Cell Cycle Regulation in the Budding Yeast

Cuong Nguyen, Chang No Yoon, Seung Kee Han*

Department of physics, Chungbuk National University, Cheongju, Korea
Email: skhan@chungbuk.ac.kr

ABSTRACT: Cell cycle is regulated cooperatively by several genes. The dynamic regulatory mechanism of protein interaction network of cell cycle will be presented taking the budding yeast as a sample system. Based on the mathematical model developed by Chen et al. (MBC, 11, 369), at first, the dynamic role of the feedback loops is investigated. Secondly, using a bifurcation diagram, dynamic analysis of the cell cycle regulation is illustrated. The bifurcation diagram is a kind of 'dynamic road map' with stable and unstable solutions. On the map, a stable solution denotes a 'road' attracting the state and an unstable solution 'a repelling road'. The 'START' transition, the initiation of the cell cycle, occurs at the point where the dynamic road changes from a fixed point to an oscillatory solution. The 'FINISH' transition, the completion of a cell cycle, is returning back to the initial state. The bifurcation analysis for the mutants could be used uncovering the role of proteins in the cell cycle regulation network.

1. INTRODUCTION

The cell cycle is the sequence of events by which a growing cell duplicates all its components and then divides into two daughter cells so that they can repeat the process.

The cell cycle usually divided into 4 phases, G1, S, G2 and M. Where G1 and G2 are gaps in which cell prepares materials for the next coming phases. During S phase, cell duplicates their genetic materials and they will be separated into two daughter cells in M phase. In fact, S, G2 and M phases in budding yeast are regarded as only one phase called S/M phase in budding yeast because it's hard to distinguish them clearly. And budding yeast cell division is asymmetric by which the mother cell divides into a small "daughter" cell and a large "mother" cell.

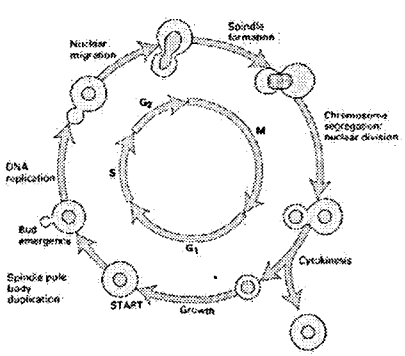

Figure 1 Budding yeast cell division is asymmetric.

Consider the small "daughter" cell in G1 phase (Fig. 1). The small cell grows up until meet the G1 checkpoint (Is the cell is big enough? Is DNA undamaged? If yes), the cell executes START. A bud emerges and keeps growing; the cell starts DNA synthesis; the spindle pole duplicates and mitosis commences. At the M checkpoint chromosome must be properly aligned on mitotic spindle and DNA synthesis is complete. If yes, the cell processes through anaphase, telophase and cell separation.

In the budding yeast, a single CDK, Cdc28, which is in conjunction with two families of cyclins: Cln1-3 and Clb1-6, control the major cell cycle events. Cln1/Cdc28 and Cln2/Cdc28 play major roles in budding and spindle pole body duplication. Cln3/Cdc28 seems to govern the size at which newborn cells execute START. Clb5/Cdc28 and Clb6/Cdc28 are essential for timely DNA replication. Clb3/Cdc28 and Clb4/Cdc28 seem to assist in DNA replication and spindle formation. Clb1/Cdc28 and Clb2/Cdc28 are necessary for proper completion of mitosis. Based on that, a protein-protein wire-diagram interaction network (Fig.2) was constructed and it was cast into a set of ordinary differential equations, 11 dynamic variables, with numbers of kinetic parameters (*Chen et al. MBC 2000, table 1 and 2*). This mathematical model is an intensive model that explains correctly wild type phenotype as well as many mutant phenotypes by doing simulation.

