Bifurcation analysis of budding yeast cell cycle

Cuong Nguyen[1], Chang No Yoon[1], Hak Yong Kim[2], Seung Kee Han[1*]

[1] Department of physics, Chungbuk National University, Cheongju, Korea
[2] Department of biochemistry, Chungbuk National University, Cheongju, Korea

[*] To whom correspondence should be addressed. Email: skhan@chungbuk.ac.kr

Abstract

Bifurcation analysis of cell cycle regulation in the budding yeast is performed basedon the mathematical model by Chen et al [*Molecular biology of cell, 11:369-391, 2000*]. On the bifurcation diagram, locations of both stable and unstable solutions of the nonlinear differential equations are presented by taking the mass of cell as a controlparameter. Based on the bifurcation diagram, dynamic mechanism underlying the '**start**' transition, initiation of a new round of cell cycle, and the **finish**' transition, completion of cell cycle and returning back to the initial state, is discussed: the**start**' transition is a transition from a stable fixed solution for a small mass and to an oscillatory state for a large mass, and the '**finish**' transition is a switching back to the stable fixed solution from the oscillatory state. To understand the role of the genes during the cell cycle regulation, bifurcation diagrams for the mutants are compared with that of the wild type.

Introduction

The cell cycle

The cell cycle is the sequence of events by which a growing cell duplicates all its components and then divides this material between two daughter cells so that they can repeat the process. Usually, a cell cycle is divided into four phases, G1 (gap), S (synthesis), G2, and M (mitosis) phase. The development and reproduction of all living organisms is based on this fundamental ability of a cell to replicate itself. Although there are many unique features of cell proliferation in different organisms, the basic events of the eukaryotic division cycle are stereotypical: first the DNA molecule within each chromosome is faithfully replicated during S phase, and then one copy of each DNA molecule is segregated to each sister cell during M phase. S and M phases alternate in time, in response to signals from a network of enzymatic reactions that is highly conserved acros all eukaryotic lineages, from fungi and plants to insects and mammals. In this paper we shall concentrate on cell cycle regulation in budding yeast, where the control system is simple.

The budding yeast cell cycle

In the case of budding yeast (Saccharomyes cerevisie), S and M phases overlap. We regard these phases as one only, called S/M phase. Budding yeast cell division is asymmetric by which the mother cell divides into a small "daughter" cell and a large "mother" cell.

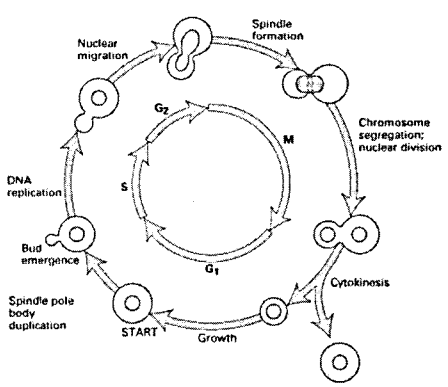

Figure 1 Budding yeast cell division is asymmetric.

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.

The mathematical model of budding yeast cell cycle

The simple mathematical model was first built up by J.J. Tyson [2] from several components based on the idea that the cell cycle is an alternation between two self-maintaining states (G1 and S-G2-M) [*K. Nasmyth "At the Heart of the budding Yeast Cell Cycle" -1996*]. By that model, the cell cycle was characterized into two states, G1 corresponds to low activity of Clb-dependent kinases and S-G2-M corresponds to high activity of Clb-dependent kinases.

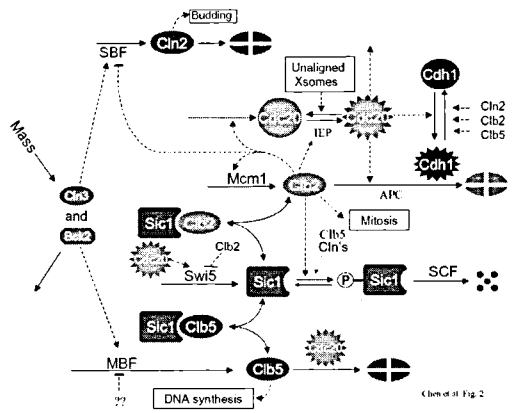

Figure 2 The protein-protein interaction network. *CDK Cdc28 is not present because it is in excess (assumption). This network can be read from left to right.*

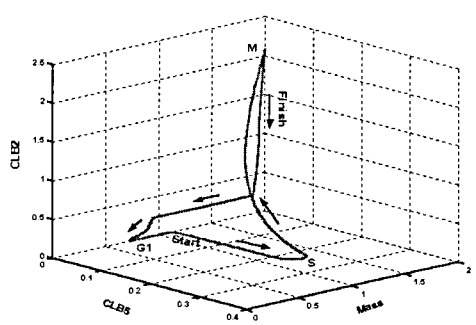

Figure 3: Phase trajectory of wild type

In the Saccharomyces cerevisie, 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*).

