Discrete cellular automaton model coupled with a biochemical ODE model to study the cell cycle

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Contents:

Abstract

A study of automaton model of the cell cycle coupled with a basic biochemical ODE model that progresses through the phases: G1, S, G2 and M and the transition between each phase controlled by the concentrations of the major molecules, total cyclin and MPF. which in turn are regulated by other molecules.

Cells on completing M phase, divide into two daughter cells which enter into the G1 phase of the cycle. Further, during the cycle each cell has a defined probability for exiting the cycle and undergo apoptosis. The exit can take place only during G1-S and G2-M transitions thus establishing the role of molecular check points in a cycle.

To achieve homeostasis and the maintenance of total number of cells, we assumed a fixed cell population with apoptosis balancing cell replication. We also assumed that the cell divides symmetrically and have not considered cell size variations.

With this crude model, we attempted to study the dynamics of the cell cycle such as the effect of the concentrations of the molecules, the number and the evolution of cell cycle phases in the cell population with time, mean duration of phases, number of cells in the population, and their steady-state behaviours corresponding to the different initial conditions.

We intend to understand and analyse how the cell cycle is regulated and also comprehend how the decisions made by a cell, whether to live in a quiescent stage, replicate or die, affect the cell population. Understanding cell cycle dynamics is crucial in learning uncontrolled cell proliferation (cancer) and diseases with unregulated apoptosis (E.g. Parkinson's disease - death of dopaminergic neurons).

Introduction

Several studies have been modelled to understand cell cycle regulation in the recent past. Few studies have predicted the dynamic properties and sought to identify the various existing and the unknown molecules and the signals responsible for them. Experimental and computational research is yet to be explored and understood in depth.

Considering the thousands of molecules and their reactions and control mechanisms, and exploring the regulation processes varying across the cell types in an individual organism and across organisms also pose a huge challenge.

Cell cycle in brief-
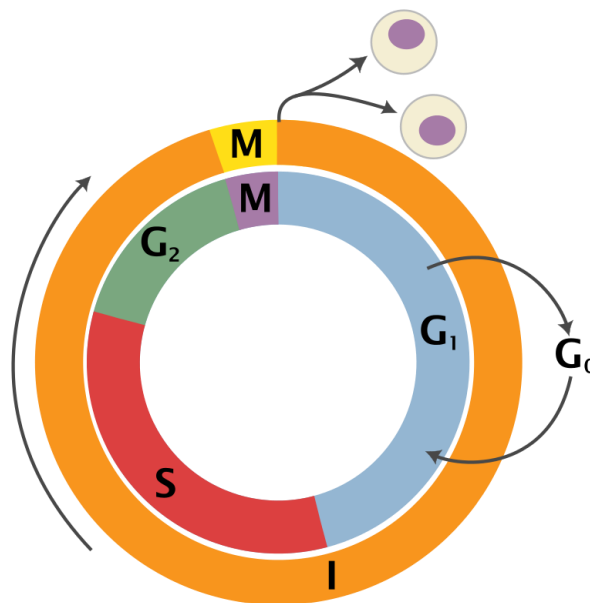
Outer ring: I = Interphase, M = Mitosis;
Inner ring: M = Mitosis, G1 = Gap 1, G2 = Gap 2, S = Synthesis;
Not in ring: G0 = Gap 0/Resting phase
Image Source: Wikipedia (https://en.wikipedia.org/wiki/Cell_cycle)

The cell cycle involves a sequence of events that leads to the duplication of cells, starting from the S-phase where the cell replicates its DNA and distributes the two copies to the two daughter nuclei during the M-phase (Mitosis).

Cdk and cyclin families are the core molecules that participate in eukaryotic cell cycle regulation. Various Cdk/cyclin pairs regulate critical transitions- G1 to S, G2- M and inhibit M-G1 transition.

The major transitions are also regulated by checkpoints which ensure that DNA synthesis takes place only in the presence of nutrients and growth factors and that the cellular transition into M phase only upon the successful DNA synthesis. In case of errors, these checkpoints inhibit further steps and in most cases the cell is marked for death and undergoes apoptosis.

