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| Bifurcation Analysis of the Biochemical Switches in the G1-S Transition of the Eukaryotic Cell Cycle  C. Liu, M. Huang  Department of Biomedical Engineering, University of Southern California |

**ABSTRACT**

**Motivation:** The G1/S transition of the mammalian cell cycle has been the center of many studies in cancer growth as its proper function is critical to ensure the cell divides without errors. Mathematical modeling of the body of knowledge regarding the regulatory molecules contained in this system can formulate testable hypothesis that can lead to targeted treatments for cancer.

**Results:** Our bifurcation analysis showed that the overexpression of constitutive synthesis rate of cyclin D and/or cyclin E resulted in a negative shift in the threshold level of mitogenic stimulation to form a saddle node bifurcation. This indicates bistability in the system – a key indicator for cell proliferation.

**Conclusion:** This suggests the use of drugs that target the inhibits the expression of Cyclin D and Cyclin E to induce cell cycle arrest on cancer cells

**1. INTRODUCTION**

The G1-S transition of cell cycling provides the go signal for DNA synthesis after the cell has grown to a sufficient stage and the requisite processes to continue into cell division have completed. Errors in this go signal have been shown to lead to uncontrolled growth of the cell - a hallmark of cancer. Thus there is a major focus to understand the molecular interactions of this regulatory system. Decades of experimental research has uncovered a range of molecules and interactions involved. Mathematical modeling is used to integrate the existing body of knowledge into a larger framework to describe experimental observations of the overall system. If successful, the model can then be reasonably used to make predictions about effects of manipulating cell regulatory molecules on cell cycling, which suggest treatments to correct cell cycling errors that lead to cancer, as well as to further scientific knowledge.

The present model of the G1-S transition by Hatzimanikatis, et al 1999 can be characterized by a phosphorylation cascade that amplifies a transcription promoter to activate a transcription factor to bring the cell cycle into DNA synthesis - the S phase [1]. This phosphorylation cascade can be characterized as a positive feedback loop that behaves as a biochemical switch to toggle the transcription factor to a higher steady state. While this model has been shown to replicate experimentally observed phenomena, this model only contains one transcription factor promoter, cyclin E. Much evidence demonstrates the overexpression of an additional transcription factor promoter, cyclin D, in several types of tumor formation. Cyclin D is established as an oncogene with an important pathogenetic role in many human tumors [2]. Thus, the mathematical model by Hatzimanikatis is oversimplified and demonstrates an omission of important components in the overall picture.

To overcome this limitation, we extend the model by incorporating the phosphorylation cascade involving cyclin D and its corresponding cyclin-dependent kinases, cdk4/6, as described by [Swat]. Next we determine if this model can be used to describe experimental observations of the effects of overexpressing the constitutive synthesis rates of cyclin D and cyclin E on cell proliferation. If successful, it increases confidence in the model’s ability to suggest drug targets to inhibit cell proliferation, and thus stop tumorigenesis.  If successful, it increases confidence in the model in its ability to suggest drug targets to inhibit proliferation, and thus stop tumorigenesis.

**2. METHODS**

**2.1 The G1/S Bistability Model**

The mathematical model of the G1/S transition in mammalian cells is modified based on a previous model proposed by Swat et al. (2004) which includes a set of proteins and their regulatory gene factors. The schematic diagram of the G1/S transition network is provided in Figure 1 and their relationship is presented as differential equations provided in the supplementary material section [Swat].

The model can be summarized by its two phosphorylation cascades involving the cyclin D, cdk4/6 complex and the cyclin E, cdk2 complex that act is biochemical switches to regulate the level of the transcription factor, E2F-1. At beginning of the cell cycle, E2F-1 is bound to the tumor suppressor (pRB). The activated cyclinD-cdk4,6 complex first promote the phosphorylation of pRB and then the activated cyclinE/CDK2 complex promote the reaction of double-phosphorylated pRB from to fully release E2F-1 [Swat]. Moreover, E2F-1 will amplify the signal of the generation of cyclin D, cyclin E, and pRB and cause them to reach a higher stable steady state. The presence of two steady states in the system that toggle from a low steady state (off) to a high steady state (on) is called bistability. The switch-like behavior indicates the start of cell proliferation.

Bistability is a common response of systems containing combination of positive and negative feedback loops. Indeed, these positive and negative feedback regulatory systems are abundant in the G1/S cycle [Bertoli 2013].