When the cell grows to a sufficiently large size, Cln3/Cdc28 and Bck2 activate SBF and MBF by phosphorylation, causing Cln2 and Clb5 to begin accumulation. At first, Clb5 accumulates in inactive trimmers, Clb5/Cdc28/Sic1, but Cln2/Cdc28 is not so inhibited. Rising Cln2/Cdc28 activity plays three important roles. *Firstly*, it initiates bud formation. *Secondly*, it phosphorylates Sic1, priming the inhibitor for ubiquitination by SCF and ultimate degradation by the proteasome. *Thirdly*, it inactivates Cdh1 (Hct1), which, in conjunction with APC, was responsible for Clb2 degradation in G1 phase.

Since Sic1 is destroyed, Clb5/Cdc28 activity rises abruptly and drives the cell into S phase. These are the major physiological events associated with the Start transition. With Sic1 gone and Cdh1 (Hct1) inactivated, Clb2-dependent kinase can begin to rise, with some lag, because Clb2/Cdc28 activates its own transcription factor, Mcm1. In addition, Clb2/Cdc28 inactivates SBF, so Cln2-dependent kinase activity begins to fall as Cln2 synthesis shuts off. At about the same time, MBF is inactivated, and the Clb5 level starts to fall. Rising Clb2/Cdc28 activity induces progress through mitosis. The metaphase–anaphase transition is regulated by a pair of proteins, Cdc20 and Hct1 that target substrates to the APC for ubiquitination. At metaphase, they are inactive, but when DNA is fully replicated and all chromosomes are aligned on the metaphase plate, Cdc20 is activated. Indirectly Cdc20 promotes: Dissociation of sister chromatids (anaphase A), activation of Hct1, which conducts Clb2 to the APC, thereby initiating anaphase B and cell separation, and activation of Swi5, the transcription factor for Sic1. With all CDK activity gone (except for a little associated with Cln3), Sic1 can make a comeback, and the cell returns to G1. This model is an intensive model that explains correctly wild type phenotype as well as many mutant phenotypes by doing simulation.

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).

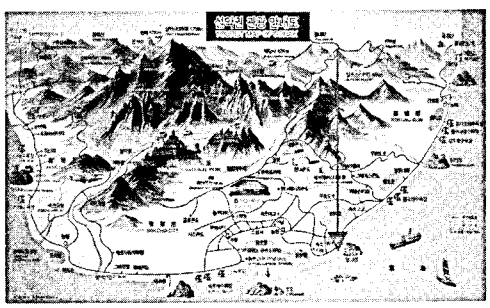

Figure 4: The dynamic road map. There are many "roads" that the system can moves along but it gets to the same destination.

As a person moves from mountain top down to the beach, his temporal trajectory is very like simulation result of the mathematical model of cell cycle. Depending on the starting point on the mountain, the initial transient behaviors are different but the final destination is very similar. The trajectories of a person could be best understood with the help of the road map as shown in fig.4. The bifurcation analysis is very like locating the dynamical road map by taking the mass m as a control parameter.

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 point.* 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.

1. Wild type

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. Base on that, the budding yeast cell cycle is characterized by two kinds of solutions, steady state and oscillatory state.

- 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).

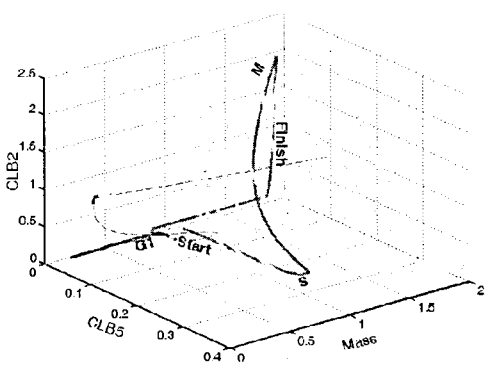

Figure 5: The underlying mechanism of budding yeast cell cycle. The cell cycle is characterized by two kinds of solutions, steady state (G1) and oscillatory state (S/M). Clbs activities are low in G1 phase, as cell growing

(mass increasing); cell cycle changes its state from steady one to oscillatory one. 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.

