Network Dynamics of Budding Yeast

Cell Cycle

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Declaration

I hereby declare that this thesis represents my own work which has been done in the past one year for the fulfillment for the degree of Bachelor of Science (Honors) in Physics major (computer science concentration) at Hong Kong Baptist University, and has not been previously included in a thesis, dissertation submitted to this or other institution for a degree, diploma or other qualification.

Signature: ____________________
Date: April 22, 2005
I would like to express my sincere gratitude towards my research project supervisor, Dr Tang Lei-han for sharing his precious time to supervise and patronize my project.

I also would like to thank Prof. S.Y Zhu as well as all the other team members for their valuable suggestion and comments.

I would thank all my friends, especially my girl friend for her continuous encouragement.

Finally, I would like to thank my family for their many years’ caring and support.
ABSTRACT

The cell cycle of budding yeast is the succession of events that lead to reproduction of daughter cells and are tightly regulated. In this project, we study a much simplified model of cell cycle progression by Tang Chao and corroborate dynamic properties of the model, such as stability and robustness, with time course gene expression data. The regulation of yeast cell cycle clock is mainly based on the transcriptional regulation of cell cycle genes which are controlled by nine known transcription factors. Expression data are used to analyze the profiles of cell cycle genes. Fourier transform method is applied to sort genes according to their expression peak time. Furthermore, by combining expression time course data with Gerstein’s regulatory network, we find three TF_orf clusters that function in different cell cycle stages.

Keywords: cell cycle, gene expression, regulation, transcription factor, regulatory network
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Chapter 1

Introduction

1.1 Yeast Biology

Budding Yeast, *Saccharomyces cerevisiae*, has been studied experimentally as a model organism of biology since the 1930’s. Its complete genomic sequence was published in 1996, the first among eukaryotic organisms. The genome of *Saccharomyces cerevisiae* is divided up into 16 chromosomes, ranging from 220 kb to 2200 kb, with a total genome size of approximately 12,000kb. 6,183 open reading frames (ORFs) on the genome have been identified, most of which are believed to encode specific proteins.

*Saccharomyces cerevisiae* is a unicellular organism which, unlike more complex eukaryotes, can grow on defined media, giving the investigator complete control of environmental parameters. Moreover, since there are substantial cellular functions which are highly conserved from yeast to mammals, sequence information obtained in the yeast genome project is extremely useful as a reference against the sequences of human, animal or plant genes. The unique properties of the yeast *Saccharomyces cerevisiae*, among some 700 yeast species and its enormous hidden potential which has been exploited for many thousands of years, made it a preferred organism for research.
1.2 Introduction to Yeast Cell Cycle

A yeast cell receives a wide variety of cellular and environmental signals, which are often processed to generate specific genetic response. Here, we explore the molecular and genetic machinery of yeast cell cycle control which forms a highly independent system and is known in great detail.

The cell cycle is the succession of events whereby a cell grows and divides into two daughter cells that each contains the information and machinery necessary to repeat the process. The basic function of the yeast cell cycle is like other eukaryotic cells which is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the two copies precisely into mother cell and daughter cell. These processes define two major phases of the yeast cell cycle – S phase and M phase. Bud emergence and DNA duplication occur during S phase (S for synthesis). After S phase, DNA is segregated into mother cell and daughter cell (mitosis). When DNA has been partitioned, the cell undergoes cell division (cytokinesis), separating mother cell from the daughter cell. These two events occur in M phase. Besides these two major events, the cell requires much time to grow and double their mass of proteins and organelles. After S phase, replicated DNA is checked for its genetic integrity to ensure there is no damage during replication, otherwise the cell cycle is halted for DNA reparation. Hence, extra gap phases are inserted into the cell cycle – G2 between S phase and M phase; while G1 between M phase and next S phase. Thus, the cell cycle is divided into four sequential phases: G1, S, G2, M.

Basic biology on yeast and its cell cycle are covered in most textbooks: Biochemistry (Mathews et al 2000), Molecular Biology of the cell cycle (Alberts et al 2002) and Gene VIII (Lewin 2003). The online databases contain publications and comprehensive information on yeast: SGD (http://www.yeastgenome.org/), SMD
and KEGG (http://www.genome.jp/kegg/).

1.3 Objectives

Yeast cell cycle is tightly regulated for proper functioning at proper time. The cell cycle control system guarantees the stability and robustness when cell goes through cell cycle progression. In the present project, Prof. Tang Chao’s dynamic model is employed and further implemented to demonstrate the global dynamic properties and stabilities of cell cycle network. Our aim is to understand how the command system, which is basically a controlled program of gene expression, is designed to regulate the cell cycle. Beyond this much simplified model that focuses on the cell-cycle progression, we wish to dig deeper into the genetic construction of regulatory circuit and the ensuing dynamics. For this purpose, we examine the expression pattern of the 792 known cell cycle genes from time course measurement. By clustering into phase-synchronized groups, we hope to decipher details of their regulatory program, including on-off switch by single or multiple TFs, and the associated dynamic process of TF binding or activation.
Chapter 2

Yeast Cell Cycle Control

2.1 Yeast Cell Cycle is Tightly Regulated

For many years, cell biologist watched the puppet show of DNA synthesis, mitosis and cytokinesis but had no idea of what lay behind the curtain controlling these events. The cell cycle control system was simply a black box inside the cell.

The cell cycle control system possesses the following features.

i. A clock, or timer, that turns on each event at a specific time, and provide a relatively fixed amount of time for the completion of each event;

ii. A mechanism for initiating events in correct order; for example, entry into mitosis must always come after DNA replication

iii. A mechanism to ensure that each event is triggered only once per cycle; for example, the DNA cannot be replicated twice during a single cell cycle

iv. Binary (on/off) switch that trigger events in a complete, irreversible fashion; It would be a disaster, if events like nuclear envelope breakdown were initiated but not completed.

v. Robustness, backup mechanism to ensure that the cycle can process smoothly even when parts of the systems malfunction

vi. Adaptability. So that the system’s behavior can be modified to suit specific environmental conditions.
What makes up the control system to regulate the cell cycle clock? A lot of surveys and studies have been done by biologists, showing that the control system is mainly based on a family of protein kinase knows as CDK (cyclin-dependent kinase), and gene regulation. In budding yeast cell cycle, there is only one CDK – Cdc28 (C Wittenberg, 2005; Mart Loog and David O. Morgan, 2005; Alberghina et al, 2004).

Much is known about Cdc28 activities and its functions (). Cdc28 associates successively with different cyclins which is also proteins (Cln1,2,3,4; Clb1,2,5,6) to trigger the different events of the cycle, and its activity is usually terminated by cyclin degradation or inhibitory phophorylation (Nash et al., 1988). The activity of the Cdc28 rises and falls as cell progress through the cycle and the oscillations lead directly to cyclical change activation of certain proteins that initiate the major events of the cell cycle, for example an increase in Clb2/Cdc28 activity at the beginning of mitosis leads to increased activation of proteins that control chromosome condensation, nuclear envelope breakdown and spindle assembly. Others like Cln2/Cdc28 is responsible for DNA replication and Clb5/Cdc28 is responsible for bud emergence (Tyers et al., 1993; Schwob and Nasmyth, 1993).

The cell cycle control obviously depends on protein-protein interactions, which is also referred to as post-transcriptional mechanism. However, transcriptional regulation provides another level of control which is more fundamental. The genes peak in different phase during cell cycle are responsible for synthesis of cell cycle specific proteins. Some cyclin levels, for example, are controlled through cyclin gene transcription, since the genes mainly code for proteins.
2.2 Yeast Cell Cycle Dynamic Model

How do physicists study cell cycle regulatory process? As they first studied the hydrogen atom before coming into the more complex atoms, physicists first focused on the yeast cell cycle with the most simplified network. Prof. Tang Chao developed a simple dynamic model with just a few nodes to investigate the global dynamic properties and stabilities of cell cycle network (Li 2004). Fig 2.1 shows the proteins and their interactions (in the sense of information flow) in Chao's network.

