Mathematical Model of the Budding Yeast Cell Cycle

Laurence Calzone

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John J. Tyson, Chair
Robert C. Rogers
Robert Wheeler

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(ABSTRACT)

The cell cycle of the budding yeast, *Saccharomyces cerevisiae*, is regulated by a complex network of chemical reactions controlling the activity of the cyclin-dependent kinases (CDKs), a family of protein kinases that drive the major events of the cell cycle. A previous mathematical model by Chen et al. (2000) described a molecular mechanism for the Start transition (passage from G1 phase to S/M phase) in budding yeast. In this thesis, my main goal is to extend Chen's model to include new information about the mechanism controlling Finish (passage from S/M phase to G1 phase). Using laws of biochemical kinetics, I transcribed the hypothetical molecular mechanism into a set of differential equations. Simulations of the wild-type cell cycle and the phenotypes of more than 60 mutants provide a thorough understanding of how budding yeast cells exit mitosis.

Acknowledgements

I would like to thank my advisor, Dr. John J. Tyson, for his patience and encouragement. Dr. Tyson has helped me learn and appreciate the wonders of mathematical biology. I am sincerely grateful to Kathy Chen from Virginia Tech, Dr. Bela Novak and my friend, Attila Csikasz-Nagy from the Technical University of Budapest, Hungary for working with me and correcting my frequent errors throughout my research. I also would like to thank other members of my committee, Dr R. Rogers and Dr. R. Wheeler for their interest in my project. I am also very grateful to all my friends for their moral support.

I dedicate this thesis to my parents, Dominique and Francoise Calzone and my sister Sandrine Calzone-Consolin. Their love and support has helped me through these years far from home. I hope you are proud of me.

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CDK: Cyclin-Dependent Kinase

APC: Anaphase Promoting Complex

RENT: Regulation of Nucleolar silencing and Telophase exit

ts: temperature sensitive

Δ: deletion

Chapter 1: Physiology of the Cell Cycle

DEFINITION

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. Between one cell division and the next, all essential components of the cell must be duplicated. The most important component is the genetic material (DNA molecules, also called chromosomes), which must be accurately replicated and the two copies carefully segregated to the two daughter cells. The processes of DNA replication and sister chromatid separation occur in temporally distinct phases of the cell cycle (called S-phase, for DNA synthesis, and M-phase for mitosis).

PHASES OF THE CYCLE

The cell cycle is usually divided in four phases (Murray & Hunt, 1993):

- G1-phase: temporal gap between cell division and DNA synthesis.
- S-phase: period of DNA replication. From one double-stranded DNA molecule (chromosome), two identical double stranded DNA molecules (called sister chromatids) are formed and held together by cohesion proteins.
- G2-phase: temporal gap between the end of replication and the beginning of mitosis, when cells are preparing for mitosis. (In budding yeast, there is no clear G2 phase, because some early events of mitosis overlap with S phase).
- M-phase (mitosis): process by which replicated DNA molecules are segregated to daughter cells. The sister chromatids separate so that the daughter cells get one copy of each chromosome.

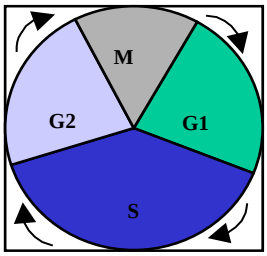


Figure 1 Four phases of the cell cycle. (Murray, p8, fig 1.4)

The cell cycle is cyclic progression through these four stages (Fig. 1).

The process of separating sister chromatids is quite complex, occurring in four different stages: prophase, metaphase, anaphase, and telophase.

In *prophase*, the chromosomes slowly condense and mitotic spindle forms. At this time of the cell cycle, each chromosome consists of two sister chromatids. The chromatids are each carefully folded up into very compact structures (condensed chromosomes), which are still held together in pairs at structures called kinetochores situated in the centromere. In early prophase, thin fibers called microtubules are assembling a bipolar spindle. The microtubules attach to the chromosomes and pull them into alignment in between the two mitotic spindles (so called metaphase plate). When aligned, one chromatid of each chromosome is attached by microtubules to one pole of the spindle, and its sister chromatid is attached by other microtubules to the other pole of the spindle. This brief state of the cell is called *metaphase*. Triggered by a specific signal, the glue that holds the sister chromatids together is dissolved allowing each chromatid, separated from its sister, to be pulled by the microtubules to one of the poles of the spindle. The cell is in *anaphase*. During telophase two nuclei are created, and the cell divides at the final step, called *cytokinesis*, in two daughter cells. The daughter cells are now back in G1 phase. The cycle repeats.

CHECKPOINTS

Cell division is a complex process for which many mistakes are fatal. In order to avoid catastrophic failure, the cell verifies that proper conditions are satisfied at crucial steps in the division process. As it progresses through the division cycle, the cell halts at three checkpoints: in G1, G2 and M phases.

Before the cell enters S-phase, it must be large enough and have undamaged DNA. If these conditions are not met, the cell arrests at the G1 checkpoint. When the conditions are satisfied, the G1 checkpoint is lifted and the cell can enter S-phase.

Before entering mitosis, at the G2 checkpoint, the cell verifies that DNA synthesis is complete, DNA is undamaged and the cell is large enough.

Before the cell executes anaphase (exit from mitosis), the chromosomes need to be properly aligned and DNA replication needs to be complete. When these conditions are verified, the metaphase checkpoint is lifted and the cell can divide.

PHYSIOLOGY OF THE BUDDING YEAST CELL CYCLE

In the case of budding yeast (*Saccharomyces cerevisiae*), cell division is asymmetric. The dividing cell separates into a small "daughter" cell, and a large "mother" cell.

S and M phases overlap. We refer to these phases as one phase only, called S/M phase. Also, there is no G2 checkpoint.

Consider a daughter cell (Fig. 2) in G1 phase. The G1 checkpoint "checks" if the cell is large enough and if the DNA is undamaged. When these conclusions are satisfied, the cell executes Start. A bud emerges and keeps growing; the cell starts DNA synthesis; the spindle pole body duplicates and mitosis commences. (Since the small yeast chromosomes never condense enough to be seen in a light microscope, it is difficult to say exactly when mitosis begins.) At the metaphase checkpoint, the cell verifies that the chromosomes are well aligned on the mitotic spindle and that DNA replication is complete. When these conditions are satisfied, the cell proceeds through anaphase, telophase and cell separation.

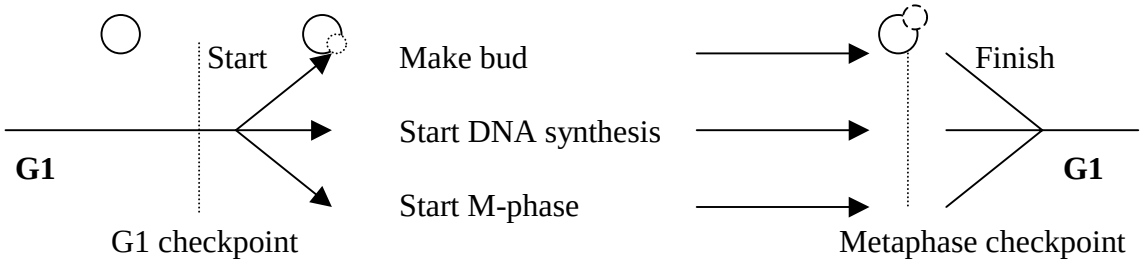


Figure 2 The Budding Yeast Cell Cycle and its checkpoints.