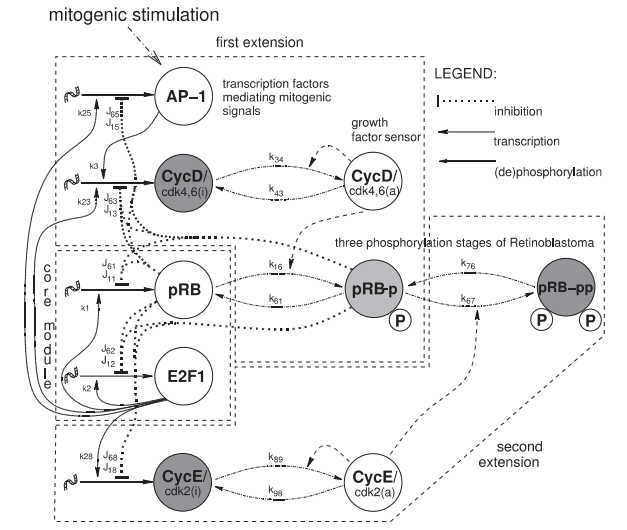


Figure The schematic diagram of cell cycle transition during G0-phase to S-phase.

In this case, two positive feedback loops trigger a two stage phosphorylation of pRB to free its inhibition onto E2F-1. Fingure X shows the rapid incensement of E2F-1 in a switch like manner to bring it to a higher steady state, and thus committing the cell cycle into cell proliferation, the S phase.



**2.2 Modified model to show cell proliferation oscillation**

This model can also be modified to show the oscillatory behavior of cell proliferation that is found in the past model by Hatzimanikatis et al. (1999) [ref Hatz]. After E2F provided a positive feedback on the generation of cyclin D, cyclin E, and pRB, it provides a negative feedback to inactivate transcription. This regulatory system coupling with both positive and negative feedback to generate oscillatory behavior has been found in several literature reviews [ref Hatz, Bertoli 2013]. Thus, by relating the degradation term of cyclin D and cyclin E with the concentration of E2F-1, we will be able to generate the oscillatory plot. The oscillations of cell cycling are generated first with a positive feedback system that brings the transcription factor signal up to commit the cell cycle into the S phase, and then a negative feedback mechanism to bring the transcription factor back down.

**2.3 Coupling to the growth signal pathway**

The analysis of bifurcations due to the strength of the mitogenic growth signal, Fm, as a bifurcation parameter is used to determine cell proliferation. Low levels of Fm are inadequate to produce CycD to trigger a phosphorylation cascade. In this case, the switching to a second steady state does not occur. As Fm increases past a threshold as indicated through a saddle node bifurcation, the system switches to generate bistability. Figure X. below performs the time course of protein concentration at G1/S transition with different Fm values. It is observed that these protein concentrations will have bistability when Fm is greater than the transcritical bifurcation (TC) around 0.0035. The study was further extended to evaluate the change of transcritical bifurcation point when overexpressed the cyclin E, cyclinD, and combination by increasing their constitutive concentration in next section.

**2.4 Constitutive Synthesis Rates for Cyclin D and Cyclin E**

For the model to describe experiments involving the constitutive overexpression of the synthesis rates of cyclin D and cyclin E [ref][ref], we added a constitutive value, CD and CE, to provide a basal rate of growth for Cyclin D and Cyclin E, respectively.



|  |  |
| --- | --- |
|  | (1) |

|  |  |
| --- | --- |
|  | (2) |

With the constitutive term added, this increases the production rate of CycD and CycE. To bring the system back down to baseline levels as seen in the original model, the E2F-1 concentration dependent constants, k23 and k28, for CycD and CycE, respectively, is decreased.

**3. RESULTS AND DISCUSSION**

**3.1 Inclusion of the Constitutive Rate for the production of CycD/CycE**

**3.2 Bifurcation Analysis of the Constitutive Overexpression in the Synthesis Rates of CycD/CycE**

By performing the bifurcation analysis on the constitutive overexpression of the synthesis rates of cyclin E, cyclin D, and both cyclin D&E, it is found that the saddle point is shifted to a smaller value of Fm shown in Figure X. This indicates that the higher synthesis rates of cyclin E and cyclin D brought the cell from quiescence to proliferation. This is experimentally observed where a constituve overexpression of cloned cyclin E can result in a transition to proliferation in serum-free medium for Chinese hamster ovary cells (Renner et al., 1995). Additionally, overexpression of cyclin D1 in rat embryo fibroblasts is shown to shorten the G1 phase by inducing a progression into the S phase (Imoto et al., 1997). For the overexpression of both cyclin D&E, the cell proliferated even more rapidly, Varying with the constitutive synthesis rates for cyclin E shown in Fingure X, the result display that the saddle point shift forward with a greater overexpression in cyclin E.