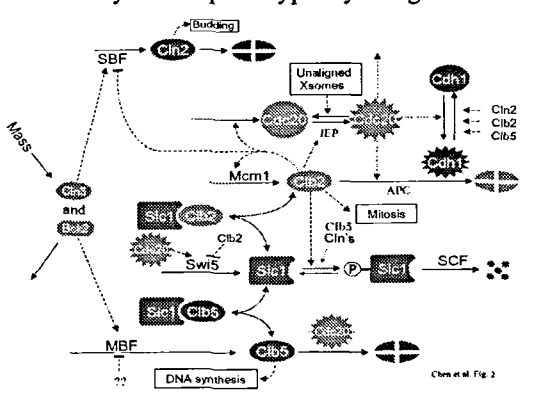

Figure 2 The protein-protein interaction network. *CDK Cdc28 is not present because it is in excess (assumption). This network can be read from left to right.*

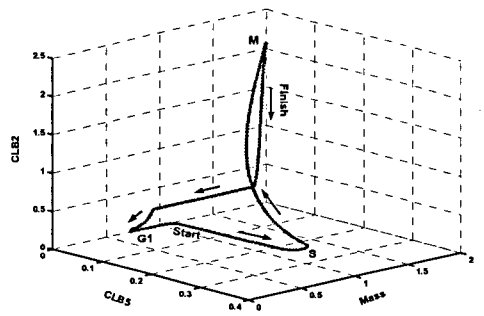

Figure 3: Phase trajectory of wild type, presents the concentrations of cyclins, CLB5 and CLB2, varying when the cell size increases with time. This can be done easily be solving system ODEs.

However, by doing simulation does not give out the underlying mechanism that controls cell cycle. Doing bifurcation analysis reveals not only the underlying mechanism but also the mechanism that controls the START and FINISH transitions. Further more, the bifurcation analysis turns out several interesting issues, is the temporal behavior transient of not? What is the abnormality at START and FINSH transitions, the dynamical organization of cell cycle?... Those issues are very like dynamic road map (fig. 4).

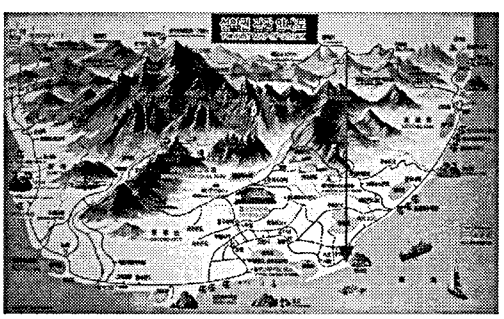

Figure 4: The dynamic road map. There are many "roads" that the system can moves along but it gets to the same destination.

2. THE CORE OF THE CELL CYCLE

There are two major and important events in budding yeast cell cycle, they are G1/S transition, START, by which the activities of cyclin dependent kinases (CDK) rise up abruptly causing budding, DNA synthesis and drive the cell into M phase, and exiting M phase, FINISH, by which the activities of a cyclin dependent kinase drop down dramatically causing separation of genetic materials (DNA) into two daughter cells then dividing a cell into two cells completely.

To generate those behaviors, CDK activities rising up abruptly and CDK activities dropping down dramatically, the best way is introduce a positive feedback loop for the former and a negative feedback loop for the later one.

Positive feedback loops

Positive feedback loops are fairly common in bacterial gene regulatory networks and have been reported in a number of eukaryotic signaling pathways (Ferrell and Machleder, 1998; Ferrel, 2002) as well as in protein-protein interaction networks (Chen 2000, Bela Novak and J.Tyson, 2001). So, why should evolution have selected for it? The answer is simple; positive feedback can be used to generate bi-stability. That is a device which can turn a graded signal into an all-or-nothing response. Bi-stability implies two states, usually a high state and a low state. At any one time, only one state can exist, although both states are accessible at the same parameter values. Both states are generally very stable and movement from one state to the other tends to be difficult.

The positive feedback shows bi-stability in the region 0.39<MASS<0.77 for a positive feedback loop MASS→CLB5→MBF→CLB5 (figure 5A, left panel). In which the system can be either low-fixed point or higher fixed point. The concentration of Clb5 is increasing continuously until the mass reaches to SN1, at this moment the concentration of Clb5 changes abruptly from low to higher level and it's hard to slip back if the MASS is slightly decrease, unless MASS decrease down to SN2. This can be easily seen from the null-cline plot, figure 5A middle panel. The mathematical model and its parameters can be found in table 1.