Molecular players-

MPF (Mitosis promoting factor or Maturation promoting factor) is crucial for cell division and phase change from G2-> M. MPF is a dimer composed of CycB (cyclinB) and Cdk (cyclin dependent kinase). Inactive MPF contain phosphate groups at Thr14 and Tyr15 and these are dephosphorylated to make active MPF.
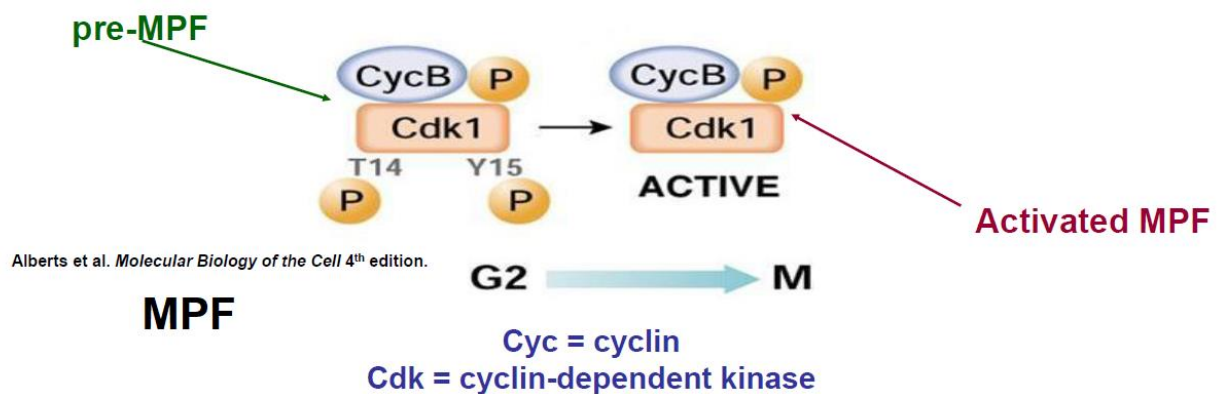
Hence, the two ways to regulate MPF activity are i) production/degradation of cyclin ii) the phosphorylation/dephosphorylation of Cdk.

Further, cyclin is alternatively synthesized and degraded in cells. Different cell phases are regulated by the synthesis and degradation of the various cyclins. In the ODE model, we consider only one of the cyclins (CyclinB) and ignore the other cyclins (S-cyclin and M-cyclin).

Cdc25 is a phosphatase, and activates MPF removing the phosphate groups. A positive feedback exists between MPF and Cdc25. MPF increases Cdc25 activity and Cdc25 in turn increases MPF activity. Thus, there is mutual activation and there can be a display of bistability.

The deactivation of MPF by phosphorylating it is performed by the protein wee1. Further MPF regulates its own deactivation through wee1.
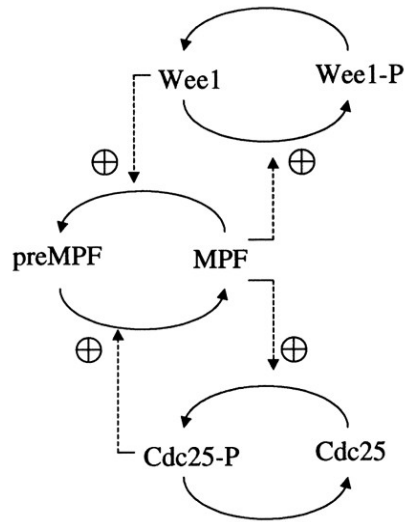
MPF destruction is regulated by the Anaphase Promoting Complex (APC). In the Novak Tyson ODE model, an intermediate enzyme (IE) was introduced to accommodate for the delay in the activation of MPF and activation of APC. Active MPF leads to phosphorylation of IE, which activates APC leading to cyclin degradation. The IE has now been identified by Chen et al (2004) as the APC core complex.

Objectives

1. To integrate a biochemical ODE model to an automaton model, and observe the time evolution of cell cycle phases and distributions.

2. To understand and analyse how the cell cycle is regulated and also comprehend how the decisions made by a cell, whether to live in a quiescent stage, replicate or die, affect the cell population.

3. To study the dynamics of the cell cycle such as the effect of the concentrations of the molecules, number and evolution of cell cycle phases in the cell population with time, mean duration of phases, number of cells in the population, and their steady-state behaviours corresponding to the different initial conditions with the above mentioned crude model.

Methods

The model was implemented using MATLAB (attached code in the zip file).