![Fig 2.1: Chao's network](image)

This regulatory network includes cell size check point and 3 classes of proteins: cyclin, which bind to the kinase Cdc28; the inhibitors, degraders, and competitors of the cyclin/Cdc28 complexes (Sic1, Cdh1, Cdc20, Cdc14) and transcription factors (SBF, MBF, Mcm1/SFF, Swi5). Green arrows represent positive regulations. For example, when the cell grows large enough, the Cln3/Cdc28 will be activated, which in turn activates a pair of transcription factor groups, SBF and MBF to activate the genes of the cyclins Cln1,2 and Clb5,6, respectively. Red arrows represent negative regulaton (inhibition, repression, or degradation). For example, the protein Sic1 can bind to the Clb/Cdc28 complex to inhibit its function, Clb1,2 phosphorylate Swi5 to prevent its entry into the nucleus, whereas Cdh1 targets Clb1,2 for degradation.
Yellow loops are added to represent ‘self-degradation’ to those nodes that are not negatively regulated by others. The degradation is modeled as a time-delayed interaction: if a protein with a self yellow arrow is active at time $t$ ($S_i(t) = 1$) and if its total input (from $t$ to $t+1$) is zero, it will be degraded at time $t+1$, i.e., ($S_i=0$). Since much of the biology seems to be reflected in the on–off characteristics of the network components, the nodes and arrows can be treated as logic-like operations in this simplified dynamic network. Hence, each node $i$ has only two states, $S_i = 1$ and $S_i = 0$, representing the active and the inactive state of the protein, respectively, with totally 11 nodes in the network. The protein states in the next time step are determined by the protein states in the present time step via the following rule:

$$S_i(t+1) = \begin{cases} 1, & \sum_j a_{ij} S_j(t) > 0 \\ 0, & \sum_j a_{ij} S_j(t) < 0 \\ S_i(t), & \sum_j a_{ij} S_j(t) = 0 \end{cases}$$

where $a_{ij} = 1$ for a green arrow from protein $j$ to protein $i$ and $a_{ij} = 1$ for a red arrow from $j$ to $i$. 
Fixed Points

We implemented the dynamic model to study the time evolution of the protein states. Following Tang Chao’s work, we start from each of the 2,048 initial states in the 11-node network. We find that all of the initial states eventually flow into one of the seven stationary states (fixed points) shown in Table 2.1. There is one big fixed point attracting 1,764 states among the seven fixed points. This super stable state is the biological G1 stationary state. The advantage for a cell’s stationary state to be a big attractor of the network is obvious: the stability of the cell state is guaranteed since under normal conditions the cell will be sitting at this fixed point, waiting for the signal for another round of division.

<table>
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<th>Cln3</th>
<th>MBF</th>
<th>SBF</th>
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<th>Cdh1</th>
<th>Swi5</th>
<th>Cdc20</th>
<th>Clb5,6</th>
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Table 2.1: Fixed points of cell-cycle network

Biological Pathway

Next, we start the cell-cycle process with the cell size signal, and observe that the system starts from G1 and goes back to the G1 stationary state. The temporal evolution of the protein states indeed follows the cell-cycle sequence, shown in Table 2.2. This is the biological trajectory or pathway of the cell-cycle network which represents the cell cycle progression.
<table>
<thead>
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Table 2.2: Time evolution of cell-cycle pathway

Newborn daughter cells grow to a critical size to have enough Cln3 to activate the transcription factors, MBF and SBF, which transcriptional activate two classes of cyclins, Cln1,2 and Clb5,6. Cln2 is primarily responsible for bud emergence and Clb5 for initiating DNA synthesis. Clb5-dependent kinase activity is not immediately evident because the G1-phase cell is full of cyclin-dependent kinase inhibitors (Sic1). After the Sic1 is phosphorylated by Cln2/Cdc28 for degradation, Clb5/Cdc28 is released to do its job.

Another class of cyclin, Clb2, are out of the picture in G1 because their transcription factor, Mcm1, is inactive, their degradation pathway, Cdh1/APC, is active, and their stoichiometric inhibitors, Sic1, are abundant. Cln2- and Clb5-dependent kinases remove Sic1 and inactivate Cdh1. Clb2 is then allowed to appear. Moreover, Clb2/Cdc28 soon activates its own transcription factor, Mcm1, which in turn further drive its synthesis.

Clb2/Cdc28 turns off SBF and MBF. As Clb2/Cdc28 drives the cell into mitosis, it also sets the stage for exit from mitosis by stimulating the synthesis of
Cdc20 which is transcriptional regulated by Mcm1. Then, Cdc20 promotes the activation of Cdc14. Cdc20&Cdc14 play several roles in mitotic exit. First, they degrade Clb5,6 and Clb1,2, remove their potency on Cdh1 inactivation. Next, they activate Cdh1, stabilizing Sic1, and activate Swi5 (the transcription factor for Sic1). As Clb2-kinase activity is quenched by Cdh1 and by Sic1 to below a threshold value, a signal for exit from mitosis is triggered, the cell divides and returns to G1 phase.

To investigate the dynamical stability of cell cycle pathway, Li et al (Li, 2004) analyzed the dynamic trajectories of all 1,764 protein states that will flow to the G1 fixed point, shown in Fig 2.2. The cell cycle pathway is colored in blue and so is the node representing the G1 stationary state. The dynamic flow of the protein states is convergent onto the biological pathway, making the pathway an attracting trajectory of the dynamics. With such a topological structure, the cell-cycle pathway is a very stable trajectory; it is very unlikely for a sequence of events, starting at the beginning (or at any other point) of the cell-cycle process, to deviate from the cell-cycle pathway.
Fig 2.2: Dynamic trajectory of 1,764 protein states

From Li et al (Li 2004), the yeast cell-cycle network is robustly designed. Furthermore, since the network is only a skeleton of a larger cell-cycle network with many "redundant" components and interactions, the complete network is expected to be even more stable.
Chapter 3

Gene Expression and Transcriptional Regulatory Network

3.1 Gene Expression

Regulation of the cell cycle clock is mainly effected through a controlled program of gene expression (Paul T. Spellman, et al, 1998). In budding yeast, there are about 800 cell cycle genes, oscillating during the cell cycle. Some of these genes encode proteins with known cell-cycle functions, such as cell cycle control, cell wall biogenesis, DNA replication and so on, but most are unknown.

One set of gene expression time course data of cell cycle category was collected from Stanford Microarray Database (http://genome-www5.stanford.edu). The expression ratio was measured under the specific condition that yeast cells were blocked in mitosis using a cdc15-2 temperature sensitive mutant at restrictive temperature which is in order to synchronize the sample cells. The mutant can prevent the release of CDC14 which will further activate CDH1. Hence, the cells are arrested in 10th time step of Tang Chao’s cell cycle pathway. The culture was then shifted to permissive temperature (25°C), and released into the cell cycle. The cell cycle then starts at M/G1. Samples were then taken every 10 mins (some are taken every 20 mins) during the course of over two full cell cycles, 290 mins (Table3.1). Then, we collected 798 cell cycle genes with some of predicted phases according to Richard Young’s work in MIT (http://web.wi.mit.edu/young/cellcycle/). After the combination of Richard Young’s cell cycle genes with those involved in the time course data, we finally have a data set of 792 cell cycle genes.
<table>
<thead>
<tr>
<th>Time point</th>
<th>Sample taken time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cdc15 010 min</td>
</tr>
<tr>
<td>2</td>
<td>cdc15 030 min</td>
</tr>
<tr>
<td>3</td>
<td>cdc15 050 min</td>
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<td>4</td>
<td>cdc15 070 min</td>
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<td>cdc15 080 min</td>
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<td>6</td>
<td>cdc15 090 min</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>cdc15 120 min</td>
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<tr>
<td>10</td>
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<td>11</td>
<td>cdc15 140 min</td>
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<td>cdc15 240 min</td>
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<td>22</td>
<td>cdc15 250 min</td>
</tr>
<tr>
<td>23</td>
<td>cdc15 270 min</td>
</tr>
<tr>
<td>24</td>
<td>cdc15 290 min</td>
</tr>
</tbody>
</table>

**Table 3.1 Time elapsed after escape from cdc15 arrest**

In order to standardize and analyze the data set, we normalized the gene expression data so that the average log2(ratio) over the course of the experiment is equal to 0 and were further divided by the standard deviation. Fig 3.1 shows the raw data set using color coding of the gene expression value during the yeast cell cycle. Genes correspond to rows, and the time points of the experiment are the columns. Yellow color means positive regulation, while blue color means negative regulation.
Fig 3.1: Raw data of all cell cycle genes

Hardly any information can be extracted from the above figure, because the genes are disorderly placed along the Y axis. In order to test if these data is good and whether we can see some periodic patterns that are expected, we applied Fourier Transform to analyze the data. We calculated Fourier Transform magnitudes for each gene in terms of different values of omega.

\[
\text{Im} = \sum \sin(\omega t_j)x(t_j) \quad (1)
\]

\[
\text{Re} = \sum \cos(\omega t_j)x(t_j) \quad (2)
\]

\[
I = A^2 + B^2 \quad (3)
\]

\[
\Phi = \tan^{-1}(\text{Im/Re}) \quad (4)
\]

Since the cell cycle period usually varies from 90mins to 120mins. The test omega value is taken as $2\pi/90$. Fig 3.2 shows the distribution of Fourier transform magnitudes, with omega ranging from 0.4*test to 1.5*test and we can easily see that
most genes peak at either position 4 or position 5, which is 115 min and 125 min respectively. Thus the periodical property of these genes under this condition is quite similar. We also found that Fourier Transform magnitudes are unstable for small variations of omega, so the magnitudes were averaged as well as phase over 115 to 125 mins with 1 min each step to get both magnitudes and phase of each gene.