Oscillation in delayed-negative feedback loop

Delayed-negative feedback loops can be found in many applications and its role is to generate oscillating behavior. Recently, delayed negative feedback loop are found in transcription-gene networks, signaling pathway, metabolic ... Delayed term keeps the system oscillating with time as shown in the figure 5B, left panel shows a delayed negative feedback loop, middle panel shows time oscillating trajectories of system by solving ODE and 1-parameter bifurcation is plotted in the right panel. Firstly, concentration of Clb2 is rising up and turns on the intermediate enzyme IEP, which lately increases and again turns on Cdc20 which will decrease concentration of Clb2 by degrading. Once Clb2 decreases, IEP is off and consequently Cdc20 is off then Clb2 increase again to make another cycle, figure 5B-middle panel. The delayed term is very important to maintain oscillating. This is shown by 1-parameter (MASS) bifurcation in the figure 5B-right panel, it shows that there is no oscillation unless the MASS is big enough (>0.56, Hopf-bifurcation point), Clb2 is big enough, to turn on IEP to start oscillating. The mathematical model and its parameters can be found in table 1.

Jump 'n' run away

The cell cycle consists 2 periods, G1 and S/G2/M. During G1, the activities (concentration) of cyclins are low meanwhile during the S/G2/M phase the activities of cyclins are firstly increasing then decreasing to get cell division. This behavior look like jump 'n' run away of cyclin's activities, it jumps from low state to a higher one and goes up and down until division.

By combining both positive feedback loop and delayed-negative feedback loop, the "jump 'n' run away" behavior can be shown, figure 5C. The middle panel shows the 1-parameter bifurcation diagram of that system. The activities of cyclins, Clb2, is gradually increasing from lower state (G1) when the MASS is increasing up to 3.29, abruptly Clb2 activities jump into limit cycle and "run away" (S/G2/M). On the figure 5C right panel, we are presenting 2-parameters bifurcation diagram of the system by taking MASS and one of feedback loop strength, positive and negative one. Firstly, fixing k_{a_IEP}, the strength of negative feedback, we were varying MASS and k_{a_MCM}, the positive feedback strength, to get bifurcation diagram (blue-solid line), it shows a bi-stability region of $0.49<k_{a\_MCM}$. As k_{a_MCM} is smaller and smaller, the MASS is larger and larger to have bi-stability. At k_{a_MCM}~0.49, the saddle node 1, SN1, and the second one, SN2 joint together and disappear, it means that the positive feedback strength is not strong enough to turn on the feedback. Similarly, fixing k_{a_MCM}, we were varying MASS and k_{a_IEP} to get the 2-parameters bifurcation diagram (red-dashed line). It turns out that what ever the negative feedback strength is, we do have oscillating behavior with the note that, at the point of ~0.5, the first hopf-bifurcation point joints with the second saddle-node, SN2. The mathematical model and its parameters can be found in table 1.