Very small cells have only one stable state of the cell cycle control system, namely G1. They must grow to a sufficiently large size before they can leave G1 phase and enter S/M phase, an oscillatory state, in which, Clbs activities increasing causes budding, DNA replication, spindle formation... and cell division. At cell division, the bifurcation parameter, mass m, drops by a factor f [Chen et al] and the control system goes back the only one stable state, G1.

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 is trigged and effected by gene mutations as the discussion following.

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 Fig. 6.

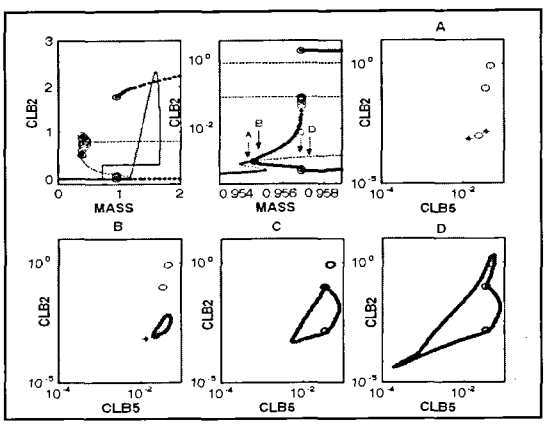

Figure 6: Wild type START transition analysis. Oscillation bifurcates from a homo-clinic connection.

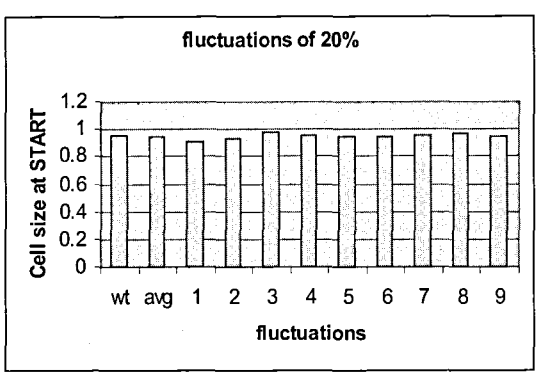

Figure 7: Comparison between wild-type and fluctuation. Average difference: 1.02%. Mean square deviation: 0.003

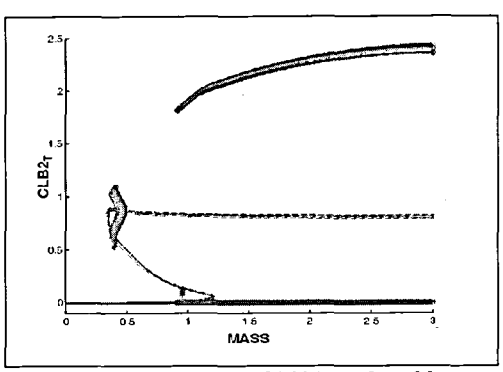

Figure 8: Fluctuation of 20%. Red: wild-type; blue: fluctuations.

At point A, there are two stable fixed points and three unstable fixed points and the control system stays on lower stable fixed point, fig.6. As mass increasing, the upper stable fixed point becomes unstable and a small limit cycle (oscillatory state) appears via hopf-bifurcation (HB), fig.6B. At soon later, the under stable fixed point collides with an unstable fixed point and both of them disappear, a saddle-node (SN). At this moment, system control jumps on the only stable oscillatory state, small limit cycle, which grows up as mass increasing and collides with another unstable fixed point and disappears (fig.6C). Simultaneously, another big limit cycle

appears homo-clinic connection, where the control system stays on (fig.6D).

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.

Assay of model robustness

To determine the model robustness, the model was modified by the following way. At the same time, all of parameters of the model were decreased or increased with a certain random fluctuation, say 20%. By getting bifurcation diagrams all fluctuations of 20% randomly modified model and comparing the bifurcation points at START, it shows that the overall structure of bifurcation diagram does not change as summarized in fig.7 and fig.8.