Fig 3.2: Fourier transform magnitudes distribution

The genes are then sorted according to their magnitudes which are their fluctuation strength, and further sorted in terms of their phase (time of peak expression). Fig 3.3 shows results of sorting the strongest 50, 100, 200, 400, 600 and all cell cycle genes respectively.
Fig 3.3: Expression of sorted genes (50, 100, 200, 400, 600, 792)
Fig 3.3 shows clearly the periodic patterns of cell cycle genes. These genes are regulated in a periodic manner coincident with the cell cycle. Such gene regulation is required for proper functioning of the control mechanism to maintain events’ order through cell cycle. For example, the occurrence of 50 genes of strongest oscillation obeys the cell cycle sequence, though the boundary is not absolute, shown in Table 3.1 and Fig 3.4.

<table>
<thead>
<tr>
<th>Orf</th>
<th>Phase</th>
<th>Orf</th>
<th>Phase</th>
<th>Orf</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>YPL187W</td>
<td>m/g1</td>
<td>YJL078C</td>
<td>g1</td>
<td>YBR009C</td>
<td>s</td>
</tr>
<tr>
<td>YJL159W</td>
<td>m/g1</td>
<td>YJL115W</td>
<td>g1</td>
<td>YDR224C</td>
<td>s</td>
</tr>
<tr>
<td>YKL185W</td>
<td>m/g1</td>
<td>YPL267W</td>
<td>g1</td>
<td>YBR010W</td>
<td>s</td>
</tr>
<tr>
<td>YKL164C</td>
<td>m/g1</td>
<td>YBL035C</td>
<td>g1</td>
<td>YBL003C</td>
<td>s</td>
</tr>
<tr>
<td>YKL163W</td>
<td>m/g1</td>
<td>YLR286C</td>
<td>g1</td>
<td>YMR003W</td>
<td>s/g2</td>
</tr>
<tr>
<td>YDR261C</td>
<td>s</td>
<td>YDR097C</td>
<td>g1</td>
<td>YML052W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YOR307C</td>
<td>g1</td>
<td>YGR189C</td>
<td>g1</td>
<td>YHL028W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YLR079W</td>
<td>m/g1</td>
<td>YIL066C</td>
<td>g1</td>
<td>YPR149W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YKR077W</td>
<td>g1</td>
<td>YBR088C</td>
<td>g1</td>
<td>YMR032W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YBR158W</td>
<td>m/g1</td>
<td>YDL003W</td>
<td>g1</td>
<td>YLR190W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YOR308C</td>
<td>g1</td>
<td>YHR143W</td>
<td>g1</td>
<td>YBR038W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YNL327W</td>
<td>m/g1</td>
<td>YAR007C</td>
<td>g1</td>
<td>YBR092C</td>
<td>g2/m</td>
</tr>
<tr>
<td>YBR108W</td>
<td>g1</td>
<td>YBR089W</td>
<td>g1</td>
<td>YDR033W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YLR049C</td>
<td>g1</td>
<td>YOL090W</td>
<td>g1</td>
<td>YDR225W</td>
<td>s</td>
</tr>
<tr>
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<td>g1</td>
<td>YPL256C</td>
<td>g1</td>
<td>YBR054W</td>
<td>g2/m</td>
</tr>
<tr>
<td>YGR044C</td>
<td>g1</td>
<td>YOL007C</td>
<td>g1</td>
<td>YNL160W</td>
<td>m/g1</td>
</tr>
<tr>
<td>YCL024W</td>
<td>g1</td>
<td>YNL030W</td>
<td>s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2: 50 strongest oscillating genes
Besides the differences among their peak expression time, genes may have distinct duration of activation. Some genes are turned on for a short period and then turned off again, while others are turned on for a longer period. This may be related to their functionality. For example, genes responsible for cell wall biosynthesis and integrity should be active during the course of mitosis (YHL028C) while others required for cell cycle check points only need to function over a short period of time (YCL024W), shown in Fig 3.5.
The experiment as mentioned previously starts at late M phase and then the cell enters G1 very quickly. The literature review of yeast cell cycle elucidates G1 and M phases both generally take up about 1/3 cell cycle time, while S phase takes up over 1/2 of the remaining time (Dien BS, Srienc F, 1991). Since time duration of the cell cycle in this experiment is around 120 mins, we can assign each time point with a hypothetic phase description, i.e. 0 mins to 10 mins – M, 10 mins to 50 mins – G1, 50 mins to 80 mins – S, 80 mins to 90 mins – G2 and 90 mins to 120 mins – M, as shown in Table 3.3. Gene expression is further plotted along with the hypothetical phase description of first round of cell cycle in Fig 3.6. The result is supported by the 50 genes that have the strongest oscillations, as shown in Fig 3.4. The time for peak expression of m/g1, g1, s and g2/m genes is 5 mins to 20 mins, 20 mins to 40 mins, 50 mins to 60 mins and 90 mins to 110 mins respectively.

<table>
<thead>
<tr>
<th>Time point</th>
<th>Sample taken time</th>
<th>Hypothetical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>cdc15 010 min</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>cdc15 030 min</td>
<td>G1</td>
</tr>
<tr>
<td>3</td>
<td>cdc15 050 min</td>
<td>G1</td>
</tr>
<tr>
<td>4</td>
<td>cdc15 070 min</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>cdc15 080 min</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>cdc15 090 min</td>
<td>G2</td>
</tr>
<tr>
<td>7</td>
<td>cdc15 100 min</td>
<td>M</td>
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<td>8</td>
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<td>cdc15 250 min</td>
<td>M</td>
</tr>
<tr>
<td>23</td>
<td>cdc15 270 min</td>
<td>G1</td>
</tr>
</tbody>
</table>
Table 3.3 Cell cycle progression time with phase description

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>cdc15</td>
<td>290 min</td>
</tr>
</tbody>
</table>

Fig 3.6: Hypothetic phase description

First round of cell cycle progression
2.2 Transcriptional Regulatory Network

Gene activation and repression events are initiated by a special class of proteins known as transcription factors (TF) which bind to short DNA sequence (known as binding motifs or TF binding sites) upstream the transcription start site of a given gene. The collection of TF’s and their target ORF’s forms a transcriptional regulatory network that constructs the backbone of the genetic regulatory program for all cellular process. The yeast cell cycle gene expression program can be viewed as outcome of such a program in action. Nine known cell cycle transcriptional factors are involved, each regulating a group of genes and functioning during one stage of cell cycle. Furthermore, these nine transcription factors are divided into three groups, as illustrated in Fig 3.7. Mbp1, Swi4 and Swi6 control the late G1 genes. Mcm1, Fkh2 (or Fkh1) and Ndd1 control the transcription of G2/M genes. Mcm1 is also involved in the transcription of genes at the end of m and early G1 together with Swi5 and Ace2 (Itamar Simon, et al, 2001).

Fig 3.7: Groups of transcription factors function in different cell cycle stages
In 2004, a genome-scale transcriptional regulatory network, involving 7074 regulatory interactions between 142 transcription factors and 3420 target genes, was developed by Mark Gerstein and his collaborators (Nicholas M. Luscombe, et al., 2004), one of the most complete so far. 409 out of 792 cell cycle genes were extracted from the network and drawn into three sub-networks using Pajek (http://vlado.fmf.uni-lj.si/pub/networks/pajek/). Fig 3.8 to Fig 3.10 show sub-networks correspond to G1/S, G2/M and M/G1 transitions, with big green nodes and red nodes representing the transcription factors and target genes respectively. The arrows indicate the direction of information flow. Yellow nodes were added to indicate regulators of the transcription factors.

**Fig 3.8: G1/S sub-network**
(TF: Mbp1(YDL056W), Swi4(YER111C) and Swi6(YLR182W))
Fig 3.9: G2/M sub-network  
(TF: Fkh2(YNL068C), Ndd1(YOR372C) and Mcm1(YMR043W))

Fig 3.10: M/G1 sub-network  
(TF: Mcm1(YMR043W), Swi5(YDR146C) and Ace2(YLR131C))
2.3 Cluster

Not all of the interactions between transcription factors and genes contained in the Gerstein’s network have been experimental verified. Therefore, comparing the regulating network with time course micro-array data will not only serve to confirm the proposed genetic interactions, but also yield information on the execution of the regulatory program in a dynamic context.