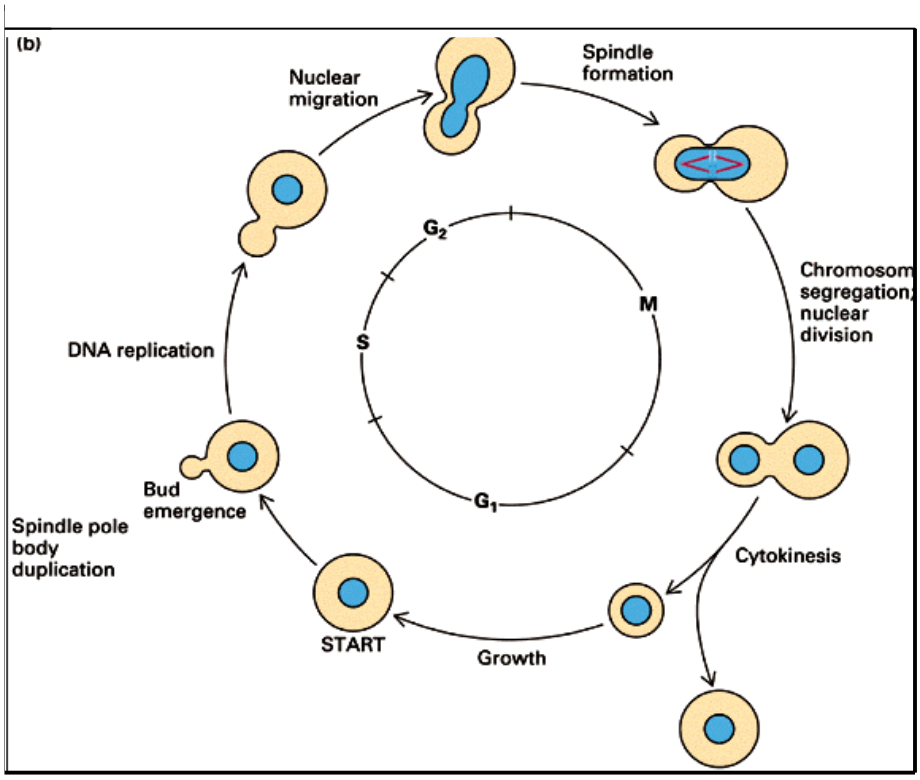


Figure 3. The budding yeast cell cycle. *Saccharomyces cerevisiae* cells divide asymmetrically. The daughter cell grows to a size that allows it to execute Start. A bud emerges and keeps growing until two nuclei are created and cytokinesis occurs. Read text for details.

At cytokinesis, the cell has just divided asymmetrically into a small daughter cell and a large mother cell. Soon after division, the mother cell executes Start, but the daughter cell must grow for some time before they are large enough to Start. Within a few minutes of the Start transition, a bud emerges and DNA replication commences. The bud keeps on growing, and the cell prepares for mitosis. Mitosis divides the nucleus in two nuclei containing identical copies of DNA information. At this point, there are two nuclei in the cell. The nuclei migrate to opposite poles of the cell. The cell constricts at the neck and finally divides into mother and daughter cells. The newborn daughter cell follows the same process.

Chapter 2: Molecular Biology

The following model of exit from mitosis was developed in collaboration with John Tyson and Kathy Chen (at Virginia Tech), and Bela Novak and Attila Csikasz-Nagy (at the Technical University of Budapest).

THE COMPONENTS OF THE MODEL

Cyclin-Dependent Kinases

Some of the major players in cellular regulation are protein kinases, enzymes that activate or inactivate other proteins by transferring a phosphate group from ATP to the target protein. For instance, protein kinases play a major role in relating messages from the cell's environment to its nucleus, to regulate gene expression.

The protein kinases that coordinate the cell cycle are called cyclin-dependent kinases (CDKs). They are composed of a sequence of approximatively 300 amino acids. They bind to cyclins and become active under specific conditions (e.g., a phosphate must be added to a specific threonine side chain).

Progress through the cell cycle requires successive activation and inactivation of CDKs. For instance, at Start, CDKs are activated and at Finish, they are shut off.

In the case of budding yeast, the only essential CDK is Cdc28, always present in excess. Cdc28 binds with specific cyclin partners to form CDK/Cyclin dimers that phosphorylate specific target proteins. The identity of the cyclin partner determines, to a major extent, which target proteins get phosphorylated by the CDK subunit.

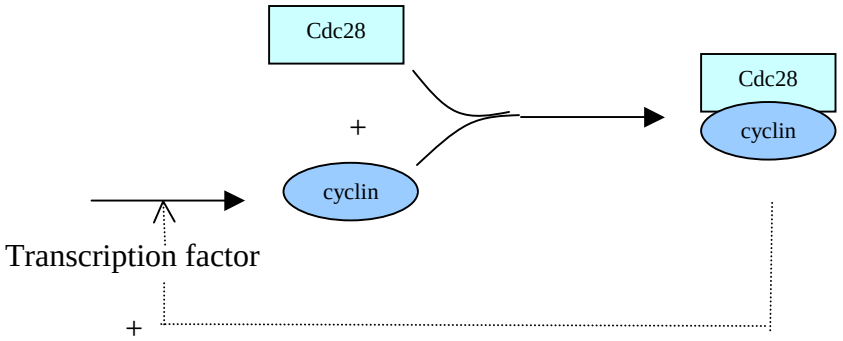


Figure 4 Synthesis of the cyclins, combination with CDK. When referring to the activity of CDK, we will ignore Cdc28 in the complex and consider only the comings and goings of the cyclins.

The cyclin partners that determine the targets of the CDKs are the following:

- Cdc28/Cln1 and Cdc28/Cln2 induce budding.
- Cdc28/Cln3 initiates Start events when cells grow to a critical size.
- Cdc28/Clb1 and Cdc28/Clb2 are essential for successful mitosis.
- Cdc28/Clb5 and Cdc28/Clb6 are responsible for timely DNA replication.

In the paper, we will lump together Cln1 and Cln2 (calling the pair "Cln2"), Clb1 and 2 (calling the pair "Clb2"), and Clb5 and 6 (calling the pair "Clb5").

What is the role of the CDKs in the cell cycle?

At Start, CDK triggers S phase by phosphorylating proteins at origins of replication on the DNA. Simultaneously, it phosphorylates cytoskeletal proteins that determine the location of a new bud. CDK/Cyclin dimers are also responsible for initiating mitotic events, spindle formation and the capture of replicated chromosomes.

To understand the timing of these cell cycle events, we must first understand the sequential activation of CDK/Cyclin dimers.

How are CDK/Cyclin dimers controlled?