**CONCLUSIONS**

**ACKNOWLEDGEMENTS**

**G1S\_Func**

function dydt=G1S\_Func(t,y,pars)

RB = y(1);

E2F = y(2);

CycD = y(3);

CycD\_a = y(4);

AP = y(5);

RB1 = y(6);

RB2 = y(7);

CycE = y(8);

CycE\_a = y(9);

k1 = pars(1);

k2 = pars(2);

k3 = pars(3);

k16 = pars(4);

k34 = pars(5);

k43 = pars(6);

k61 = pars(7);

k67 = pars(8);

k76 = pars(9);

k23 = pars(10);

k25 = pars(11);

k28 = pars(12);

k89 = pars(13);

k98 = pars(14);

a = pars(15);

J11 = pars(16);

J12 = pars(17);

J15 = pars(18);

J18 = pars(19);

J61 = pars(20);

J62 = pars(21);

J65 = pars(22);

J68 = pars(23);

J13 = pars(24);

J63 = pars(25);

Km1 = pars(26);

Km2 = pars(27);

Km4 = pars(28);

Km9 = pars(29);

kp = pars(30);

phi\_RB = pars(31);

phi\_E2F = pars(32);

phi\_CycD = pars(33);

phi\_CycD\_a = pars(34);

phi\_AP = pars(35);

phi\_RB1 = pars(36);

phi\_RB2 = pars(37);

phi\_CycE = pars(38);

phi\_CycE\_a = pars(39);

Fm = pars(40);

C=pars(41); %Constitutive Term

dydt(1,1) = k1\*E2F\*J11\*J61/((Km1+E2F)\*(J11+RB)\*(J61+RB1))-k16\*RB\*CycD\_a+k61\*RB1-phi\_RB\*RB;

dydt(2,1) = kp+k2\*(a^2+E2F^2)\*J12\*J62/((Km2^2+E2F^2)\*(J12+RB)\*(J62+RB1))-phi\_E2F\*E2F;

dydt(3,1) = k3\*AP+k23\*E2F\*J13\*J63/((J13+RB)\*(J63+RB1))+k43\*CycD\_a-k34\*CycD\*CycD\_a/(Km4+CycD\_a)-phi\_CycD\*CycD;

dydt(4,1) = k34\*CycD\*CycD\_a/(Km4+CycD\_a)-k43\*CycD\_a-phi\_CycD\_a\*CycD\_a;

dydt(5,1) = Fm + k25\*E2F\*J15\*J65/((J15+RB)\*(J65+RB1))-phi\_AP\*AP;

dydt(6,1) = k16\*RB\*CycD\_a-k61\*RB1-k67\*RB1\*CycE\_a+k76\*RB2-phi\_RB1\*RB1;

dydt(7,1) = k67\*RB1\*CycE\_a-k76\*RB2-phi\_RB2\*RB2;

dydt(8,1) = C+k28\*E2F\*J18\*J68/((J18+RB)\*(J68+RB1))+k98\*CycE\_a-k89\*CycE\*CycE\_a/(Km9+CycE\_a)-phi\_CycE\*CycE;

dydt(9,1) = k89\*CycE\*CycE\_a/(Km9+CycE\_a)-k98\*CycE\_a-phi\_CycE\_a\*CycE\_a;

end

**G1S\_Drive\_Bifurcation**

clc

clear all;

close all;

tspan = 0:1:1000;

%k1 k2 k3 k16 k34 k43 k61 k67 k76 k23 k25 k28 k89 k98 a

k=[1 1.6 0.05 0.4 0.04 0.01 0.30 0.7 0.1 0.3 0.9 0.06 0.07 0.01 0.04];

%J11 J12 J15 J18 J61 J62 J65 J68 J13 J63

J=[0.5 5.00 0.001 0.6 5.0 8.0 6.0 7 0.002 2.0];

%Km1 Km2 Km4 Km9 kp

Km=[0.5 4.0 0.3 0.005 0.05];

%phiRB phiE2F phicycD phicycDa phiAP phiPRBp phiPRBpp phiCycE phiCyca Fm

phi=[0.005 0.1 0.02300 0.030000 0.0100 0.06000 0.040000 0.06000 0.05000 0.044];

C=0;

pars = [k J Km phi C];

initial = [5 0 0 0.01 0 0 0 0 0.01];

figure;

%Solve for ODE at different Fm

for i=1:16

pars(40)=i\*0.0005;

options = odeset('reltol',1e-6);

[t,result{i}]=ode45(@G1S\_Func,tspan,initial,options,pars);

subplot(4,4,i);plot(t,result{i}); axis([0 1000 0 15]);

end

r1=result{1};r2=result{2};r3=result{3};r4=result{4};r5=result{5};

r6=result{6};r7=result{7};r8=result{8};r9=result{9};r10=result{10};

r11=result{11};r12=result{12};r13=result{13};r14=result{14};r15=result{15};r16=result{16};

Stability\_Low=[r1(200,2),r2(200,2),r3(200,2),r4(200,2),r5(200,2),r6(200,2),r7(200,2),r8(200,2),r9(200,2),r10(200,2),r11(200,2),r12(200,2),r13(200,2),r14(200,2),r15(200,2),r16(200,2)];

