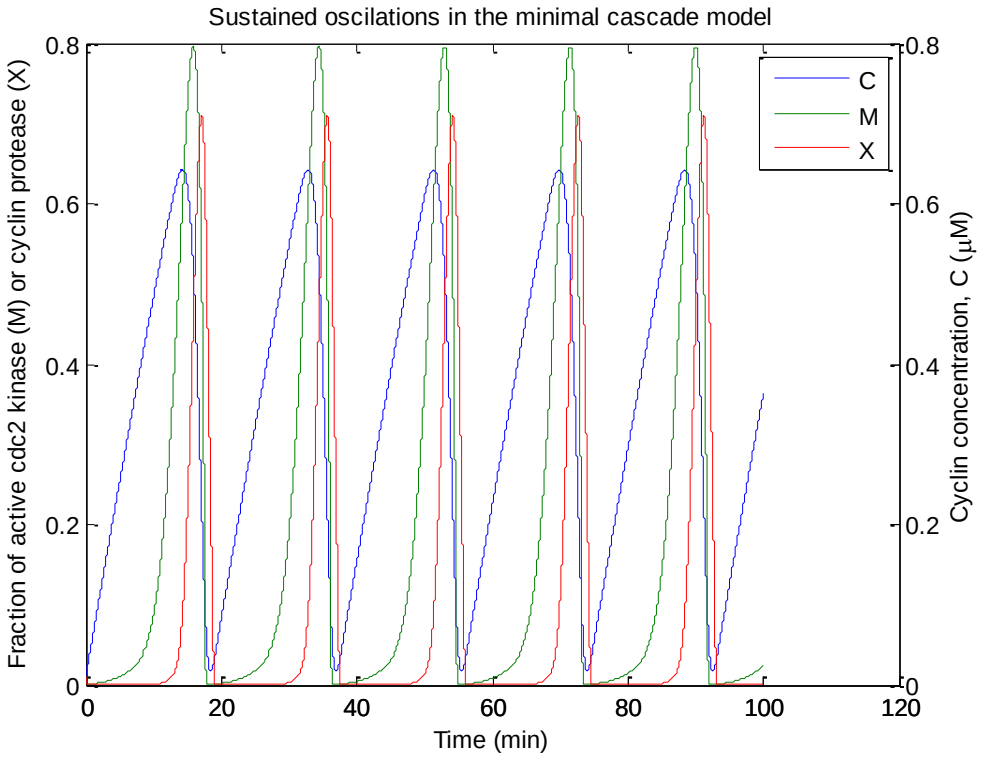


Figure 8. Sustained oscillations in the minimal cascade model involving CyclinB and Cdc2 kinase with Initial conditions same as that in Figure, but v_i and v_d are doubled.

Figure shows the result obtaining by doubling CyclinB synthesis rate and degradation rate. While other variables remain the same, the result suggests that we can have a shorter period. This, in fact, implies that if there is an increase in chemical reactivity in CyclinB synthesis, instead of being unstable, it will simply increase the synthesis rate of active Cdc2 kinase M as well as active CyclinB protease X.

Conclusion

In this project, we presented a part of the mitosis cycle in which after the rise of CyclinB beyond a threshold triggers the activation of cdc2. With cdc2 being activated, it brings up the activation of protease and then in turn causes CyclinB to be degraded. (Fig 3)

In order to understand the mathematical aspect of this biological scenario, we built a model which relies on the cascade of phosphorylation dephosphorylation cycles. So we focused on the oscillations in CyclinB and cdc2. Also, we constructed a model to analyze protein activation and the control of cell cycle by regulating protein.

While solving the problem, what are the properties that we should take into consideration when the system reaches to a steady state? First of all, the initial conditions should be independent. Secondly, the numerical model shouldn't be at an equilibrium state. Instead, the simulation by Matlab should be at periodic waveforms, which means the oscillations will be established so that the system can be self-stabilized.

The checkpoint of cell cycle is driven by continuous oscillations. Now the question is at the applicability of the model to cell cycles subject to more complex regulation. For example, in yeast, an additional control occurs at the start point before DNA replication. Two different states of cdc2 kinase are involved in the checkpoints before the start point and mitosis, corresponding to two different forms of covalently modified cdc2 per cycle. Each of these two forms could be involved in an oscillator similar to our model, with its own CyclinB and protease. The alteration between the two forms of active cdc2 kinase would result from the coupling of the two oscillators. The minimum oscillating cascade of the cycle as we showed could then provide a building block for more complex mechanisms. In conclusion, it is safe to say that our model can reproduce the synthesis and degradation of enzymes in the cells.

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