1. ODE model-

Obtained from the Novak Tyson (1993) model on Xenopus oocyte. As described by Silbe & Tyson (2007)
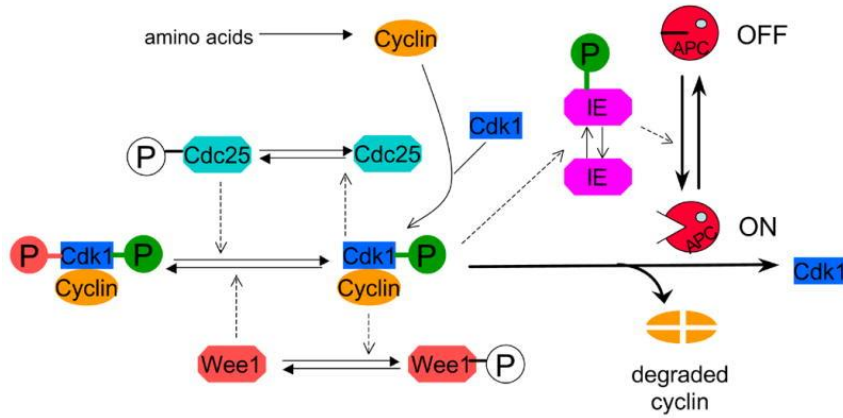
1. $\dfrac{d}{dt}[\text{Cyclin}] = k_1 - k_2[\text{Cyclin}] - k_3[\text{Cyclin}][\text{Cdk}]$

2. $\dfrac{d}{dt}[\text{MPF}] = k_3[\text{Cyclin}][\text{Cdk}] - k_2[\text{MPF}] - k_\text{wee}[\text{MPF}] + k_{25}[\text{preMPF}]$

3. $\dfrac{d}{dt}[\text{preMPF}] = -k_2[\text{preMPF}] + k_\text{wee}[\text{MPF}] - k_{25}[\text{preMPF}]$

4. $\dfrac{d}{dt}[\text{Cdc25P}] = \dfrac{k_a[\text{MPF}]([\text{total Cdc25}] - [\text{Cdc25P}])}{K_a + [\text{total Cdc25}] - [\text{Cdc25P}]} - \dfrac{k_b[\text{PPase}][\text{Cdc25P}]}{K_b + [\text{Cdc25P}]}$

5. $\dfrac{d}{dt}[\text{Wee1P}] = \dfrac{k_e[\text{MPF}]([\text{total Wee1}] - [\text{Wee1P}])}{K_e + [\text{total Wee1}] - [\text{Wee1P}]} - \dfrac{k_f[\text{PPase}][\text{Wee1P}]}{K_f + [\text{Wee1P}]}$

6. $\dfrac{d}{dt}[\text{IEP}] = \dfrac{k_g[\text{MPF}]([\text{total IE}] - [\text{IEP}])}{K_g + [\text{total IE}] - [\text{IEP}]} - \dfrac{k_h[\text{PPase}][\text{IEP}]}{K_h + [\text{IEP}]}$

7. $\dfrac{d}{dt}[\text{APC}] = \dfrac{k_c[\text{MPF}]([\text{total APC}] - [\text{APC}])}{K_c + [\text{total APC}] - [\text{APC}]} - \dfrac{k_d[\text{PPase}][\text{APC}]}{K_d + [\text{APC}]}$

8. $[\text{Cdk}] = [\text{Total Cdk}] - [\text{MPF}] - [\text{preMPF}]$

9. $k_{25} = V_{25}'([\text{Total Cdc25}] - [\text{Cdc25P}]) + V_{25}''[\text{Cdc25P}]$

10. $k_\text{wee} = V_\text{wee}'[\text{Wee1P}] + V_\text{wee}''([\text{Total Wee1}] - [\text{Wee1P}])$

11. $k_2 = V_2'([\text{Total APC}] - [\text{APC*}]) + V_2''[\text{APC*}]$

Image   source: Silbe & Tyson (2007)

k1 = 1; k3 = 0.005; ka = 0.02; Ka = 0.1; kb = 0.1; Kb = 1; kc = 0.13; Kc = 0.01; kd = 0.13;
Kd = 1; ke = 0.02; Ke = 1; kf = 0.1; Kf = 1; kg = 0.02; Kg = 0.01; kh = 0.15; Kh = 0.01;
v2_1 = 0.005; v2_2 = 0.25; v25_1 = 0.5*0.017; v25_2 = 0.5*0.17; vwee_1 = 0.01;
vwee_2 = 1;
CDK_total = 100; cdc25_total = 5; wee1_total = 1; IE_total = 1; APC_total = 1; PPase = 1;

Initial conditions:
cyclin = 0; MPF = 0; preMPF = 0; cdc25P = 0; wee1P = 0; IEP = 1; APC = 1;
cyclin_tot = cyclin + MPF + preMPF;