Stability\_High=[r1(800,2),r2(800,2),r3(800,2),r4(800,2),r5(800,2),r6(800,2),r7(800,2),r8(800,2),r9(800,2),r10(800,2),r11(800,2),r12(800,2),r13(800,2),r14(800,2),r15(800,2),r16(800,2)];

Fm=zeros(1,16);

for i=1:16

Fm(i)=i\*0.0005;

end

%Constitutive Overspression of Cyclin E

C=0.2;

k(12)=0.04;

pars = [k J Km phi C];

figure;

for i=1:16

pars(40)=i\*0.0005;

options = odeset('reltol',1e-6);

[t,result{i}]=ode45(@G1S\_Func,tspan,initial,options,pars);

subplot(4,4,i);plot(t,result{i}); axis([0 1000 0 15]);

end

r1=result{1};r2=result{2};r3=result{3};r4=result{4};r5=result{5};

r6=result{6};r7=result{7};r8=result{8};r9=result{9};r10=result{10};

r11=result{11};r12=result{12};r13=result{13};r14=result{14};r15=result{15};r16=result{16};

Stability\_Low1=[r1(200,2),r2(200,2),r3(200,2),r4(200,2),r5(200,2),r6(200,2),r7(200,2),r8(200,2),r9(200,2),r10(200,2),r11(200,2),r12(200,2),r13(200,2),r14(200,2),r15(200,2),r16(200,2)];

Stability\_High1=[r1(800,2),r2(800,2),r3(800,2),r4(800,2),r5(800,2),r6(800,2),r7(800,2),r8(800,2),r9(800,2),r10(800,2),r11(800,2),r12(800,2),r13(800,2),r14(800,2),r15(800,2),r16(800,2)];

%Generate Bifurcation plot

figure; axis([0 0.0005\*16 0 15]);

subplot(2,1,1);plot(Fm,Stability\_Low,Fm(7:16),Stability\_High(7:16));

xlabel('Fm');ylabel('E2F1');grid on;title('Bifurcation without Overexpression of Cyclin E')

subplot(2,1,2);plot(Fm,Stability\_Low1,Fm(4:16),Stability\_High1(4:16));

xlabel('Fm');ylabel('E2F1');grid on;title('Bifurcation with Overexpression of Cyclin E at C=0.2')

SUPPLEMENTARY MATERIALS

Original case









When C=0.2, K28=0.4







When C=0.4, K28=0.2





When D=0.02, k23=0.28



REFERENCES

Alao, J. P. (2007). The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Molecular Cancer*, *6*(1), 24.

Bardon, S., Picard, K., & Martel, P. (1998). Monoterpenes Inhibit Cell Growth, Cell Cycle Progression, And Cyclin D1 Gene Expression In Human Breast Cancer Cell Lines. *Nutrition and Cancer*, *32*(1), 1-7.

Basso, A. D., Solit, D. B., Munster, P. N., & Rosen, N. (2002). Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. *Oncogene*, *21*(8), 1159-1166.

Hatzimanikatis, V., Lee, K. H., & Bailey, J. E. (1999). A mathematical description of regulation of the G1‐S transition of the mammalian cell cycle. *Biotechnology and Bioengineering*, *65*, 631-637.

He G, Thuiller P, Fischer SM, (2004). Troglitazone inhibits cyclin D1 expression and cell cycling independently of PPARgamma in normal mouse skin keratinocytes. *J Invest Dermatol*, 2004 Dec;123(6):1110-9

Imoto, M. (1997). Effects of Cyclin D1 Overexpression on G1 Progression-Related Events. *Experimental Cell Research*, *236*(1), 173-180.

Koroxenidou, L., Ohlson, L., & Hallstrom, I. P. (2005). Long-term 17alpha-ethinyl estradiol treatment decreases cyclin E and cdk2 expression, reduces cdk2 kinase activity and inhibits S phase entry in regenerating rat liver. *J Hepatol*, *43*(3), 478-84.

Renner W, Lee KH, Hatzimanikatis V, Bailey JE, Eppenberger H. 1995. Recombinant cyclin E expression activates proliferation and obviates surface attachment of Chinese hamster ovary (CHO) cells in proteinfree medium. Biotechnol Bioeng 47:476–482

Stamatakos, M., Palla, V., Karaiskos, I., Xiromeritis, K., Alexiou, I., Pateras, I., et al. (2010). Cell cyclins: triggering elements of cancer or not?. *World Journal of Surgical Oncology*, *8*, 111.

Brikci, F., Chiorino, G., & Boushaba, K. (2009). G1/S transition and cell population dynamics. *Networks and Heterogeneous Media*, *4*, 67-90.

Swat, M., Kel, A., & Herzel, H. (2004). Bifurcation analysis of the regulatory modules of the mammalian G1/S transition. *Bioinformatics*, *20*, 1506-1511.