2.3.1 G1/S Cluster

![Expression of genes in G1/S sub-network](image)

**Fig 3.11: Expression of genes in G1/S sub-network**

The cluster of gene 47 to 90 is clearly visible

Fig 3.11 shows gene expression controlled by G1/S transcription factors according to Gerstein’s work. From the phase sorted expression pattern, we find that gene 47 to 90, form a synchronized cluster and hence are good candidate for targets of the three G1/S transcription factors alone. The other genes behavior differently could
be co-regulated by other transcription factors. Checked with Richard Young’s cell cycle phase description, genes in this cluster are almost exclusively ‘g1’ genes, while the others are categorized into ‘s’, ‘g2/m’ or ‘m/g1’. To verify our guess, we searched the binding motifs of several genes (http://jura.wi.mit.edu/fraenkel/regcode/).

Fig 3.12 shows the binding motifs of ‘YJL158C’ (No12 gene in Fig 3.11) YHR061C (No13 gene in Fig 3.11) and ‘YNR044W’ (No45 gene in Fig 3.11) that have different oscillation properties from the ‘cluster’. The result indicates that ‘YJL158C’ seems to have no binding site in the promoter region. ‘YHR061C’ has two binding sites for fkh2 that function in next stage G2/M. ‘YNR044W’ could not be regulated by G1/S transcription factors at all.

![Fig 3.12_A: YJL158C](image.png)
Fig 3.12_B: YHR061C

Fig 3.12_C: YNR044W

Fig 3.13 shows the binding motifs of ‘YBR071W’ (No49 gene in Fig 3.11), ‘YJL073W’ (No61 gene in Fig 3.11) and ‘YDL003W’ (No80 gene in Fig 3.11) in the ‘cluster’. All have at least one binding site for G1/S transcription factors.

Fig 3.13_A: YBR071W
We have checked against the binding data for all the genes in Fig 3.11 when available. The sample genes shown here are representative of the situation for the majority of genes in this group. Their functional annotation is given in table 3.4.

<table>
<thead>
<tr>
<th>ORF No</th>
<th>ORF</th>
<th>Young's phase</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>YNL273W</td>
<td>g1</td>
<td>TOF1 topoisomerase I interacting factor 1</td>
</tr>
<tr>
<td>48</td>
<td>YOR315W</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>49</td>
<td>YBR071W</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>50</td>
<td>YDL127W</td>
<td>g1</td>
<td>PCL2 cyclin, G1/S-specific</td>
</tr>
<tr>
<td>51</td>
<td>YDL102W</td>
<td>g1</td>
<td>CDC2 DNA-directed DNA polymerase delta, catalytic 125 KD subunit</td>
</tr>
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<td>52</td>
<td>YJR030C</td>
<td>g1</td>
<td>N/A</td>
</tr>
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<td>SPT21 required for normal transcription at a number of loci</td>
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<td>YAR008W</td>
<td>g1</td>
<td>SEN34 tRNA splicing endonuclease gamma subunit</td>
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<td>CLB6 cyclin, B-type</td>
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<td>YDR309C</td>
<td>g1</td>
<td>GIC2 Cdc42 GTPase-binding protein</td>
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<td>YGR041W</td>
<td>g1</td>
<td>BUD9 budding protein</td>
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<td>YGL038C</td>
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<td>Gene ID</td>
<td>Strain</td>
<td>Function</td>
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<td>g1</td>
<td>SAT2 osmotolerance protein</td>
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<tr>
<td>YNL289W</td>
<td>g1</td>
<td>PCL1 cyclin, G1/S-specific</td>
<td></td>
</tr>
<tr>
<td>YKL045W</td>
<td>g1</td>
<td>PRI2 DNA-directed DNA polymerase alpha, 58 KD subunit (DNA primase)</td>
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</tr>
<tr>
<td>YPL267W</td>
<td>g1</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>YGR221C</td>
<td>g1</td>
<td>TOS2 Target of SBF, localizes to the bud neck and bud tip</td>
<td></td>
</tr>
<tr>
<td>YGR153W</td>
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<td>N/A</td>
<td></td>
</tr>
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<td>CTS1 endochitinase</td>
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</tr>
<tr>
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<td>g1</td>
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<td></td>
</tr>
<tr>
<td>YDR097C</td>
<td>g1</td>
<td>MSH6 DNA mismatch repair protein</td>
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</tr>
<tr>
<td>YOR075W</td>
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<td>UFE1 syntaxin (T-SNARE) of the ER</td>
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<tr>
<td>YGR152C</td>
<td>g1</td>
<td>RSR1 GTP-binding protein</td>
<td></td>
</tr>
<tr>
<td>YDR528W</td>
<td>g1</td>
<td>N/A</td>
<td></td>
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<tr>
<td>YGR189C</td>
<td>g1</td>
<td>CRH1 family of putative glycosidases might exert a common role in cell wall organization</td>
<td></td>
</tr>
<tr>
<td>YML100W</td>
<td>g1</td>
<td>TSL1 alpha,alpha-trehalose-phosphate synthase, 123 KD subunit</td>
<td></td>
</tr>
<tr>
<td>YHR149C</td>
<td>g1</td>
<td>SKG6 similarity to hypothetical protein YGR221c</td>
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</tr>
<tr>
<td>YPR120C</td>
<td>g1</td>
<td>CLB5 cyclin, B-type</td>
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</tr>
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<td>YNL231C</td>
<td>g1</td>
<td>PDR16 protein involved in lipid biosynthesis and multidrug resistance</td>
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<tr>
<td>YDR501W</td>
<td>g1</td>
<td>PLM2 PLasmid Maintenance mutant shows 2mu-m plasmid instability</td>
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</tr>
<tr>
<td>YOL019W</td>
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<td>N/A</td>
<td></td>
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<td>g1</td>
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<td>g1</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>YAR007C</td>
<td>g1</td>
<td>RFA1 DNA replication factor A, 69 KD subunit</td>
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<tr>
<td>YLR103C</td>
<td>g1</td>
<td>CDC45 required for minichromosome maintenance and initiation of chromosomal DNA replication</td>
<td></td>
</tr>
<tr>
<td>YDL018C</td>
<td>g1</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>YER095W</td>
<td>g1</td>
<td>RAD51 DNA repair protein</td>
<td></td>
</tr>
<tr>
<td>YJL074C</td>
<td>g1</td>
<td>SMC3 required for structural maintenance of chromosomes</td>
<td></td>
</tr>
<tr>
<td>YCR065W</td>
<td>g1</td>
<td>HCM1 transcription factor</td>
<td></td>
</tr>
<tr>
<td>YNL102W</td>
<td>g1</td>
<td>POL1 DNA-directed DNA polymerase alpha, 180 KD subunit</td>
<td></td>
</tr>
<tr>
<td>YPL256C</td>
<td>g1</td>
<td>CLN2 cyclin, G1/S-specific</td>
<td></td>
</tr>
<tr>
<td>YDL101C</td>
<td>g1</td>
<td>DUN1 protein kinase</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4: G1/S Cluster (Gene 47 to 90 in Fig 3.9)

In conclusion, the G1/S cluster contains 44 genes. The well studied G1 cyclin genes Clb5,6 and Cln2 are included as well as budding protein BUD9. Others with similar behavior in the experiment were added to form a cluster of G1/S coregulated genes.
Fig 3.14: Expression of genes in G2/M sub-network