There are different ways to regulate the activity of the CDKs in the cell.

1. Availability of cyclin subunits

Cyclin supply is controlled by synthesis and degradation.

a) Synthesis of cyclins

At the beginning of the cell cycle, the transcription factors that control cyclin synthesis are inactive. When the transcription factors turn on, cyclin levels increase considerably. There is a direct correlation between the activity of transcription factors and the cyclins.

For example, SBF controls the synthesis of Cln2 (hypothesis).

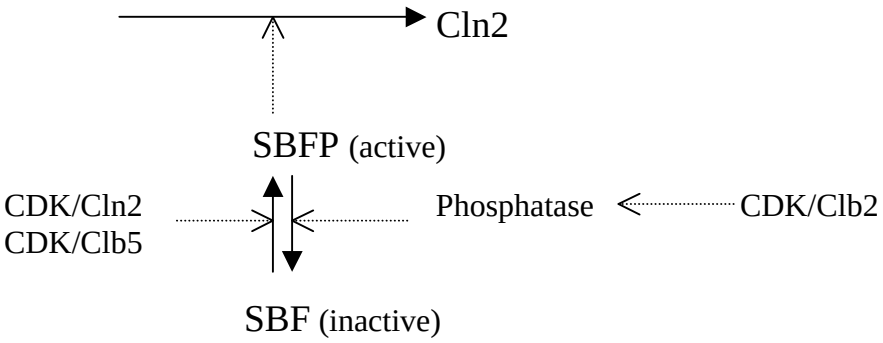


Figure 5. The transcription factor SBF induces the synthesis of Cln2. SBF is phosphorylated by the dimers Cdc28/Cln2 and 3, Cdc28/Clb5, and dephosphorylated by a phosphatase controlled by Cdc28/Clb2.

b) Degradation of the cyclins

Cyclin level drops dramatically at cytokinesis. The cyclins are degraded suddenly as the cell exits from mitosis. An ubiquitin-dependent, proteolytic pathway (Ubiquitin is a small protein, it is a "destroy me" label for the protein degradation machinery) is known to drive the cell out of mitosis.

Different players are involved in the process of ubiquitination and proteolysis. The protein complexes, APC and SCF, catalyze the ubiquitination of substrate proteins. Adaptor proteins assist the APC or SCF by recognizing appropriate substrates and presenting them for ubiquitination. The adaptors are specific to each protein target. Once a protein has been ubiquitinated by the APC or SCF, it is quickly recognized by proteasomes and degraded.

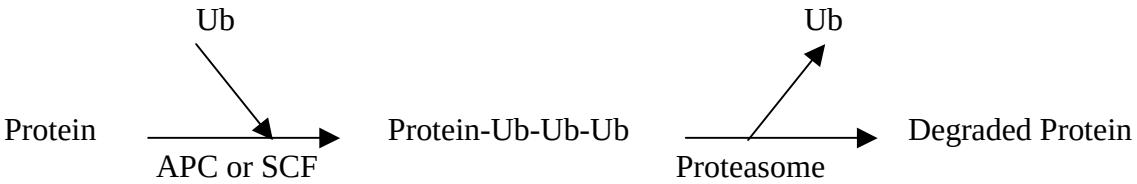


Figure 6. Ubiquitination and proteolysis. The protein is brought to the APC or SCF, where it is tagged by a poly-ubiquitin tail. The tagged protein is then captured by the proteasome and degraded.

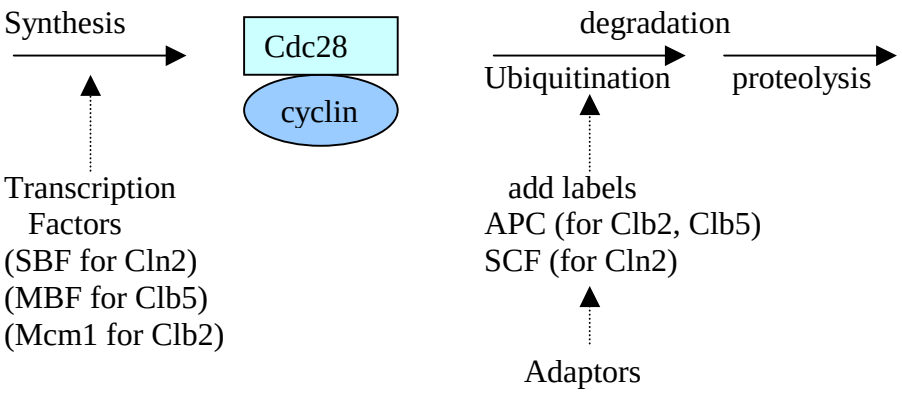


Figure 7. Synthesis and degradation of cyclins

c) Adaptors (Morgan, 1999)

- Cdh1

Cdh1 is an adaptor protein of the APC. Cdh1 recognizes Clb2 and brings it to the APC to be ubiquitinated. It appears in two forms, inactive when phosphorylated by the dimers Cdc28/Clb5 and Cdc28/Cln2 and 3, and active when dephosphorylated by the phosphatase Cdc14. When Cdh1 is active, the level of Clb2 drops considerably.

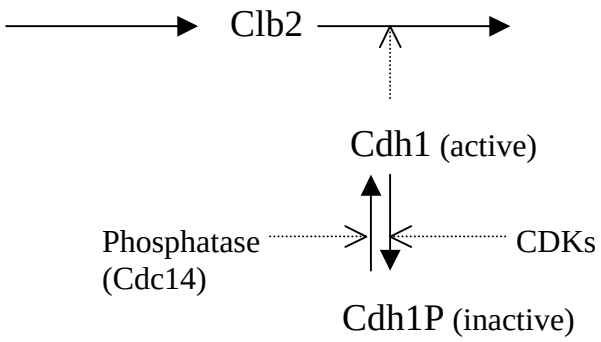


Figure 8. Degradation of cyclin by Cdh1.

- Cdc20

Cdc20 is another adaptor protein of the APC. It targets a number of proteins for degradation, including Clb2, Clb5, and Pds1.

2. Binding to stoichiometric inhibitors

Some Cdc28/Cyclin dimers associate with a third partner to form an inactive trimer.

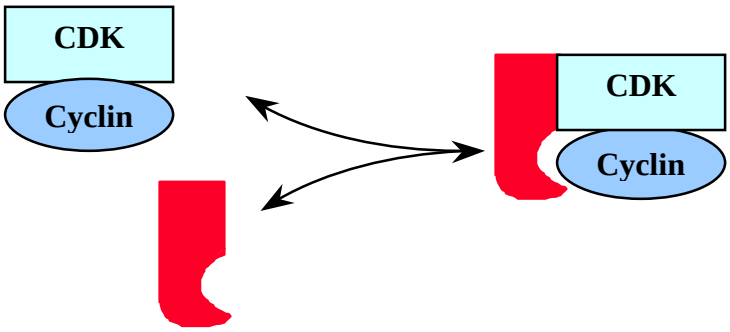


Figure 9. The dimer associates with a CKI (red) to form an inactive trimer.