CDK = CDK_total - MPF - preMPF;
k25 = v25_1*(cdc25_total - cdc25P) + v25_2*cdc25P;
kwee = vwee_1*wee1P + vwee_2*(wee1_total - wee1P);
k2 = v2_1*(APC_total - APC) + v2_2*APC;

From the Novak Tyson ODE model, we obtained a rapid oscillations in MPF and cyclin, which is analogous to the rapid divisions observed in frog oocytes.
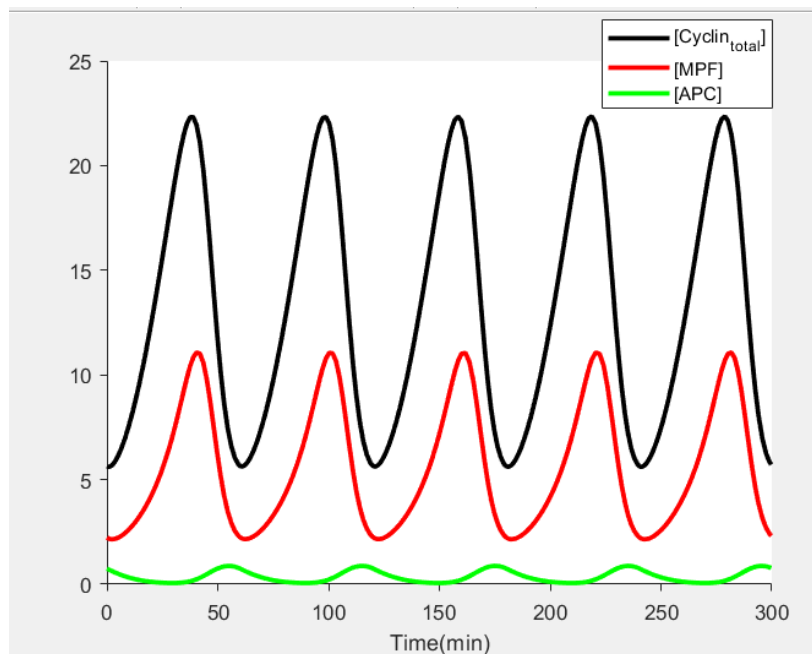

Figure: From the ODE simulation, MPF is active when cyclin is active, APC responsible for the cyclin degradation, is maximum when cyclin is minimum.
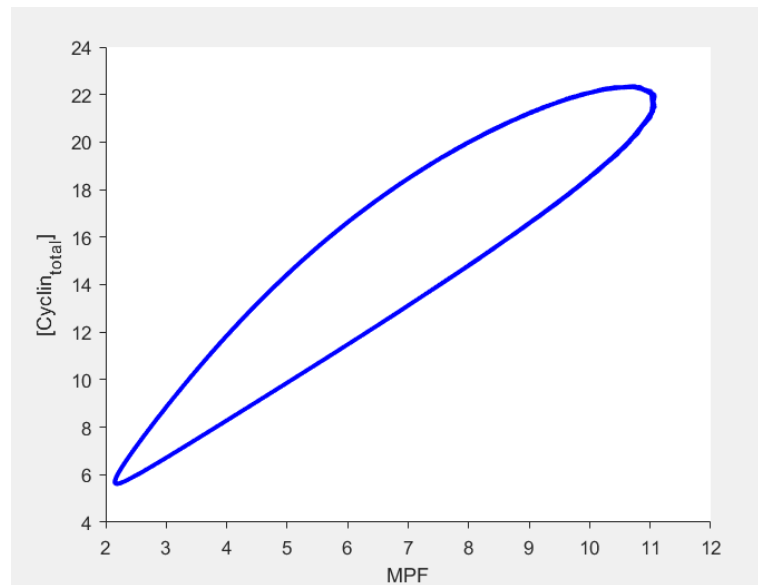
Figure: The ODE model leads to stable limit cycle oscillations.

The inclusion of too many molecules might become cumbersome, therefore as per the Novak Tyson model only the most important players were considered and used to control the phase transition.