The cluster of gene 17 to 56 is clearly visible

Fig 3.14 shows gene expression controlled by G2/M transcription factors according to Gerstein’s work. Similarly, we believe that gene 17 to 56 is within the same cluster that is most possibly controlled by M/G1 transcription factors. We also checked with Richard Young’s cell cycle phase description and genes in the ‘cluster’ are almost ‘g2/m’ genes, while the others do not have such a regulation. However, there seems to be a small cluster between gene 70 and 90 regulated by Mcm1 in M/G1.
<table>
<thead>
<tr>
<th>ORF No</th>
<th>ORF</th>
<th>Young’s phase</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>YML064C</td>
<td>g2/m</td>
<td>TEM1 GTP-binding protein of the RAS superfamily</td>
</tr>
<tr>
<td>18</td>
<td>YDR150W</td>
<td>s/g2</td>
<td>NUM1 nuclear migration protein</td>
</tr>
<tr>
<td>19</td>
<td>YPL111W</td>
<td>g2/m</td>
<td>CAR1 arginase</td>
</tr>
<tr>
<td>20</td>
<td>YOR247W</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>21</td>
<td>YPL155C</td>
<td>s/g2</td>
<td>KIP2 kinesin-related protein</td>
</tr>
<tr>
<td>22</td>
<td>YBR133C</td>
<td>s/g2</td>
<td>HSL7 adapter in a regulatory pathway that relieves tyrosine phosphorylation of Cdc28</td>
</tr>
<tr>
<td>23</td>
<td>YIL158W</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>24</td>
<td>YGL255W</td>
<td>g2/m</td>
<td>ZRT1 Zinc transporter I</td>
</tr>
<tr>
<td>25</td>
<td>YNL192W</td>
<td>m/g1</td>
<td>CHS1 chitin synthase I</td>
</tr>
<tr>
<td>26</td>
<td>YMR001C</td>
<td>g2/m</td>
<td>CDC5 protein kinase, involved in regulation of DNA replication</td>
</tr>
<tr>
<td>27</td>
<td>YML052W</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>28</td>
<td>YLR131C</td>
<td>g2/m</td>
<td>ACE2 metallothionein expression activator</td>
</tr>
<tr>
<td>29</td>
<td>YHL028W</td>
<td>g2/m</td>
<td>WSC4 Cell wall integrity and stress response component 4</td>
</tr>
<tr>
<td>30</td>
<td>YGR108W</td>
<td>g2/m</td>
<td>CLB1 cyclin, G2/M-specific</td>
</tr>
<tr>
<td>31</td>
<td>YAL053W</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>32</td>
<td>YKL096W-A</td>
<td>s/g2</td>
<td>CWP2 cell wall mannoprotein</td>
</tr>
<tr>
<td>33</td>
<td>YPL141C</td>
<td>s/g2</td>
<td>N/A</td>
</tr>
<tr>
<td>34</td>
<td>YOR025W</td>
<td>g2/m</td>
<td>HST3 silencing protein</td>
</tr>
<tr>
<td>35</td>
<td>YMR144W</td>
<td>s</td>
<td>N/A</td>
</tr>
<tr>
<td>36</td>
<td>YLR084C</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>37</td>
<td>YDR146C</td>
<td>g2/m</td>
<td>SWI5 transcription factor</td>
</tr>
<tr>
<td>38</td>
<td>YMR002W</td>
<td>s/g2</td>
<td>N/A</td>
</tr>
<tr>
<td>39</td>
<td>YBR138C</td>
<td>g2/m</td>
<td>HDR1 High-Dosage Reduclional segregation defective</td>
</tr>
<tr>
<td>40</td>
<td>YPR149W</td>
<td>g2/m</td>
<td>NCE102 involved in non-classical protein export pathway</td>
</tr>
<tr>
<td>41</td>
<td>YPR119W</td>
<td>g2/m</td>
<td>CLB2 cyclin, G2/M-specific</td>
</tr>
<tr>
<td>42</td>
<td>YGL008C</td>
<td>g2/m</td>
<td>PMA1 H+-transporting P-type ATPase</td>
</tr>
<tr>
<td>43</td>
<td>YOR129C</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>44</td>
<td>YLR190W</td>
<td>g2/m</td>
<td>MMR1 protein localized to bud sites and tips, mother-bud junction</td>
</tr>
<tr>
<td>45</td>
<td>YCR024C-A</td>
<td>g2/m</td>
<td>PMP1 H+-ATPase subunit, plasma membrane</td>
</tr>
<tr>
<td>46</td>
<td>YHR151C</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>47</td>
<td>YGL162W</td>
<td>g2/m</td>
<td>SUT1 hypoxic protein involved in sterol uptake</td>
</tr>
<tr>
<td>48</td>
<td>YNL172W</td>
<td>g2/m</td>
<td>APC1 subunit of anaphase-promoting complex (cyclosome)</td>
</tr>
<tr>
<td>49</td>
<td>YOR023C</td>
<td>g2/m</td>
<td>AHC1 component of the ADA histone acetyltransferase complex</td>
</tr>
<tr>
<td>50</td>
<td>YNL056W</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
<tr>
<td>51</td>
<td>YJR092W</td>
<td>g2/m</td>
<td>BUD4 budding protein</td>
</tr>
<tr>
<td>52</td>
<td>YDL048C</td>
<td>g2/m</td>
<td>STP4 involved in pre-rRNA splicing and in uptake of branched-chain amino acids</td>
</tr>
<tr>
<td>53</td>
<td>YBR038W</td>
<td>g2/m</td>
<td>CHS2 chitin synthase II</td>
</tr>
<tr>
<td>54</td>
<td>YER070W</td>
<td>g1</td>
<td>RNR1 ribonucleoside-diphosphate reductase, large subunit</td>
</tr>
<tr>
<td>55</td>
<td>YBR092C</td>
<td>g2/m</td>
<td>PHO3 constitutive acid phosphatase precursor</td>
</tr>
<tr>
<td>56</td>
<td>YMR253C</td>
<td>g2/m</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3.5: G2/M Cluster (Gene 17 to 56 in Fig 3.13)
In conclusion, the G2/M cluster contains 40 genes. The well studied G2/M cyclin genes Clb1,2 are involved and others with similar behavior in the experiment were added to form a cluster of G2/M coregulated genes.

### 2.3.3 M/G1

![Expression of genes in M/G1 sub-network](image)

**Fig 3.15: Expression of genes in M/G1 sub-network**

*The cluster of gene 43 to 90 is clearly visible*

Fig 3.15 shows gene expression controlled by M/G1 transcription factors according to Gerstein’s work. Similarly, we believe that gene 43 to 90 is within the same cluster that is most possibly controlled by M/G1 transcription factors. We also checked with Richard Young’s cell cycle phase description. Genes in the ‘cluster’ are almost exclusively ‘m/g1’ or ‘g1’ genes, while the others do not have such a
regulation. However, there also seems to be a small cluster between gene 20 and 30 which is regulated by Mcm1 in G2/M.