Such a third partner is called a cyclin-dependent kinase inhibitor (CKI). In budding yeast, the major CKI is a protein called Sic1. The abundance of Sic1 in a cell is also controlled by synthesis and degradation.

a) Synthesis of Sic1

The transcription factor of Sic1 is Swi5. Swi5 appears in two forms, unphosphorylated (active) and phosphorylated (inactive) form.

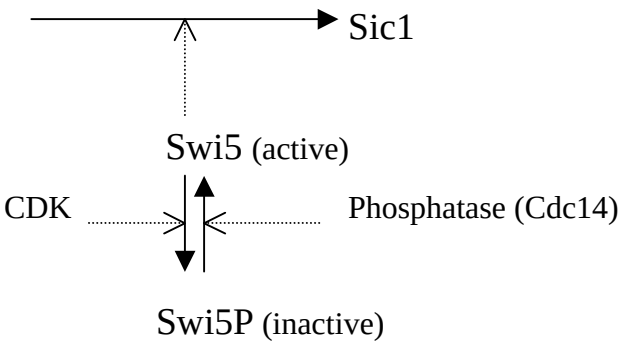


Figure 10. Swi5 functions like SBF. Here, the active form is the unphosphorylated form and the inactive form is phosphorylated. It is activated by Cdc14 and shut off by the CDKs.

b) Phosphorylation and degradation of Sic1

To be degraded, Sic1 needs to be phosphorylated by CDK/Cyclin dimers. Then, an adaptor brings Sic1P to SCF that recognizes the protein and can ubiquitinate it. Degradation follows.

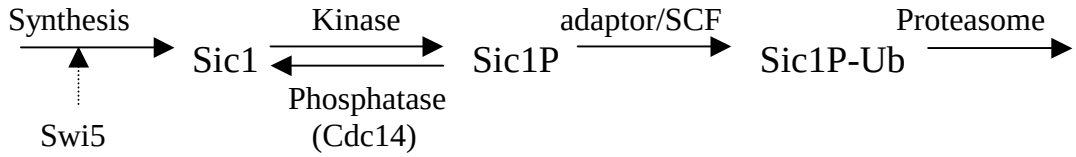


Figure 11. Degradation of Sic1 requires its phosphorylation.

What is the role of the Cdc14 Phosphatase?

The activities of both the cyclin degradation machinery (Cdh1) and the CDK inhibitor (Sic1) seem to be regulated by the phosphatase Cdc14. Visintin (1998) showed that Cdc14 is a major player in helping cells exit from mitosis.

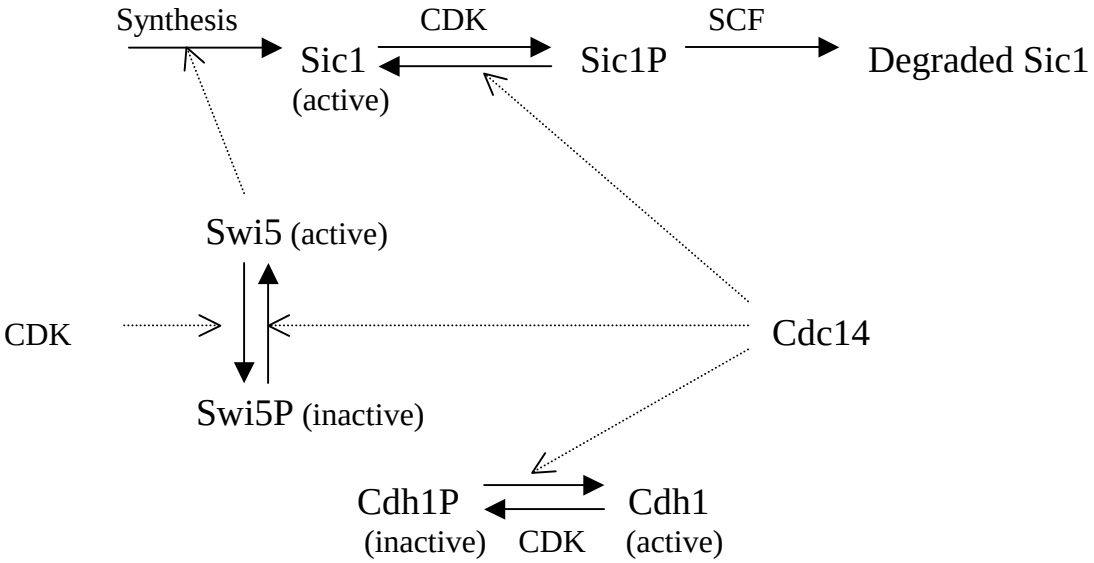


Figure 12. Cdc14 is the phosphatase that activates Cdh1, Swi5, and Sic1.

How is the phosphatase Cdc14 regulated?

1. Cdc14 localization

Cdc14 is synthesized in the cytoplasm and its total level (averaged over the cell) remains constant during the cycle. It becomes active in the nucleus through different pathways involving Cdc5 and Cdc15 (Visintin, 1998). Very little is known about the role of Cdc5 in the activation of Cdc14. We assume in our model that Cdc5 is responsible for the transport of Cdc14 from the cytoplasm to the nucleus.

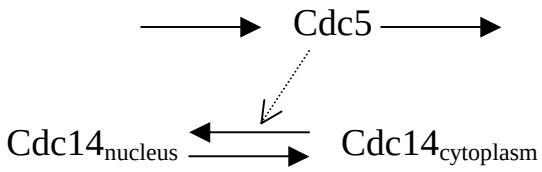


Figure 13. Transport of Cdc14 from the cytoplasm to the nucleus may be promoted by Cdc5.

2. Inactivation of nuclear Cdc14 by association with Net1

As soon as Cdc14 enters the nucleus, it tends to bind with the inhibitor Net1 to form an inactive complex called RENT (Shou 1999; Visintin 1999). It has been proven that Net1 is an inhibitor of Cdc14 by looking at *net1^{ts}*, *net1Δ*, and *NET1^{op}* mutants. Cdc14 rises in anaphase, when it is released from RENT complexes.

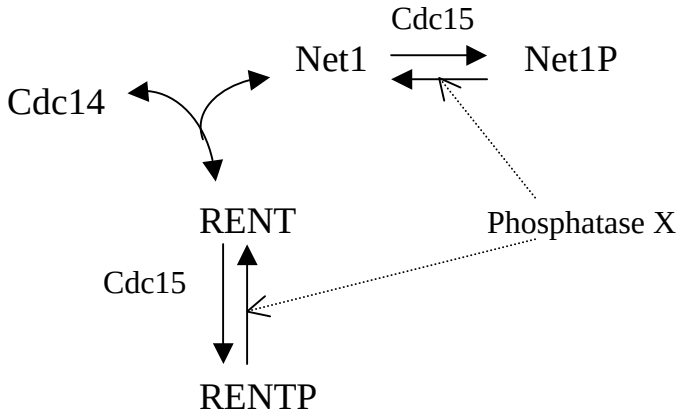


Figure 14. Inactivation of Cdc14. Cdc14 binds to the nuclear protein Net1 to form an inactive complex called RENT (REgulation of Nucleolar silencing and Telophase exit).