2. Automaton model with the phase changes determined by concentration changes as obtained from the ODE model.

a. Original cell populations were initialized in the G1, S, G2, M phases.

b. Death probabilities were set and in addition, the cell exit could occur only in the G1- S and G2- M phases. This was done to mimic the role of molecular check-points as effected by Atilla et al.

c. The cells on completing M phase, divide into two cells which enter into the G1 cycle.
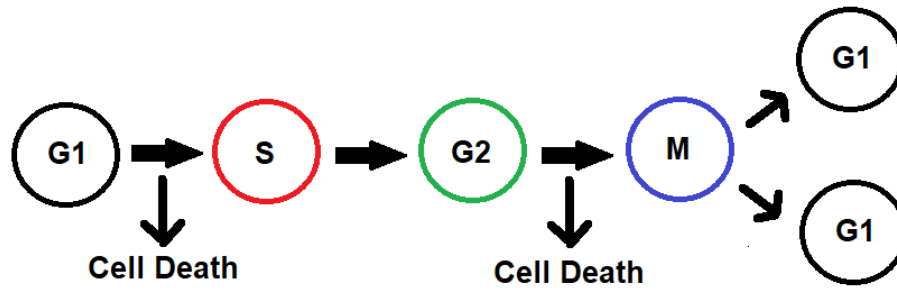
Figure: Automaton Model

d. In the current model, the transition between each phase was determined according to concentration of the Cdk and total cyclin at the particular time point.

e. A rough estimate of MPF and cyclin concentration was set between the various cell phases similar to the study by Srividhya et al.
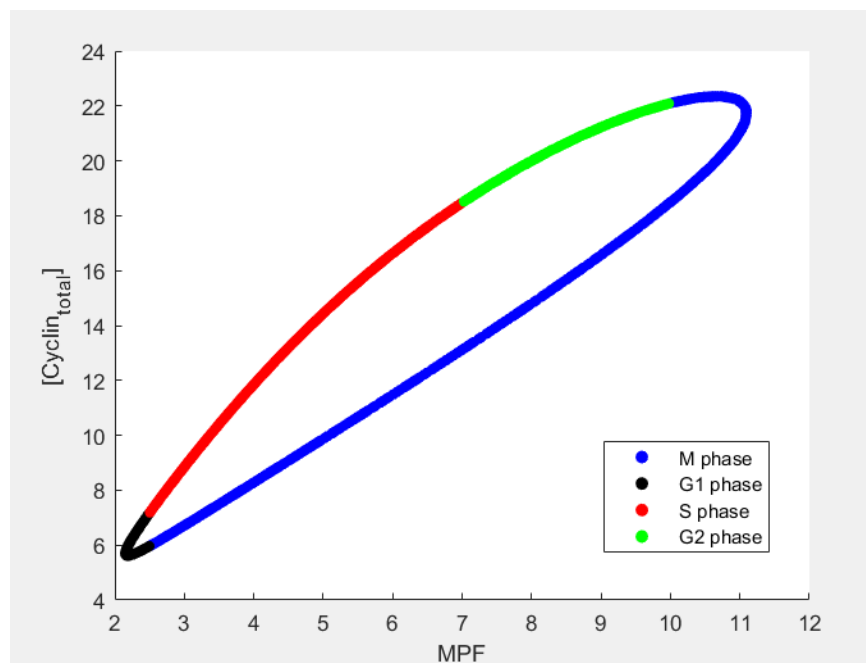

Figure:
G1 phase -> cyclin_tot <10 & MPF <2.5
S phase -> cyclin_tot >10 & MPF <7
G2 phase -> cyclin_tot >16 & 7<= MPF <10
M phase -> MPF >= 2.5

The low cyclin state is considered as the G1 phase and the rest of the cycle with rise and fall of cyclin is the S, G2, M phase. M phase is determined by the rise of MPF, which increases with cyclin concentration. After which APC is activated leading to cyclin degradation and further initialization of the daughter cells to G1 phase. Further, when the simulation of actual cell behaviour was attempted, the M phase was short-lived and cell division occurred rapidly and the cell spent more time during interphase.

Since cell death can occur at any point of time independent of previous cell history, it is a Markov process. Further this is combined with a non-Markovian process- cell phase duration as governed by the corresponding concentrations of the cyclin and cdk.

Results & Discussion

We obtained the final cell distribution- steady state values for different values of initial parameters.

The cell population in the model has been observed for t= 1 day (1440 minutes).
With logistic regulation of death.

$$P(death) = P_0 + ks * (N/ Ns - 1)$$

We have considered,
ks = 0.01.
Ns = Number of cells at steady state= 30000
Total number of cells that can be tracked = 60000

The initial cell populations have been distributed randomly in the 4 phases with equal probability.

Further, initial cell distribution, final steady state count, death probability, and time of observation can be changed to study the dynamics.