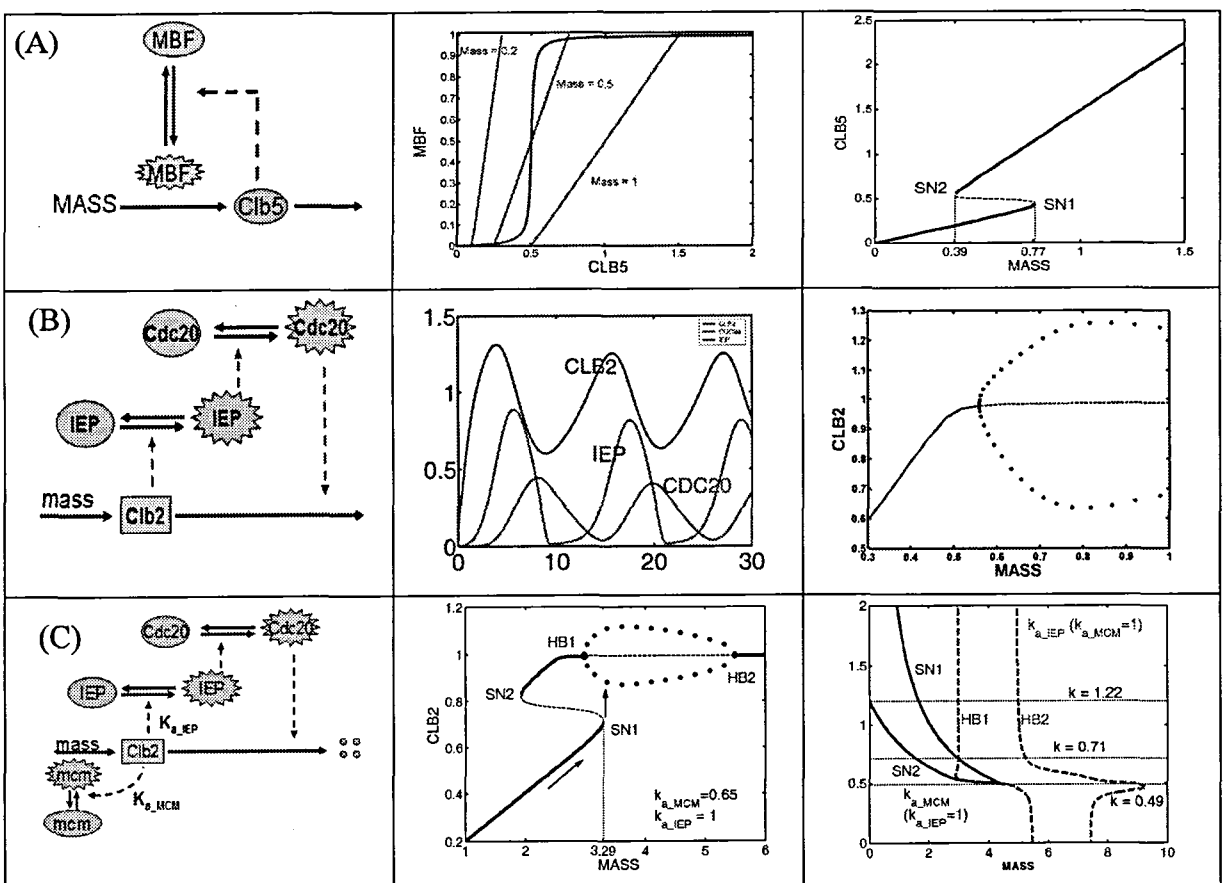

Figure 5. Wire diagrams and bifurcation analysis of feedback loops. In this tableau, the rows correspond to (A) positive feedback loop, (B) delayed-negative feedback loop and (C) Jump 'n' run away. The columns present wiring diagrams (left); null-cline (A), time trajectory (B) and 1-parameter bifurcation (C) (middle); and 1-parameter bifurcation (A,B) and 2-parameters bifurcation (C, D, F) (right).

3. BIFURCATION ANALYSIS

A primary goal of dynamical systems theory is to characterize the kinds of solutions one can expect to find for a system of nonlinear differential equations. We are primarily interested in "recurrent" solutions: both steady states (where variables are unchanging in time) and oscillatory states (where variables repeat themselves periodically in time). Recurrent solutions can be either stable or unstable. Stable steady states correspond to conditions of cell cycle arrest, e.g., G1 state. Stable oscillatory solutions correspond to unmonitored cell divisions, e.g., S/M state.

A bifurcation is a qualitative change in the behavior of solutions of a dynamics system as one or more parameters are varied. The parameter values at which these changes occur are called *bifurcation points*. If the qualitative change occurs in a neighborhood of a fixed point or periodic solution, it is called a *local bifurcation*. Another way to think of a bifurcation is the following: As one or more control parameters are varied, a fixed point may become non-hyperbolic for a certain parameter value. If the state space portraits are qualitatively different before and after this location then this point is called a bifurcation point and the qualitative change is called a bifurcation. And it is also possible to define

a concept of structural stability (robustness) of the system itself. A system is said to be structurally stable is for any small change in parameter space, the qualitative features of the new system are equivalent to the initial system. The XPPAUTO is a very powerful tool to find out bifurcation diagram, which we are using.

By taking the mass of cell as a controlling parameter, mass is varying, we found bifurcation diagram for the wild type budding yeast cell cycle, see fig. 6. Base on that, the budding yeast cell cycle is characterized by two kinds of solutions, steady state (solid blue line, MASS<1) and oscillatory state (green cycle, M>1). The lower steady state corresponds to G1 state, where the activities of Clbs cyclin are not fluctuating in time. The oscillatory state corresponds S/M state, where the increasing of Clb5 activity executes START, the increasing of Clb2 activity causes spindle formation (M phase) and the decreasing of Clb2 activity drives the cell division (FINISH).