2. Abnormality in START transition

To analyze the START transition, we mainly investigated the mutation of CLN3 gene, by suppressing and over-expressing its activity. Mutation is a change of properties of some gene (inhibition, promotion...) by accident causing strange and different cell's behavior from its wild type.

Cln3 is a cyclin which in combination with Cdc28 activates the transcription factor of Cln2, SBF, for budding and degrades the Clbs inhibitor SIC1, therefore releases Clb5 cyclin for DNA replication. CLN3 plays a major role in START execution.

By suppressing or over-expressing CLN3 activities, the cell cycle changes correspondingly, in case of CLN3 suppression, START is delayed causing elongation of G1 phase. Mean while, CLN3 over-expression cause G1 disappearance.

By doing similarly to the case of wild-type, the synthesis rate of Cln3 was taken as a control parameter, the bifurcation diagrams of CLN3 over-expression (fig. 10) and suppression (fig. 11) were found and they are consistent with experimental results (Chen et al).

Why do we get these phenomena? Well, to recover, we fixed the mass m at the START transition and took the parameter that is synthesis rate of CLN3, D_{n3}, as a second control parameter to find bifurcation diagram (fig. 12A). Since the synthesis rate, D_{n3}, is small, (suppression), the cell cycle stays on a stable fixed point (G1 phase), meanwhile it is on an oscillatory state (S/M phase) in case of D_{n3} is large (Over-expression). It shows that, an unstable fixed point (START transition, fig. 12B) in case of wild-type becomes a stable fixed point (G1 phase, fig. 12C) when D_{n3} is small and it becomes a big oscillatory state in case of CLN3 over-expression. (S phase, fig. 12D).

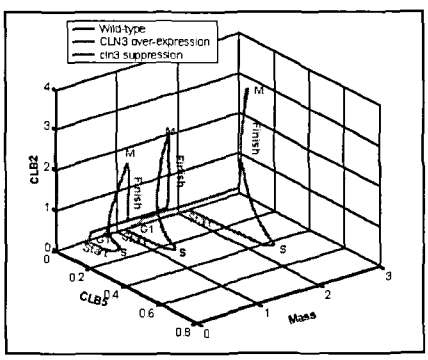

Figure 9: Phase trajectory. (Green) CLN3 over-expression, no G1 phase. (Red) wild type. (Dark-Green) CLN3 suppression, G1 is elongated.

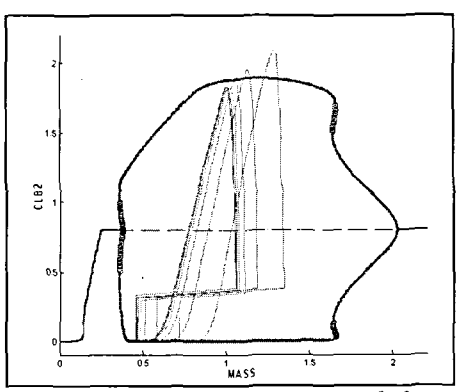

Figure 10: CLN3 over-expression bifurcation diagram, no G1 phase.

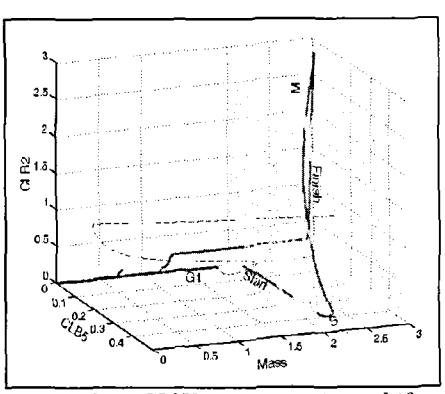

Figure 11: CLN3 suppression bifurcation diagram, G1 is elongated as twice as its wild-type.