3. Activation of Net1

a) Kinase (Cdc15)

We propose that Cdc15 is a protein kinase that phosphorylates and inactivates Net1. When Net1 is phosphorylated, Cdc14 is released from the RENT complex.

b) Phophatase (PPX)

The phosphatase that activates Net1 has not yet been identified with a gene in budding yeast. We shall call it PPX. PPX plays a crucial role in exit from mitosis. We know that Cdc15 activity is constant throughout the cycle, so we assume that Net1 phosphorylation state depends on PPX and that PPX is degraded by Cdc20/APC at anaphase, rendering Net1 inactive and Cdc14 released from the RENT complex.

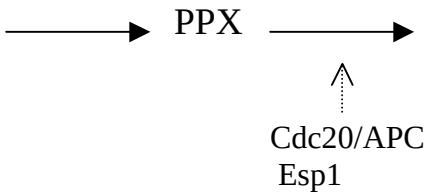


Figure 15. Activity of the hypothetical phosphatase PPX (Morgan, 1999).

c) The protease Esp1

PPX is also degraded, we assume, by Esp1, a protease involved in dissolving the glue that holds sister chromatids together. Total Esp1 level is constant throughout the cycle. However, during S/M phase it is kept inactive by binding to Pds1 (Koshland, 1996).

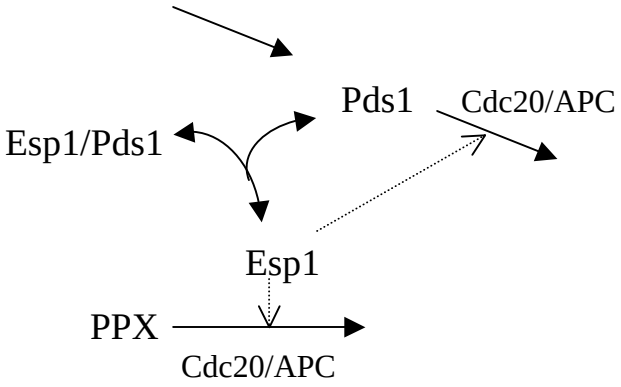


Figure 16. Degradation of PPX by Esp1. Esp1 is kept in complex with Pds1 until Pds1 is degraded. Esp1 is then released and can degrade PPX.

When Cdc20 is activated, Pds1 is degraded, releasing Esp1 which then dissolves the glue holding the sister chromatids together. We assume that Esp1 helps Cdc20 to degrade the hypothetical phosphatase, PPX, that was keeping Net1 in its unphosphorylated (active) form. As PPX disappears, Net1 is phosphorylated by Cdc15 and releases Cdc14 from the RENT complex.

How is Cdc20 activated at the Finish transition?

Cdc20 is missing during G1 phase and accumulates during S/M phase. However, it is not activated until the metaphase-anaphase transition. Prinz (1998) showed that Cdc28/Clb2 stimulates the synthesis of Cdc20 in an inactive form. Cdc20 does not activate until the cell reaches metaphase. In her model, Chen introduced a strong inhibitory signal on Cdc20 until the chromosomes are fully aligned on the metaphase plate. In our current model, we assume that Cdc28/Clb2 activates Cdc20 through an intermediary enzyme (IE), allowing a time delay for the chromosomes to align. IE is active when phosphorylated by Clb2 (IE becomes IEP, the active form of the enzyme). When IE becomes active, it competes with another signal coming from a switch turned on and off by the checkpoint protein Mad2, which is a sensor of unaligned chromosomes. As Clb2 goes away, IE is dephosphorylated and Cdc20 inactivates.

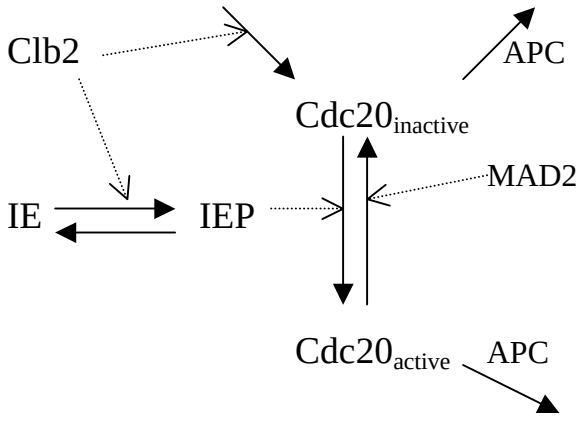


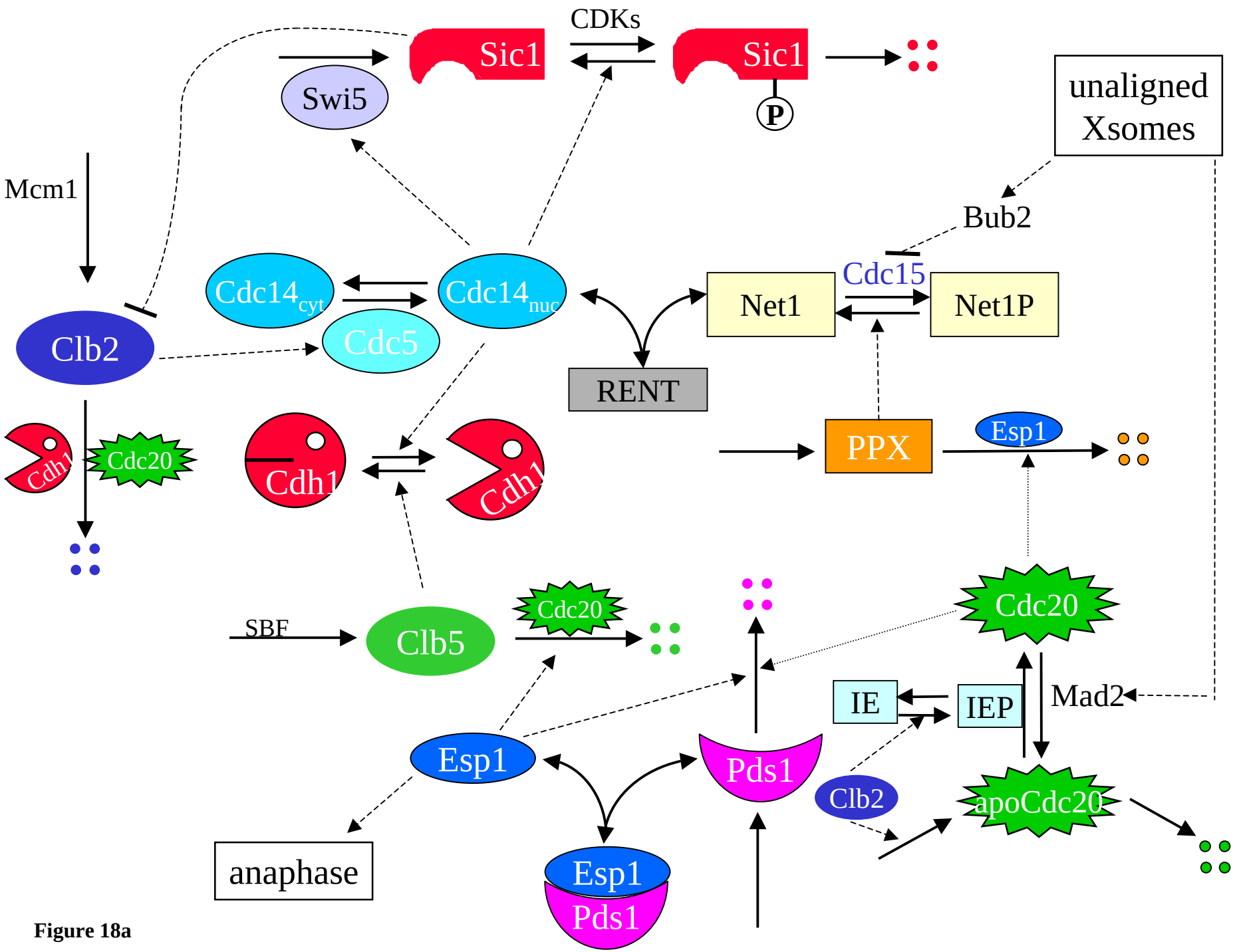
Figure 17. Activation of Cdc20. Details in the text.