<table>
<thead>
<tr>
<th>ORF No</th>
<th>ORF</th>
<th>Young's phase</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>YJL194W</td>
<td>m/g1</td>
<td>N/A</td>
</tr>
<tr>
<td>44</td>
<td>YKL178C</td>
<td>m/g1</td>
<td>MMR1 protein localized to bud sites and tips, mother-bud junction</td>
</tr>
<tr>
<td>45</td>
<td>YOR066W</td>
<td>m/g1</td>
<td>PMP1 H+-ATPase subunit, plasma membrane</td>
</tr>
<tr>
<td>46</td>
<td>YLR274W</td>
<td>m/g1</td>
<td>N/A</td>
</tr>
<tr>
<td>47</td>
<td>YPL187W</td>
<td>m/g1</td>
<td>SUT1 hypoxic protein involved in sterol uptake</td>
</tr>
<tr>
<td>48</td>
<td>YBR202W</td>
<td>g2/m</td>
<td>APC1 subunit of anaphase-promoting complex (cyclosome)</td>
</tr>
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<td>49</td>
<td>YLR273C</td>
<td>m/g1</td>
<td>AHC1 component of the ADA histone acetyltransferase complex</td>
</tr>
<tr>
<td>50</td>
<td>YJL159W</td>
<td>m/g1</td>
<td>N/A</td>
</tr>
<tr>
<td>51</td>
<td>YJL196C</td>
<td>m/g1</td>
<td>BUD4 budding protein</td>
</tr>
<tr>
<td>52</td>
<td>YKL185W</td>
<td>m/g1</td>
<td>STP4 involved in pre-tRNA splicing and in uptake of branched-chain amino acids</td>
</tr>
<tr>
<td>53</td>
<td>YKL164C</td>
<td>m/g1</td>
<td>CHS2 chitin synthase II</td>
</tr>
<tr>
<td>54</td>
<td>YNL328C</td>
<td>m/g1</td>
<td>RNR1 ribonucleoside-diphosphate reductase, large subunit</td>
</tr>
<tr>
<td>55</td>
<td>YPL158C</td>
<td>m/g1</td>
<td>PHO3 constitutive acid phosphatase precursor</td>
</tr>
<tr>
<td>56</td>
<td>YIL009W</td>
<td>m/g1</td>
<td>N/A</td>
</tr>
<tr>
<td>57</td>
<td>YKL163W</td>
<td>m/g1</td>
<td>PDS5 precocious dissociation of sister chromatids</td>
</tr>
<tr>
<td>58</td>
<td>YGR234W</td>
<td>m/g1</td>
<td>CDC20 cell division control protein</td>
</tr>
<tr>
<td>59</td>
<td>YDR055W</td>
<td>m/g1</td>
<td>MRH1 membrane protein related to Hsp30p</td>
</tr>
<tr>
<td>60</td>
<td>YNR044W</td>
<td>m/g1</td>
<td>IAH1 isoamyl acetate hydrolytic enzyme</td>
</tr>
<tr>
<td>61</td>
<td>YEL040W</td>
<td>m/g1</td>
<td>N/A</td>
</tr>
<tr>
<td>62</td>
<td>YER189W</td>
<td>g1</td>
<td>STE2 pheromone alpha-factor receptor</td>
</tr>
<tr>
<td>63</td>
<td>YFL064C</td>
<td>g1</td>
<td>KIN3 ser/thr protein kinase</td>
</tr>
<tr>
<td>64</td>
<td>YER152C</td>
<td>g1</td>
<td>AGA2 a-agglutinin binding subunit</td>
</tr>
<tr>
<td>65</td>
<td>YLR079W</td>
<td>m/g1</td>
<td>DBF2 ser/thr protein kinase related to Dbf20p</td>
</tr>
<tr>
<td>66</td>
<td>YGR086C</td>
<td>m/g1</td>
<td>MFA2 mating pheromone a-factor 2</td>
</tr>
<tr>
<td>67</td>
<td>YEL077C</td>
<td>g1</td>
<td>BNS1 Bypasses Need for Spo12p</td>
</tr>
<tr>
<td>68</td>
<td>YBR158W</td>
<td>m/g1</td>
<td>RGA1 RHO-type GTPase-activating protein for Cdc42p</td>
</tr>
<tr>
<td>69</td>
<td>YER190W</td>
<td>g1</td>
<td>SPO12 sporulation protein</td>
</tr>
<tr>
<td>70</td>
<td>YKL151C</td>
<td>m/g1</td>
<td>SWI4 transcription factor</td>
</tr>
<tr>
<td>71</td>
<td>YOR315W</td>
<td>g2/m</td>
<td>CLN3 cyclin, G1/S-specific</td>
</tr>
<tr>
<td>72</td>
<td>YNL327W</td>
<td>m/g1</td>
<td>CDC6 cell division control protein</td>
</tr>
<tr>
<td>73</td>
<td>YGL089C</td>
<td>g1</td>
<td>STE3 pheromone a-factor receptor</td>
</tr>
<tr>
<td>74</td>
<td>YDL127W</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>75</td>
<td>YPL283C</td>
<td>m/g1</td>
<td>CDC46 cell division control protein</td>
</tr>
<tr>
<td>76</td>
<td>YLR049C</td>
<td>g1</td>
<td>MF(ALPHA)1 mating pheromone alpha-1 precursor</td>
</tr>
<tr>
<td>77</td>
<td>YGR296W</td>
<td>g1</td>
<td>CDC47 cell division control protein</td>
</tr>
<tr>
<td>78</td>
<td>YGL028C</td>
<td>g1</td>
<td>PIG1 putative type 1 phosphatase regulatory subunit</td>
</tr>
<tr>
<td>79</td>
<td>YLR194C</td>
<td>m/g1</td>
<td>HSP150 member of the Pir1p/Hsp150p/Pir3p family</td>
</tr>
<tr>
<td>80</td>
<td>YGR044C</td>
<td>g1</td>
<td>ELO1 fatty acid elongation protein</td>
</tr>
<tr>
<td>81</td>
<td>YGR041W</td>
<td>g1</td>
<td>PIR1 required for tolerance to heat shock</td>
</tr>
<tr>
<td>82</td>
<td>YJL157C</td>
<td>g2/m</td>
<td>CLN1 cyclin, G1/S-specific</td>
</tr>
<tr>
<td>83</td>
<td>YJL051W</td>
<td>g2/m</td>
<td>KEL2 involved in cell fusion and morphogenesis</td>
</tr>
<tr>
<td>84</td>
<td>YJL078C</td>
<td>g1</td>
<td>PIR3 member of the Pir1p/Pir2p/Pir3p family</td>
</tr>
</tbody>
</table>
Table 3.6: M/G1 Cluster (Gene 43 to 90 in Fig 3.14)

<table>
<thead>
<tr>
<th></th>
<th>Gene</th>
<th>Type</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>YNL289W</td>
<td>g1</td>
<td>EXG2 exo-beta-1,3-glucanase minor isoform</td>
</tr>
<tr>
<td>86</td>
<td>YLR286C</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>87</td>
<td>YDR528W</td>
<td>g1</td>
<td>N/A</td>
</tr>
<tr>
<td>88</td>
<td>YGR189C</td>
<td>g1</td>
<td>AGA1 a-agglutinin anchor subunit</td>
</tr>
<tr>
<td>89</td>
<td>YDR461W</td>
<td>g1</td>
<td>UTR2 cell wall protein</td>
</tr>
</tbody>
</table>

In conclusion, the M/G1 cluster contains 48 genes. Cdc20, Cdc6 and Cln3 are involved where Cdc20 and Cdc6 trigger the exit from mitosis and Cln3 is the cell cycle signal for cell cycle division. Others with similar behavior in the experiment were added to form a cluster of M/G1 coregulated genes.

These clusters provide a foundation for understanding the transcriptional mechanism of cell cycle regulation. Fig 3.16 shows the entire cluster-gram of the transcription factor target genes. The corresponding network is shown in Fig 3.17. As discussed previously, these identified corregulated genes share common binding sites. Presumably the genes within the same group somehow have related functions.

The 44 G1/S cluster genes include CLN2, CLB5,6, CDC2, PRI2, SEN34, RFA1, CDC45 and many other genes involved in DNA replication. Many genes known to be involved in mitosis is found in G2/M cluster which contains 40 genes, such as CDC5, CLB1,2, SWI5, APC1 and BUD4. The G2/M cluster which contains 48 genes includes 4 cell division control proteins CDC20, CDC6, CDC46 and CDC47.
Fig 3.16: the clusters of cell cycle stage specific TF_orf groups

Fig 3.17: Simplified cell cycle TF_orf network
Chapter 4

Achievements and Further Work

The purpose of the present project is to study the regulatory program of yeast cell cycle. Implementation of Prof. Tang Chao’s model gives us a good demonstration of cell cycle’s robustness and stability. By digging into gene expression program and further grouping the genes into phase synchronized clusters, we confirmed the proposed genetic interactions.

Even though the cell cycle regulatory proteins are few and their roles well-characterized, the execution of the dynamic program is rather complex and many of the details are yet to be understood. In the thesis, we have only examined the gene expression data from one time course experiment. With more data under various experimental conditions, along with good binding data, one may overcome some of the intrinsic issues with noise and obtain a more complete picture of cell cycle gene regulation. Furthermore, beyond the static interactions between the transcription factors and target genes, we would like to understand in more detail the turning on/off process of a given gene by one or more transcription factors, and how such processes at the single gene level are fine tuned to meet the global demands of cell growth, replication, and division.
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Appendix -- C programming code for cell cycle model implementation

// Implementation of Chao's model used to investigate the fixed points and biological pathway under various dynamic rules
// Andy Cai
// 11/2004

#include <stdlib.h>
#include <stdio.h>
#include <math.h>

void main()
{
    int option, option1, option2, option3;
    int Sn[] = {0,0,0,0,0,0,0,0,0,0,0};
    int So[] = {0,0,0,0,0,0,0,0,0,0,0};
    int Temp[] = {0,0,0,0,0,0,0,0,0,0,0};
    int Sum[] = {0,0,0,0,0,0,0,0,0,0,0};
    int S[] =  {0,0,0,0,0,0,0,0,0,0,0};
    int Selfdeg[] = {1,1,1,1,1};
    int Basinsize[] = {0,0,0,0,1,0};
    int i, j, count, firstentry;
    int Ag, Ar, td;
    int timeinterval;

    int PF1[] = {0,0,0,0,1,0,0,0,1,0,0};
    int PF2[] = {0,0,1,1,0,0,0,0,0,0,0};
    int PF3[] = {0,1,0,0,0,0,0,0,0,0,0};
    int PF4[] = {1,0,0,0,0,0,0,0,0,0,0};
    int PF5[] = {0,1,0,0,0,0,0,0,0,0,0};
    int PF6[] = {0,0,0,0,0,0,0,0,0,0,0};
    int PF7[] = {0,0,0,0,0,0,0,0,0,0,0};

    printf("To investigate the trajectories in state space; please enter 1. \n");
    printf("To do statistics of the big fixed points of the cell-cycle network; please enter 2. \n");
    printf("To quit the program; please enter 3. \n");
    printf("Please select one option: ");
    scanf("%d", &option);

    while(option != 3) { } 
    
    for(i=0;i<=10;i++)
    { 
        Sn[i] = So[i] = Temp[i] = Sum[i] = S[i] = 0;
    }

    for(i=0;i<=4;i++)
    { 
        Selfdeg[i] = 1;
    }

    for(i=0;i<=6;i++)
    { 
        Basinsize[i] = 0;
        Basinsize[5] = 1;
    }

    if (option == 1) 
    { 
        printf("As back to main list, please enter 0; to continue, please press 1: ");
        scanf("%d", &option1);