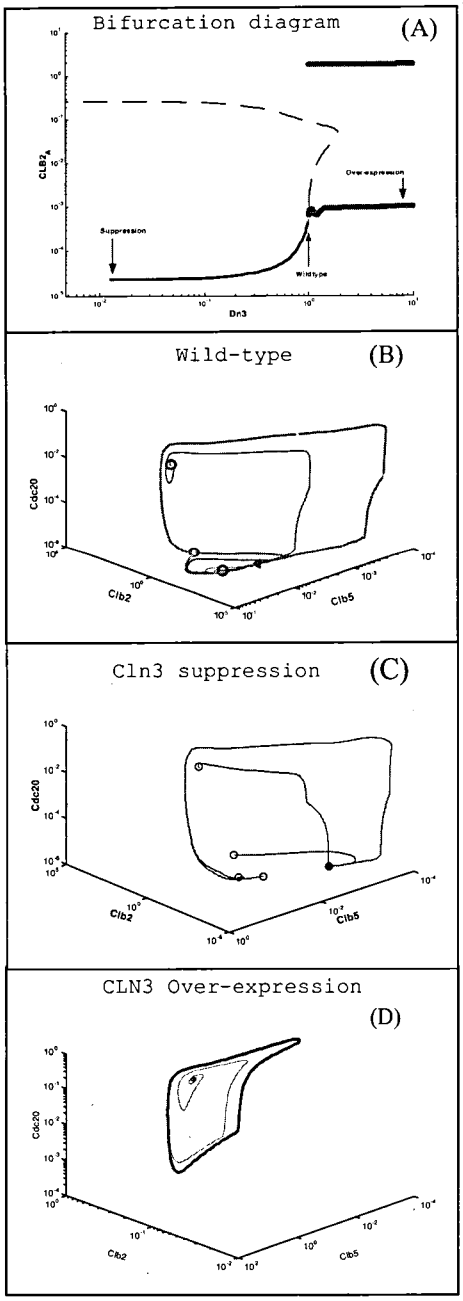

Figure 12: START transition analysis. (A) Bifurcation diagram of CLN3 mutation. START transition of wild type (B), CLN3 suppression (C) and CLN3 over-expression (D).

3. Abnormality in FINISH transition

The FINISH of cell cycle is activated by the raise up of CDC20 activity, which is controlled by an enzyme, IEP (Fig 2). To test this idea, we fixed the mass M at FINISH, took synthesis rate of enzyme IEP as a control parameter and sough for bifurcation diagram. It shows that the big oscillatory state becomes a stable fixed point (fig 13-C, D) if the IEP synthesis rate is small (IEP mutation) (fig. 13-B), therefore FINISH does not occur.

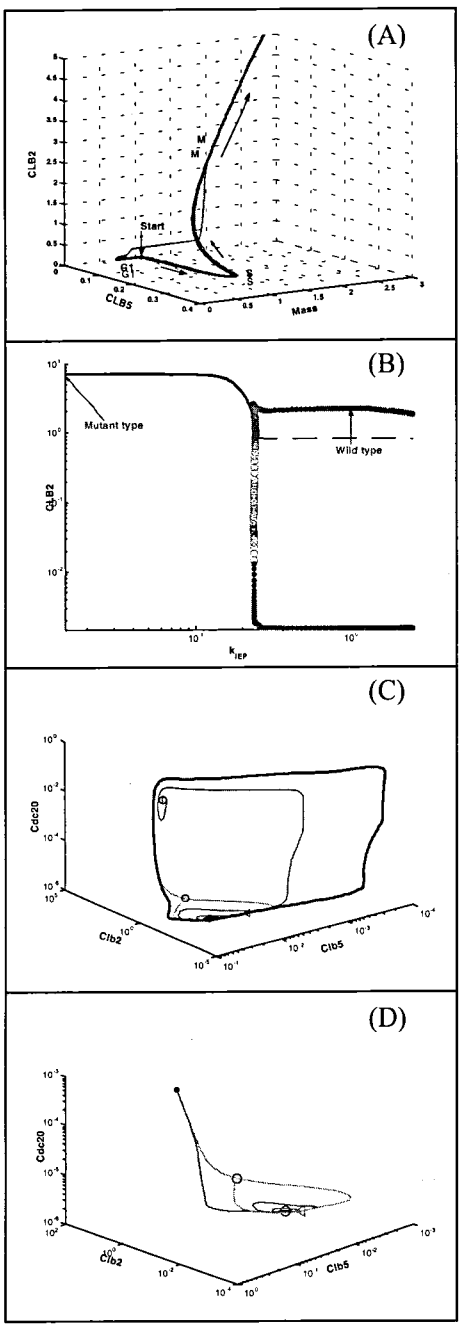

Figure 13: FINISH transition analysis. (A) Phase trajectory of wild-type (red) and IEP mutation (Blue). (B) Bifurcation diagram IEP mutation. FINISH transition of wild-type (C) and IEP mutation (D)

Discussion

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 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).

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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