Once Cdc20 is activated and the mechanism described earlier is enabled, Cdc14 is eventually released from the RENT complexes and pushes the cells out of mitosis by dephosphorylating Cdh1, Sic1 and Swi5.

THE FULLY ASSEMBLED MODEL

All the pieces are put together into a hypothetical mechanism (Fig. 18a) for the Finish transition in budding yeast. For the Start transition, we use the mechanism proposed by Chen et al. (Fig. 18b). Chen's model was adapted slightly to conform with our mechanism for the Cdc20-Cdc14 pathway in Fig. 18a. Unlike Chen et al., we now assume that Clb5 plays a major role in keeping Cdh1 inactive.

Figure 18 A comprehensive model for the budding yeast cell cycle. **(a)** Finish. Cdc10 activates Cdc14 indirectly, by degrading a hypothetical phosphotase PPX. See text for details. **(b)** Start. See Chen et al. (2000) for details.


CDKs
Sic1
Sic1
P
Swi5
unaligned
Xsomes
Mcm1
Bub2
Cdc14cyt
Cdc14nuc
Cdc15
Net1
Net1P
Clb2
Cdc5
RENT
Cdh1
Cdc20
Cdh1
Cdh1
PPX
Esp1
SBF
Clb5
Cdc20
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Mad2
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Pds1
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apoCdc20
anaphase
Esp1
Pds1

Figure 18a

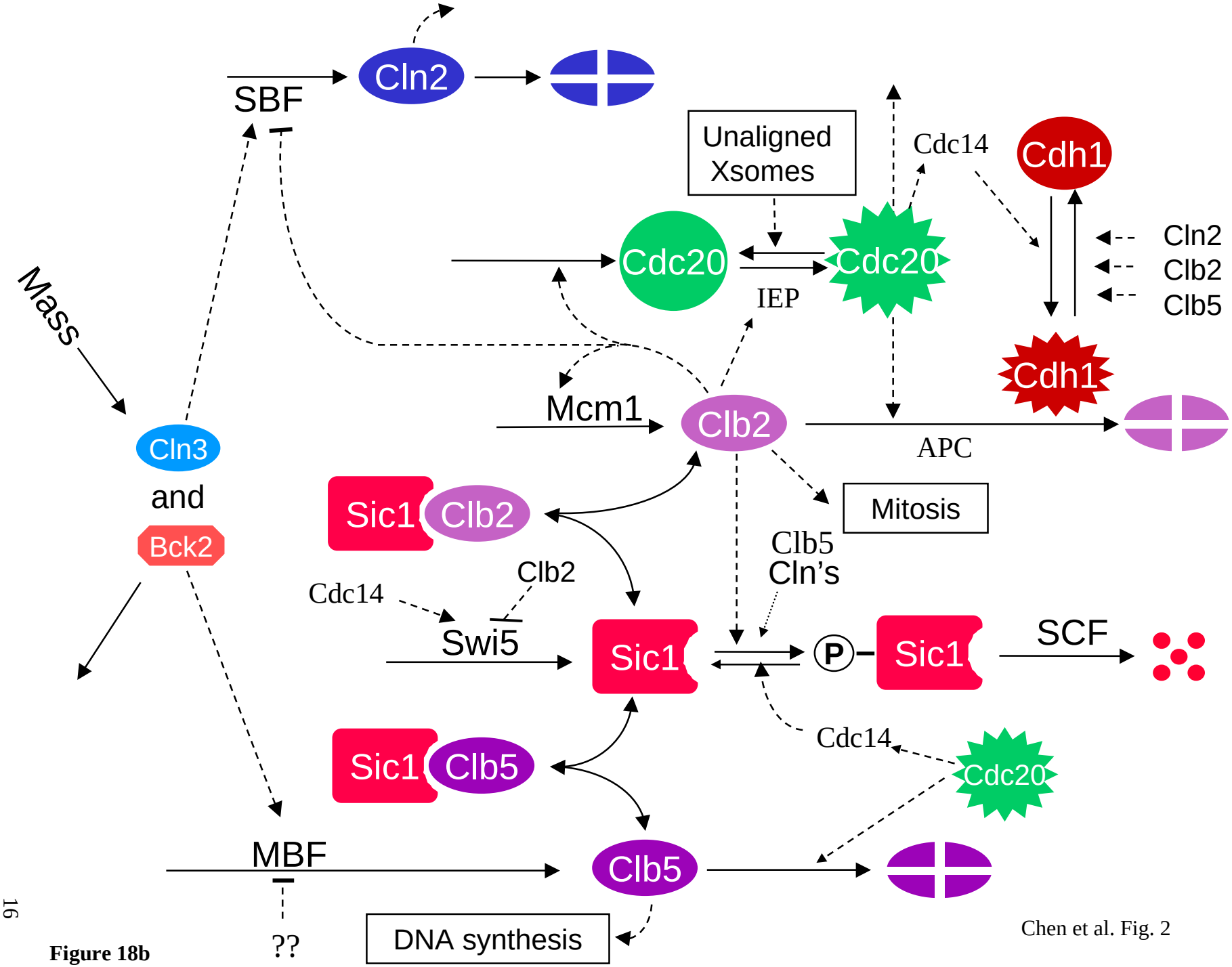

Figure 18b

Chen et al. Fig. 2

Chapter 3: Nasmyth proposal

In his papers "Evolution of the cell cycle" (1995) and "At the Heart of the Budding Yeast Cell Cycle" (1996), Nasmyth proposed the idea of two alternating, self-maintaining stable states of the cell cycle: G1 and S/M. What characterizes these two states?

- In the G1 state, CDK activity is low, cyclin synthesis is off, cyclin degradation is on, and CKI is present. The G1 stable steady state is self-maintaining because the degradation machinery keeps cyclin level low and the presence of CKI keeps CDK activity inhibited.
- In the S/M state, CDK activity is high, cyclin synthesis is turned on, cyclin degradation is off, and CKI is rapidly degraded. The S/M stable steady state is also self-maintaining because CDK activity turns Cdh1 off (and cyclin degradation machinery off) and phosphorylates Sic1, which causes its rapid degradation.