Table 1. Mathematical models of feedback loops

Figure 5A. Positive feedback loop

$$\frac{dClb5}{dt} = (k_s' + k_s''MBF)\cdot mass - k_d Clb5$$

$$\frac{dMBF}{dt} = \frac{k_a CLB5(1-MBF)}{J_a + 1 - MBF} - \frac{k_i MBF}{J_i + MBF}$$

Parameters (min^{-1}): k'_s=0.1, k''_s=0.2, k_d=0.2, k_a=0.8, k_i=0.5

Dimensionless parameters: $J_a = J_i = 0.01$

Figure 5B. Delayed-negative feedback loop

$$\frac{dClb2}{dt} = k_{sb2}MASS - (k_{db2} + k_{dc20b2}Cdc20)Clb2$$

$$\frac{dCdc20}{dt} = \frac{(k_{ac20}IEP)(1-Cdc20)}{J_{ac20} + 1 - Cdc20} - \frac{k_{ic20}Cdc20}{J_{ic20} + Cdc20}$$

$$\frac{dIEP}{dt} = \frac{k_{aiep}Clb2(1-IEP)}{J_{aiep} + 1 - IEP} - \frac{k_{iiep}IEP}{J_{iiep} + IEP}$$

Parameters (min^{-1}): k_{sb2}=1, k_{db2}=0.5, k_{dc20b2}=2, k'_{ac20}=0.01, k''_{ac20}=0.25, k_{ic20}=0.1, k_{aiep}=1,k_{iiep}=1

Dimensionless parameters: J_{ac20}= J_{ic20}= J_{aiep}= J_{iiep} =0.01

Figure 5C. Jump 'n' run away

$$\frac{dClb2}{dt} = k'_{sb2}MASS + k''_{sb2} * MCM - (k_{db2} + k_{dc20b2}Cdc20)Clb2$$

$$\frac{dCdc20}{dt} = \frac{(k_{ac20}IEP)(1-Cdc20)}{J_{ac20} + 1 - Cdc20} - \frac{k_{ic20}Cdc20}{J_{ic20} + Cdc20}$$

$$\frac{dIEP}{dt} = \frac{k_{aiep}Clb2(1-IEP)}{J_{aiep} + 1 - IEP} - \frac{k_{iiep}IEP}{J_{iiep} + IEP}$$

$$\frac{dMCM}{dt} = \frac{k_{amcm}Clb2(1-MCM)}{J_{amcm} + 1 - MCM} - \frac{k_{imcm}MCM}{J_{imcm} + MCM}$$

Parameters (min^{-1}): k_a=1, k_i=0.5, k_{sb2}=0.4, k''_{sb2}=1, k_{db2}=2, k_{dc20b2}=2, k_{ac20}=0.25, k_{ic20}=0.1, k_{aiep}=1, k_{iiep}=1

Dimensionless parameters: J_{aiep}=J_{iiep} =J_{ac20}=J_{ic20} =0.005, J_{amcm}= J_{imcm} =0.01

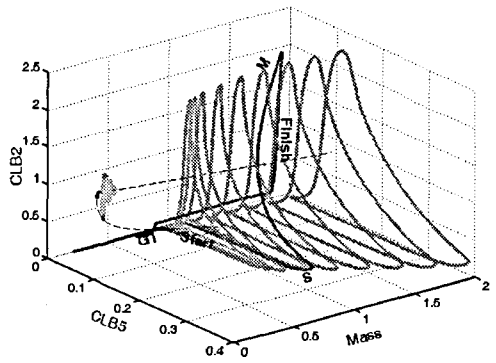

Figure 6: The underlying mechanism of budding yeast cell cycle. The cell cycle is characterized by two kinds of solutions, steady state-solid blue line, MASS<1 (G1) and oscillatory state-green cycle, M>1 (S/M). Clbs activities are low in G1 phase, as cell growing (mass increasing); cell cycle changes its state from steady one to oscillatory one, jump 'n' run away. At first, Clb5 activity raises up (START), following is rising up of Clb2 activity (M phase) and then Clb2 activity drops under a certain threshold causing cell division. As soon after division, cell get to G1 state and make another cycle. Solid-red line is cyclins time-trajectory computed by doing simulation.