        while(option1 != 0)
        { 
            for(i=0;i<=10;i++)
            {
                
            }
        }
    }
{ 
    Sn[i] = So[i] = Temp[i] = Sum[i] = S[i] = 0;
}

printf("nPlease assign values for Ag, Ar and td(Note: The value of 
Ar must larger or equal to that of Ag). \n");
scanf("%d%d%d", &Ag, &Ar, &td);
printf("Ag = %d\t Ar = %d\t td = %d\n", Ag, Ar, td);
for(i=0;i<=10;i++)
{
    Sum[i] = 0;
}
for(i=0;i<=4;i++)
{
    Selfdeg[i] = 1;
}

printf("nPlease assign state statuses for 11 nodes(1 for activated 
node; 0 for deactivated node), Cln3, MBF, SBF, Cln1,2, Cdh1, Swi5, Cdc20&cdc14, Clb5,6, Sic1,
Clb1,2 and Mcm1&SFF: \n");
scanf("%d%d%d%d%d%d%d%d%d%d%d", &So[0], &So[1], &So[2], &So[3], &So[4], &So[5], &So[6], &So[7], &So[8], &So[9], &So[10]);

printf("Cln3\tMBF\tSBF\tCln1,2\tCdh1\tSwi5\tCdc20&cdc14\tClb5,6\tSic1\nClb1,2\tMcm1&SFF \
");
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n", So[0], So[1], So[2], So[3], So[4], So[5], So[6], So[7], So[8], So[9], So[10]);
timeinterval = 0;
while(timeinterval!=td)
{
    for(i=0;i<=10;i++)
    {
        Sum[i] = 0;
    }
    if (firstentry == 1)
    {
        for(i=0;i<=10;i++)
        {
            Sn[i] = So[i];
        }
        firstentry = 0;
    }
    if (Sn[0] == 1)//self-degradation
    {
        if(Selfdeg[0] == td)
            Sum[0] = -1;
        else Selfdeg[0]++;
    }
    if (Sn[1] == 1)
    {
    }
    if (Sn[2] == 1)
\{ 
\}

if (Sn[3] == 1)/self-degradation 
{ 
    if (Sn[2] == 0) 
    { 
        if (Selfdeg[1] == td) 
            Sum[3] = -1; 
        else Selfdeg[1]++;
    }
    else if (Sn[2] == 1) 
    { 
        Selfdeg[1] = 1;
    }
}

if (Sn[4] == 1) 
{ 
}

if (Sn[5] == 1)/self-degradation 
{ 
    { 
        if (Selfdeg[2] == td) 
            Sum[5] = -1; 
        else Selfdeg[2]++;
    }
    { 
        Selfdeg[2] = 1;
    }
}

if (Sn[6] == 1)/self-degradation 
{ 
    if (Sn[9] + Sn[10] == 0) 
    { 
        if (Selfdeg[3] == td) 
            Sum[6] = -1; 
        else Selfdeg[3]++;
    }
    else if (Sn[9] + Sn[10] > 0) 
    { 
    }
}
Selfdeg[3] = 1;
}
}
if (Sn[7] == 1)
{
}
if (Sn[8] == 1)
{
}
if (Sn[9] == 1)
{
}
if (Sn[10] == 1)/self-degradation
{
    if (Sn[9] == 0)
    {
        if(Selfdeg[4] == td)
            Sum[10] = -1;
        else Selfdeg[4]++;
    }
    else if(Sn[9] == 1)
    {
    }
} //update the value for status conditions for all nodes
for(i=0;i<=10;i++)
{
    Temp[i] = Sn[i];
} //Store the old statuses in a Temp array
for(i=0;i<=10;i++)
{
    So[i] = Temp[i];
} //update the old statuses for 11 nodes
for(i=0;i<=10;i++)
{
    if (Sum[i] > 0)
        Sn[i] = 1;
    else if (Sum[i] < 0)
Sn[i] = 0;  
else Sn[i] = So[i];  

//The new statuses of all 11 nodes
{
    timeinterval++;
}
else timeinterval = 0;

printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n", Sn[0], Sn[1], Sn[2], Sn[3], Sn[4], Sn[5], Sn[6], Sn[7], Sn[8], Sn[9], Sn[10]);

//end of while loop for the fixed point

printf("\nBack to main list, please enter 0; to continue, please press 1: ");
scanf("%d", &option1);

//end of the while loop for option1

}//case for option 1
else if (option == 2)
{
    printf("\nBack to main list, please enter 0; to continue, please press 1: ");
    scanf("%d", &option2);

    while(option2 != 0)
    {
        for(i=0;i<=10;i++)
        {
            Sn[i] = So[i] = Temp[i] = Sum[i] = S[i] = 0;
        }
        for(i=0;i<=4;i++)
        {
            Selfdeg[i] = 1;
        }
        for(i=0;i<=6;i++)
        {
            Basinsize[i] = 0;
            Basinsize[5] = 1;
        }

        printf("\nTo do statistics with default dynamic rule settings, please enter 1.\nTo do statistics with other dynamic rule settings, please enter 2.\nPlease make a choice: ");
        scanf("%d", &option3);

        if (option3 == 1)
        {
            for(count=1;count<=2048;count++)
            {
                for(j=0;j<=10;j++)
                {
                    if((count%(int)pow(2,j+1))<(int)pow(2,j))
                        Sn[j] = 0;
                    else
                        if((count%(int)pow(2,j+1))>(int)pow(2,j))
                            Sn[j] = 1;
            }
    for(i=0;i<=10;i++)
        Sum[i] = 0;
    if (firstentry == 1)
        for(i=0;i<=10;i++)
            Sn[i] = So[i];
    firstentry = 0;
    if (Sn[0] == 1)//self-degradation
        Sum[0] = -1;
    if (Sn[1] == 1)
    if (Sn[2] == 1)
    if (Sn[3] == 1)//self-degradation
        if (Sn[2] == 0)
            Sum[3] = -1;
        else if(Sn[2] == 1)
    if (Sn[4] == 1)
    if (Sn[5] == 1)//self-degradation
            Sum[5] = -1;
            
}
\begin{verbatim}
if (Sn[6] == 1) /* self-degradation */
{
    if (Sn[9] + Sn[10] == 0)
    {
        Sum[6] = -1;
    }
    else if (Sn[9] + Sn[10] > 0)
    {
    }
}
if (Sn[7] == 1)
{
}
if (Sn[8] == 1)
{
}
if (Sn[9] == 1)
{
}
if (Sn[10] == 1) /* self-degradation */
{
    if (Sn[9] == 0)
    {
        Sum[10] = -1;
    }
    else if (Sn[9] == 1)
    {
    }
}
\end{verbatim}
//update the value for status conditions for all

for(i=0;i<=10;i++)
{
    Temp[i] = Sn[i];
} //Store the old statuses in a Temp array

for(i=0;i<=10;i++)
{
    So[i] = Temp[i];
} //update the old statuses for 11 nodes

for(i=0;i<=10;i++)
{
    if (Sum[i] > 0)
        Sn[i] = 1;
    else if (Sum[i] < 0)
        Sn[i] = 0;
    else Sn[i] = So[i];
} //The new statuses of all 11 nodes

{
    if(Sn[0] == PF1[0] && Sn[1] ==
        Basinsize[0]+=;
    else if(Sn[0] == PF2[0] && Sn[1] ==
        Basinsize[1]+=;
    else if(Sn[0] == PF3[0] && Sn[1] ==
        Basinsize[2]+=;
    else if(Sn[0] == PF4[0] && Sn[1] ==
        Basinsize[3]+=;
    else if(Sn[0] == PF5[0] && Sn[1] ==
        Basinsize[4]+=;
    else if(Sn[0] == PF6[0] && Sn[1] ==
        Basinsize[5]+=;
    else if(Sn[0] == PF7[0] && Sn[1] ==
        Basinsize[6]+=;
}

;//end of while loop for the fixed point

printf("Cln3,tMBF,tSBF,tCln1,2,tCdh1,tSwi5,tCdc20&
Cdc14,tC1b5,6,tSic1,tC1b1,2,tMcm1&
SFF\tBasinsize \n");

printf("%d,%d,%d,%d,%d,%d,%d,%d,%d,%d,%d,%d\n", PF1[0], PF1[1], PF1[2],
    PF1[3], PF1[4], PF1[5], PF1[6], PF1[7], PF1[8], PF1[9], PF1[10], Basinsize[0]);
printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF2[0], PF2[1], PF2[2], PF2[3], PF2[4], PF2[5], PF2[6], PF2[7], PF2[8], PF2[9], PF2[10], Basinsize[1]);

printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF3[0], PF3[1], PF3[2], PF3[3], PF3[4], PF3[5], PF3[6], PF3[7], PF3[8], PF3[9], PF3[10], Basinsize[2]);

printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF4[0], PF4[1], PF4[2], PF4[3], PF4[4], PF4[5], PF4[6], PF4[7], PF4[8], PF4[9], PF4[10], Basinsize[3]);

printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF5[0], PF5[1], PF5[2], PF5[3], PF5[4], PF5[5], PF5[6], PF5[7], PF5[8], PF5[9], PF5[10], Basinsize[4]);

printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF6[0], PF6[1], PF6[2], PF6[3], PF6[4], PF6[5], PF6[6], PF6[7], PF6[8], PF6[9], PF6[10], Basinsize[5]);

printf("%d	%d	%d	%d	%d	%d	%d	%d	%d	%d	%d\n", PF7[0], PF7[1], PF7[2], PF7[3], PF7[4], PF7[5], PF7[6], PF7[7], PF7[8], PF7[9], PF7[10], Basinsize[6]);