The passage from G1 to S/M, called the "Start" transition, and the reverse, from S/M to G1, called the "Finish" transition.

Start is driven by cell growth. A cell must be large enough to start a new cycle; the G1 checkpoint keeps the cell in G1 phase until it reaches an acceptable size. It is important that daughter cells must grow to a size threshold. Because cells can replicate their genetic information more quickly than all the other cellular components, if there were no size threshold, cells would simply divide at a smaller and smaller size, until they die. From a molecular point of view, at Start, cyclin synthesis is turned on, cyclin degradation is turned off and CKI is destroyed.

Finish is governed by chromosome alignment on the mitotic spindle. A cell halts in metaphase, until its chromosomes are all aligned on the metaphase plate. Then the glue that holds sister chromatids together is dissolved by the APC and the chromatids are pulled to the opposite poles of the spindle. At Finish, cyclin synthesis is turned off, cyclin degradation is turned on and CKI is produced.

We begin with a simple model (Tyson and Novak, 1995) that shows two stable steady states and what triggers transitions between them.

MATHEMATICAL MODEL

The following wiring diagram describes the interactions between the two proteins CycB and Cdh1.

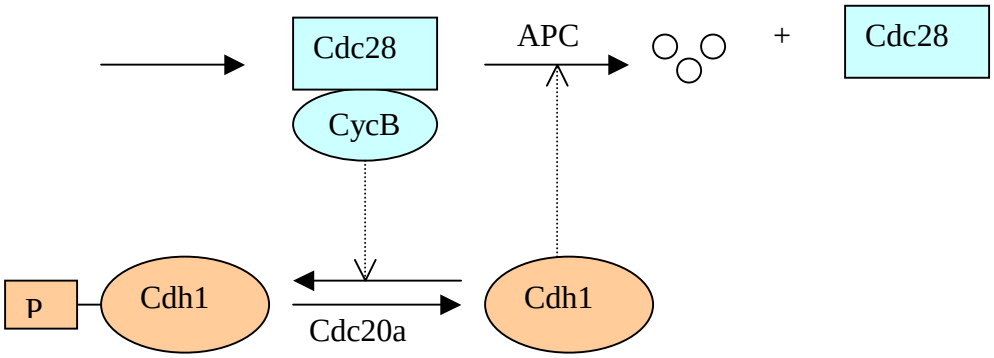

Figure 19. Simple model illustrating the fundamental antagonism between Cyclin B and Cdh1.

In this simple model, the B-cyclin is synthesized at a constant rate. Its degradation is regulated by the protein, Cdh1. Cdh1 appears in two states, active and inactive. When Cdh1 is phosphorylated by the B-cyclin, it becomes inactive. In this simple model, Cdh1 is activated by Cdc20a, whose activity is considered as a parameter.

The following differential equations allow us to follow the activity of each protein.

$$\frac{d[CycB]}{dt} = k_1 - (k_2 + k_3 \cdot [Cdh1]) \cdot [CycB]$$

$$\frac{d[Cdh1]}{dt} = \frac{(k_5 + [Cdc20a]) \cdot (1 - [Cdh1])}{J_5 + 1 - [Cdh1]} - \frac{k_6 \cdot m \cdot [CycB] \cdot [Cdh1]}{J_6 + [Cdh1]}$$

Where [CycB] = concentration of the Cyclin B, averaged over the whole cell.

[Cdh1] = relative activity of Cdh1 in nucleus.

m·[CycB] = concentration of cyclin B in the nucleus (where it accumulates).

and $m = \frac{\text{Volume of the cytoplasm}}{\text{Volume of the nucleus}}$

Parameters	Values	Parameters	Values
k_1	0.07	k_6	0.64
k_2	0.05	J_5, J_6	0.05
k_3	2.95	m	1.5
k_5	0.17	[Cdc20a]	0

We assume that the total amount of Cdh1 is 1. In the equation, Cdh1 represents the active form of the protein. When phosphorylated, it becomes inactive. The activity of Cdh1 is constructed as an ultra sensitive-switch between its two forms (Goldbeter and Koshland, 1981). At the beginning of the cycle, Cdh1 is active, i.e., [Cdh1] ≈1. When [CycB] is high enough to compete with the constant [Cdc20a], the system changes quickly to [Cdh1] ≈ 0.

We simulate this mathematical model with WinPP. In Fig. 20, we plot [Cdh1] as a function of [CycB]. The green curve represents the nullcline of Cdh1 and the red one, of CycB. To determine the equations of the nullclines, we set the derivatives equal to 0 to obtain:

$$[\text{CycB}] = \frac{k_1}{k_2 + k_3 \cdot [\text{Cdh1}]} \quad \text{(red curve)}$$

$$[\text{CycB}] = \frac{J_6 + [\text{Cdh1}]}{J_5 + 1 - [\text{Cdh1}]} \cdot \frac{1 - [\text{Cdh1}]}{k_6 \cdot R \cdot [\text{Cdh1}]} \quad \text{(green curve)}$$

$$\text{where } R = \frac{m}{k_5 + [\text{Cdc20a}]}$$

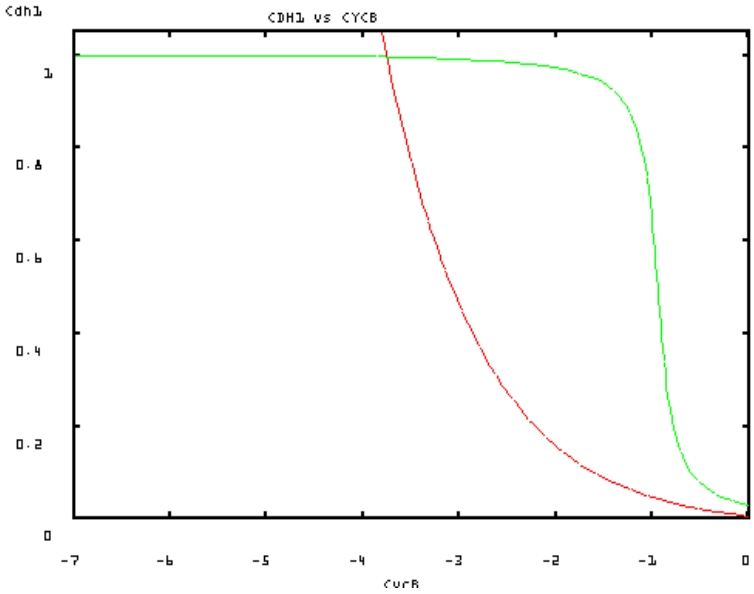

Figure 20. Phase plane portrait. CycB, on the abscissa is expressed in a $\log_e$ scale. The two curves cross at a stable steady state called G1. In this case, R=4.