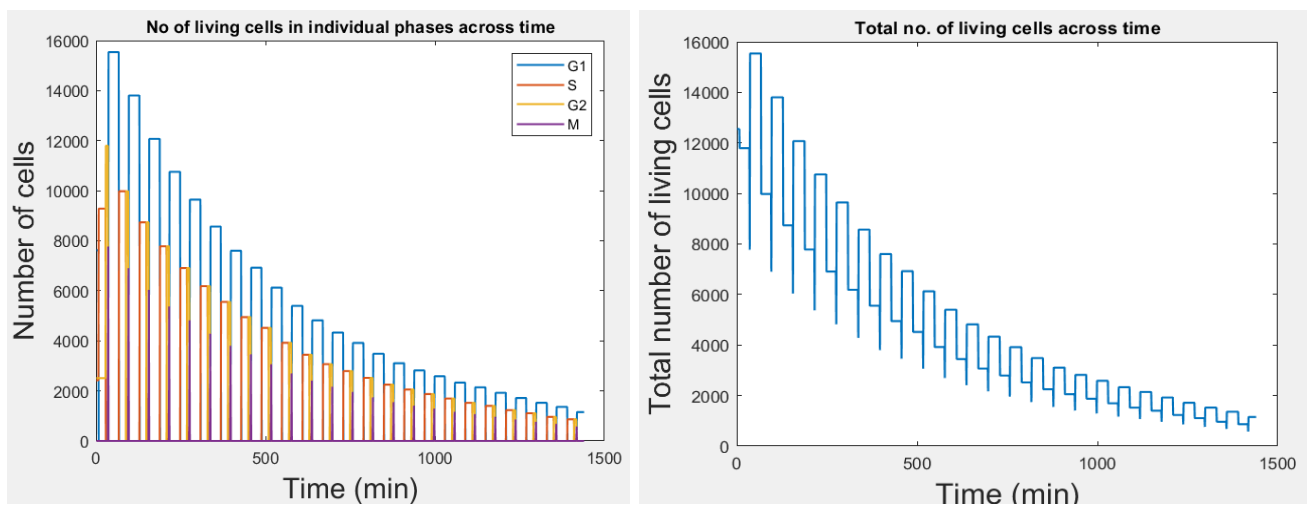Initial cell Number=1, death probability= 0.015
Final number of living cells at t=1day: 35024
Total number of dead cells across t=1day: 19502
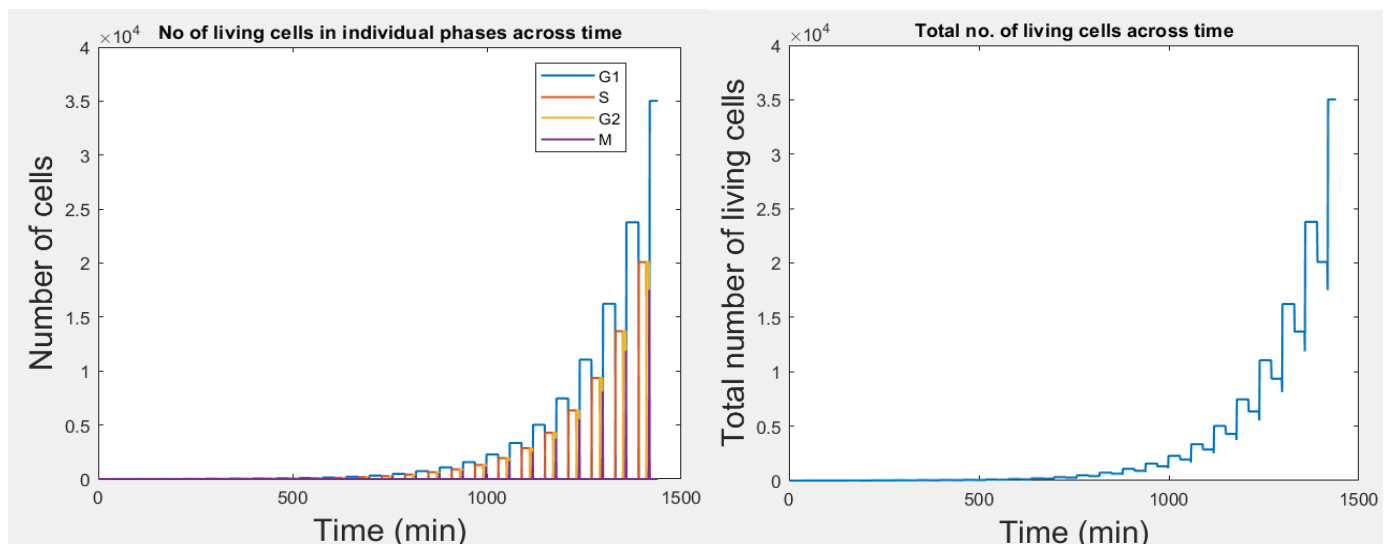
We observed how a single cell rapidly divides, producing a cell population distribution. We also observed that all the cells are synchronised i.e. have the same phase at a given point in time.