By analyzing bifurcation diagram, we do not only understand the underlying mechanism of cell cycle but also we can recover how the START and FINISH transitions are trigged as discussed below.

START transition

How the cell gets into an oscillatory state from a stable steady state (fixed point)? The answer is that the oscillations bifurcate from a "homo-clinic connection". See the bifurcation diagram and phase plane in Fig. 7.

Projecting the whole bifurcation diagram on 2 dimension space of MASS and CLB2, and zoon in at the moment of START, see fig 7. right panel, it shows that be cause of stability disappearance after colliding of an stable fixed point (lower solid line) and a unstable fixed point (a little bit higher dashed line) at the first saddle node bifurcation point, SN1, the cell cycle **jumps** from a lower state (low activities of CLB2) up to a small limit cycle (emerging from HB1, filled-cycle) then **"run"** along the small limit cycle to big limit cycle **away**.

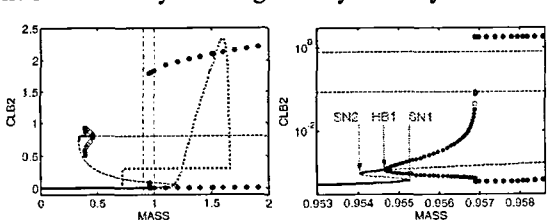

Figure 7: Oscillation bifurcates from a homo-clinic connection. The left panel is bifurcation diagram of cell cycle. The right one is zoomed part of the left one. Showing "jump and run away" behavior.

So as mass m increasing, the control system changes from the stable fixed point to a small limit cycle via a saddle-node (SN), after that, it gets into a big limit cycle via a homo-clinic connection.

Abnormality at FINISH transition

The best way to test whether the math model of the cell cycle is properly constructed is taking into account for all available mutations of the cell. Chen was successful to explain about 50 mutations through her math model, one of them is deleting the gene which directly involve into the negative feedback loop at FINISH transition. As it is predicted, once the negative feedback loop is broken down, there is no oscillating behavior therefore the cell can not get to the division. This is confirmed through out the IEP mutant bifurcation diagram, see figure 9C. The cell start from lower stable state (lowest solid line) then jump up to higher state (top solid line) and never turn back because negative feedback was broken.

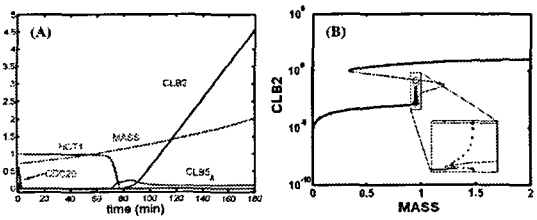

Figure 9: IEP mutant, cell is dead. (A) IEP mutation cyclins trajectories, simulated from the cell cycle math model. (C) 1-parameter bifurcation diagram of IEP mutation, cause of no big oscillation, cyclins activities can not get back to lower state, cell is dead.

4. DISCUSSION

The whole cell cycle model is well constructed modularly. To well understand the whole system, it'd better to take into account for each modules rather than whole one at a time. We were successful to explain the whole cell cycle model by doing bifurcation analysis of positive, delayed-negative and cascade of both feedback loops. By that the whole cell cycle is characterized by two states, a steady state, in which the activities of Clbs proteins are very low and unchanged, and a stable oscillatory state, in which the activities of Clbs proteins are varying, increasing of Clb5 activity causes DNA replication, increasing of Clb2 activity causes the formation of spindle (M phase) and Clb2 activity decreasing causes the cell division, fig.5. START and FINISH transitions was also investigated. It turns out that, the underlying mechanism is changing of properties of solutions, from a unstable fixed point to a stable fixed point (CLN3 suppression, IEP mutation), from an unstable fixed point to a stable oscillatory state (CLN3 over-expression).

By doing bifurcation analysis, the underlying mechanism of controlling of cell cycle, switch-like mechanism between two phases G1 and S/M, the mechanism of START and FINISH transition, changing dynamical properties of solutions, were discovered.

The bifurcation analysis is a useful mathematical tool that helps us understand more clearly about not only the cell cycle but also the dynamic systems described by ODEs. Especially, it's useful for biologists, who carry out experiments.

Acknowledgements

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