} // case for default dynamic rule

else if (option3 == 2) {

printf("Please assign values for Ag, Ar and td(Note: The value of Ar must larger or equal to that of Ag).\n");
scanf("%d%d%d", &Ag, &Ar, &td);

printf("Ag = %d	 Ar = %d	 td = %d\n", Ag, Ar, td);

for(i=0;i<=10;i++)
{
    Sum[i] = 0;
}

for(i=0;i<=4;i++)
{
    Selfdeg[i] = 1;
}

for(count=1;count<=2047;count++)
{
    for(j=0;j<=10;j++)
    {
        if((count%(int)pow(2,j+1))<(int)pow(2,j))
            Sn[j] = 0;
        else if((count%(int)pow(2,j+1))>=(int)pow(2,j))
            Sn[j] = 1;
    }

    for(i=0;i<=4;i++)
    {
        Selfdeg[i] = 1;
    }

    timeinterval = 0;

    while(timeinterval != td)
    {
        for(i=0;i<=10;i++)
        {
            Sum[i] = 0;
        }

        if (firstentry == 1)
        {
            firstentry = 0;
        }

        for(j=0;j<=10;j++)
        {
            if((count%(int)pow(2,j+1))<(int)pow(2,j))
                Sn[j] = 0;
            else if((count%(int)pow(2,j+1))>=(int)pow(2,j))
                Sn[j] = 1;
        }

        for(i=0;i<=4;i++)
        {
            Selfdeg[i] = 1;
        }

        timeinterval = 0;
    }

    // case for default dynamic rule
}

else if (option3 == 2) {

printf("Please assign values for Ag, Ar and td(Note: The value of Ar must larger or equal to that of Ag).\n");
scanf("%d%d%d", &Ag, &Ar, &td);

printf("Ag = %d	 Ar = %d	 td = %d\n", Ag, Ar, td);

for(i=0;i<=10;i++)
{
    Sum[i] = 0;
}

for(i=0;i<=4;i++)
{
    Selfdeg[i] = 1;
}

for(count=1;count<=2047;count++)
{
    for(j=0;j<=10;j++)
    {
        if((count%(int)pow(2,j+1))<(int)pow(2,j))
            Sn[j] = 0;
        else if((count%(int)pow(2,j+1))>=(int)pow(2,j))
            Sn[j] = 1;
    }

    for(i=0;i<=4;i++)
    {
        Selfdeg[i] = 1;
    }

    timeinterval = 0;

    while(timeinterval != td)
    {
        for(i=0;i<=10;i++)
        {
            Sum[i] = 0;
        }

        if (firstentry == 1)
        {
            firstentry = 0;
        }

        for(j=0;j<=10;j++)
        {
            if((count%(int)pow(2,j+1))<(int)pow(2,j))
                Sn[j] = 0;
            else if((count%(int)pow(2,j+1))>=(int)pow(2,j))
                Sn[j] = 1;
        }

        for(i=0;i<=4;i++)
        {
            Selfdeg[i] = 1;
        }

        timeinterval = 0;
    }

    // case for default dynamic rule
}
for(i=0;i<=10;i++)
{
    Sn[i] = So[i];
}
firstentry = 0;

if (Sn[0] == 1)//self-degradation
{
    Sum[0] = -1;
}

if (Sn[1] == 1)
{
}

if (Sn[2] == 1)
{
}

if (Sn[3] == 1)//self-degradation
{
    if (Sn[2] == 0)
    {
        Sum[3] = -1;
    }
    else if(Sn[2] == 1)
    {
    }
}

if (Sn[4] == 1)
{
}

if (Sn[5] == 1)//self-degradation
{
    {
        Sum[5] = -1;
    }
    {
    }
}

if (Sn[6] == 1)//self-degradation
{
    if (Sn[9] + Sn[10] == 0)
    {
    }
Sum[6] = -1;

}

if (Sn[7] == 1)
{
}

if (Sn[8] == 1)
{
}

if (Sn[9] == 1)
{
}

if (Sn[10] == 1) //self-degradation
{
    if (Sn[9] == 0)
    {
        Sum[10] = -1;
    }
    else if(Sn[9] == 1)
    {
    }
}

//update the value for status conditions for all nodes
for(i=0;i<=10;i++)
{
    Temp[i] = Sn[i];
    //Store the old statuses in a Temp array
}

for(i=0;i<=10;i++)
{
    So[i] = Temp[i];
    //update the old statuses for 11 nodes
for(i=0;i<=10;i++)
{
  if (Sum[i] > 0)
    Sn[i] = 1;
  else if (Sum[i] < 0)
    Sn[i] = 0;
  else Sn[i] = So[i];
}//The new statuses of all 11 nodes

{
  timeinterval++;
}
else timeinterval = 0;

}//end of while loop for the fixed point

{
  if(Sn[0] == PF1[0] && Sn[1] == PF1[1]
    Basinsize[0]++;
  else if(Sn[0] == PF2[0] && Sn[1] ==
    Basinsize[1]++;
  else if(Sn[0] == PF3[0] && Sn[1] ==
    Basinsize[2]++;
  else if(Sn[0] == PF4[0] && Sn[1] ==
    Basinsize[3]++;
  else if(Sn[0] == PF5[0] && Sn[1] ==
    Basinsize[4]++;
  else if(Sn[0] == PF6[0] && Sn[1] ==
    Basinsize[5]++;
  else if(Sn[0] == PF7[0] && Sn[1] ==
    Basinsize[6]++;
  }

printf("Cln3\tMBF\tSBF\tCln1,2\tCdhl1\tSwi5,6\tCdc20\tCdc14\tCib5,6\tSc1\tClb1,2\tMcm1\tSFF\tbasinsize \n");
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n", PF1[0], PF1[1],
PF1[2], PF1[3], PF1[4], PF1[5], PF1[6], PF1[7], PF1[8], PF1[9], PF1[10], Basinsize[0]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n", PF2[0], PF2[1],
PF2[2], PF2[3], PF2[4], PF2[5], PF2[6], PF2[7], PF2[8], PF2[9], PF2[10], Basinsize[1]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\t%d\n", PF3[0],
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t	%d\t%d\t%d\t%d\t	%d\n", PF3[0], PF3[1], PF3[2], PF3[3], PF3[4], PF3[5], PF3[6], PF3[7], PF3[8], PF3[9], PF3[10], Basinsize[2]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t	%d\t%d\t%d\t%d\t	%d\n", PF4[0], PF4[1], PF4[2], PF4[3], PF4[4], PF4[5], PF4[6], PF4[7], PF4[8], PF4[9], PF4[10], Basinsize[3]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t	%d\t%d\t%d\t%d\t	%d\n", PF5[0], PF5[1], PF5[2], PF5[3], PF5[4], PF5[5], PF5[6], PF5[7], PF5[8], PF5[9], PF5[10], Basinsize[4]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t	%d\t%d\t%d\t%d\t	%d\n", PF6[0], PF6[1], PF6[2], PF6[3], PF6[4], PF6[5], PF6[6], PF6[7], PF6[8], PF6[9], PF6[10], Basinsize[5]);
printf("%d\t%d\t%d\t%d\t%d\t%d\t%d\t	%d\t%d\t%d\t%d\t	%d\n", PF7[0], PF7[1], PF7[2], PF7[3], PF7[4], PF7[5], PF7[6], PF7[7], PF7[8], PF7[9], PF7[10], Basinsize[6]);

} //cases for other dynamic rules
else printf("Wrong Number!!\n");

printf("\nBack to main list, please enter 0; to continue, please press 1: ");
scanf("%d", &option2);
} //end of the while loop for option2

}
else
{
    printf("\nNo such option!\n");
    printf("To investigate the trajectories in state space; please enter 1. \n");
    printf("To do statistics of the big fixed points of the cell-cycle network; please enter 2. \n");
    printf("To quit the program; please enter 3. \n");
    scanf("%d", &option);
}

printf("\nTo investigate the trajectories in state space; please enter 1. \n");
printf("To do statistics of the big fixed points of the cell-cycle network; please enter 2. \n");
printf("To quit the program; please enter 3. \n");
scanf("%d", &option);

} //the end of the biggest while loop
}