For Initial cell Number=10000, death probability= 0.02
Final number of living cells at t=1day: 1152
Total number of dead cells across t=1day: 78220

We initially observed a sudden increase in the cell population, followed by a steady decrease with small increases in the time steps (during M phase) in the population.

For Initial cell Number=10000, death probability= 0.0175
Final number of living cells at t=1day: 36658
Total number of dead cells across t=1day: 286438

We observed the steady increase in the cell distribution.

For Initial cell Number=10000, death probability= 0.018
Final number of living cells at t=1day: 17628
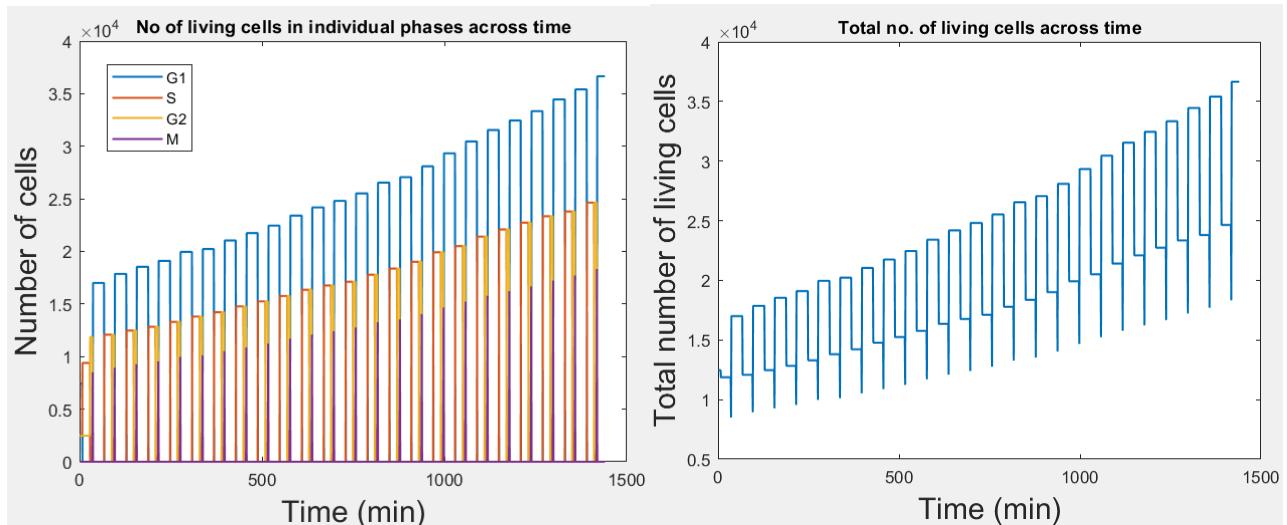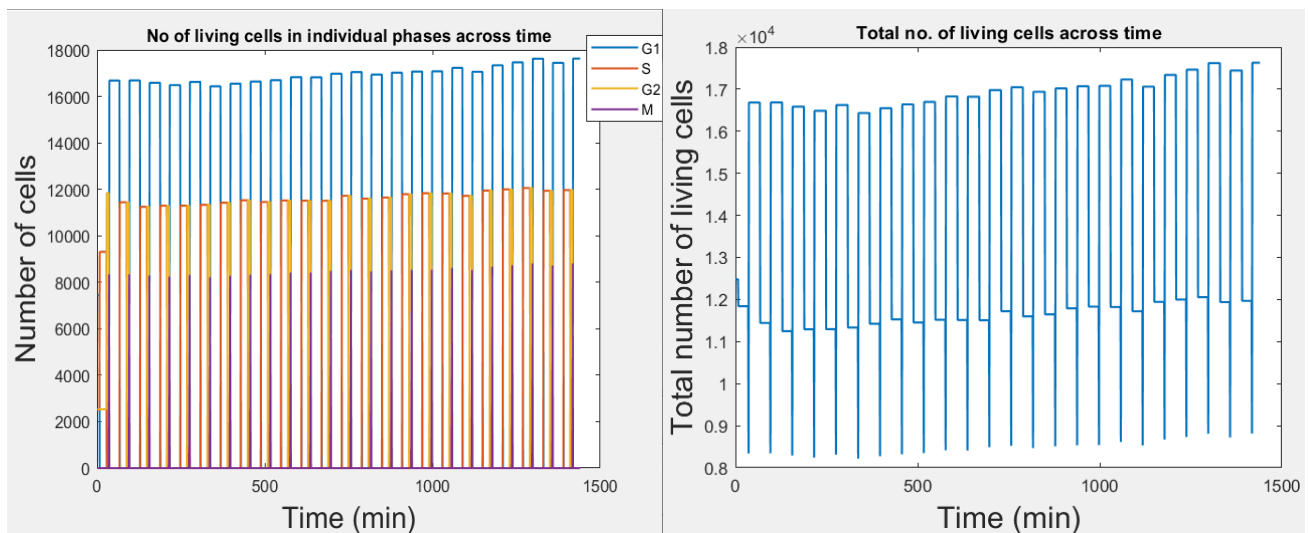Total number of dead cells across t=1day: 198327

For this death probability we found a stable distribution.

In all the simulations, we observed that even though the cells start randomly with equal probability of being in different phases, after a small time interval, they all show synchronous oscillations. All cells tend to adopt the same phase at a given time. This is somewhat similar to the oscillations as observed in Atilla et al.

Further the model was found to be very sensitive to the value of death probability. Small alterations in death probability can lead to entirely different distributions. We also observed a large decrease in cell numbers following the G1 phase.

Conclusions & Limitations

This approach of combining a biochemical ODE model to a discrete automaton model can be useful to capture the basic properties of the cell cycle and may be used to study cell populations in various phases.

This model can also be used to study the progressive synchronization of the cell populations and its response to the change in concentrations and death rate. It further provides qualitative and quantitative data that can be compared with experimental observations.

The cyclin and MPF concentrations are continuous variables coupled with the discrete value of the cell population numbers.

The non-inclusion of G0 phase (quiescent resting phase) in this study is a limitation. The G0 phase could be integrated to the model, with the known molecular players in an ODE model. Hence the transition from G0 -> G1 and G1 -> G0 can also be modelled. There are observed variations in cell populations with this transition. Non-proliferating cells in multicellular eukaryotes enter the G0 state and may remain quiescent for long periods of time. (Eg- Most neurons stay quiescent indefinitely and some enter G0 state semi-permanently- liver, kidney, stomach cells.)

Another complex issue is the spatial distribution of the participating molecules within the cell. These concentrations may vary in the nucleus (most events take place) to the cytoplasm (molecules may move in and out of the nuclear membrane). Diffusion and protein gradients may have an important role and needs consideration. However, in the current model we have assumed same cyclin and MPF concentration in all the cells, leading to the cell synchronization in phases. This may not be true in a cell population, since different cells may be subject to different cyclin and MPF concentration at a given time point.

Another major challenge would be the extensive simulation time and resources that are needed to model spatial distributions and molecular gradients.

Challenges & Future work

An agent-based model, comprising of a 2-D grid-based model of cell growth could also be implemented. This may allow division during M phase based on cell neighbours' existent condition. The ABM can provide a natural description of the system and make it easy to visualize the dynamics. The agent (cell) interactions are heterogeneous and can help us understand the network effect. This can also be extended to a 3-D grid and study interactions at a tissue level.

Simulating cell- cell interactions and proliferation at a tissue level may help us understand cell communication, regulations and tissue/organ formation. These models may also clarify how perturbations lead to tumor formation.

Better experimental data and technology, such as single cell measurements, mass spectrometry, micro-array data, fluorescent imaging that provide detailed protein and m-RNA fluctuations can identify and validate existing models better.

Models will have to be tested to fit the physiological observations not only in wild-type cells but also in various mutants. Single/ double gene deletions can further validate roles of the participating molecules.

Further this model can be extended to include external signals, and the dynamics can be further studied.

Modelling anti-cancer drugs require accurate models of cell-cycles. Such discrete automaton models might prove useful in cancer research. The way in which the mutations and drugs can inhibit/activate Cdk's can also be modelled using ODE's.

We can inculcate Boolean network models as another approach. The cell distribution in the various phases can be determined using the fluorescence-activated cell sorting (FACS). This model may also be used to simulate virtual FACS profiles.

Several unanswered questions in the field are still part of ongoing research. The cell cycle regulation plays a larger role in cell physiology, parameter of apoptosis and checkpoints and also the response of the cell to the nutrient and environmental changes.

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