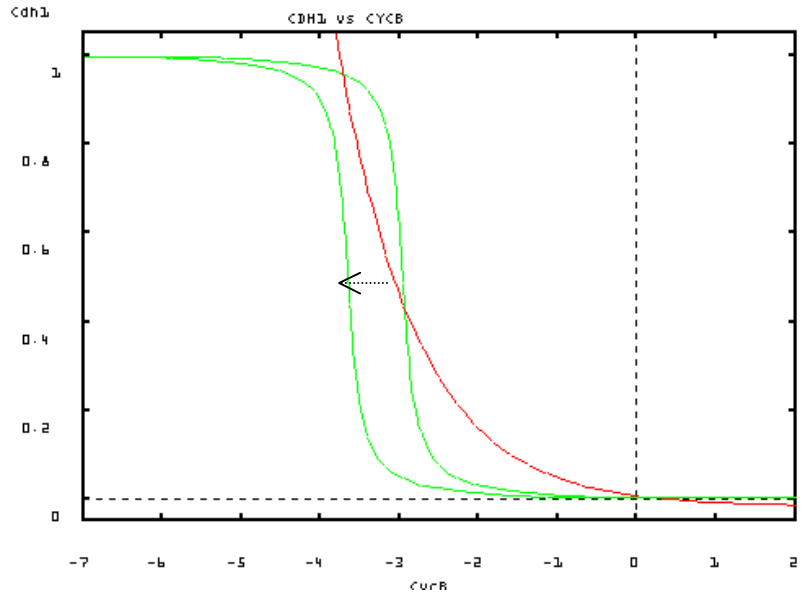

Figure 21. Phase plane portrait. CycB, on the abscissa is expressed in a $\log_e$ scale. R=30 for the green Cdh1-nullcline on the right. Here, there are two stable steady states (G1 and S/M) and one unstable steady state (middle equilibrium point). R=60 for the Cdh1-nullcline on the left. The system is in S/M phase. The red CycB-nullcline does not depend on R.

In the first phase plane (Fig. 20), we set R=4. Just after cell division, the small daughter cell is in G1 with plenty of active Cdc20. There is one stable steady state on the upper branch of the Cdh1 nullcline, keeping the system in G1 phase (Cdh1 is on and CycB is scarce). As the cell grows, *m* increases and [Cdc20a] decreases (because Cdc20 is degraded during G1 phase - not shown on the wiring diagram). It follows that the ratio R increases and the Cdh1 nullcline moves to the left (Fig. 21). The G1 checkpoint is lifted when we reach the saddle node bifurcation, leading to the first transition, Start. This transition carries the cell into a new stable steady state where Cdh1 is off and CycB is abundant. The high activity of Cdc28/CycB drives the cell into S/M phase.

The cell is now in S/M phase at a new stable steady state (the metaphase checkpoint) with Cdh1 off and high level of CycB. As chromosomes come into alignment on the metaphase plate, Cdc20 is activated and the green curve moves to the right until another saddle node bifurcation is reached. This second bifurcation lifts the metaphase checkpoint and induces the Finish transition. Cdc20a keeps on accumulating for a while until division. We are now back to Fig. 20.

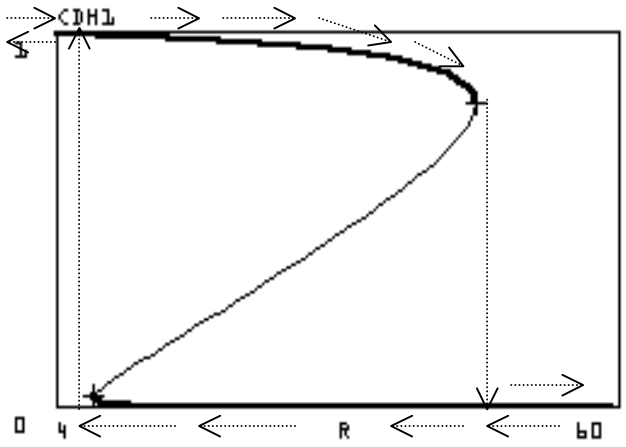

Figure 22. Bifurcation diagram of the cell cycle.
Cdh1 is expressed as a function of the parameter R.
At the two bifurcations, R=46 and R=7.8.
$R=m/(k_5+[\text{Cdc20a}])$

This bifurcation (Fig. 22) illustrates the main successive events that control the cell cycle. A newborn cell is in G1 phase with Cdh1 on and a low concentration of CycB. As the cell grows, *m* increases and [Cdc20a] decreases. As a result, R increases, and the

control system moves along the upper branch of stable steady states towards the saddle node bifurcation. As long as G1 state is stable, the cell is arrested in G1. When the G1 steady state is lost at the Saddle Node bifurcation, the checkpoint is lifted and the cell can enter S phase. The control system moves to the lower branch of the diagram (Fig. 22). This transition is called “Start”. The cell enters S/M phase. CycB is now high and switches off Cdh1 activity. Mass keeps increasing and so does *R*. Cdc20 starts accumulating but in its inactive form. After chromosome replication and chromosome alignment are complete, Cdc20 is converted to its active form, decreasing the ratio R until the system reaches the lower saddle node bifurcation. The second transition, Finish, is induced. The control system jumps to the upper branch of the diagram, in G1 phase.

As the mass divides, *R* decreases more. A newborn cell is ready for a new cycle.

In this model, checkpoints correspond to stable steady states; checkpoints are imposed or lifted by saddle node bifurcations, which create or destroy stable nodes. Start - or Finish - is the transition to a new steady state once a checkpoint is removed by a saddle node bifurcation.

Chapter 4: Budding Yeast Cell Cycle

MATHEMATICAL MODEL

In chapter 2, we formulated a mechanism for control of the budding yeast cell cycle in terms of certain regulatory molecules: cyclins, transcription factors, proteolytic enzymes. Each arrow in the mechanism (Fig. 18) denotes how the activity (or concentration) of a component changes with time. Since the activity of one component influences the activity of others and because these interactions form such a complex system, differential equations are a useful tool to explain the dynamical interactions in the molecular control system.

From Fig. 18, we composed a system of twenty-two nonlinear ordinary differential equations describing the fluctuations of the cyclins and their regulatory proteins and four auxiliary differential equations controlling different events of the budding yeast cell cycle such as growth, DNA replication, bud emergence and chromosome alignment (Appendix B). Two more functions were added to describe the activities of the transcription factors SBF and Mcm1.

Once the kinetic equations are specified, the parameters need to be defined properly and fit carefully to experiment (Appendix C). Then, given initial conditions for all components, the differential equations can be solved to predict the temporal behavior of the control system.

To simulate the model, we used a software program Solver (Borland C++ for DOS), with a variable time-step, Runge-Kutta integrator, a tolerance of 10^{-5} and a maximum step size of 10^{-3}.

SIMULATION

Wild-type cells

A simulation of the wild type budding yeast cell cycle is shown in Figure 23.

As new cells are born, at the beginning of the cycle, Cdh1 is on, and Sic1 is present. When SBF turns on, the synthesis of Cln2 and Clb5 is induced. As a result, Cdh1 turns off and Sic1 level drops at the same time as Cln2, Clb5, and Pds1 levels rise. When the transcription factor of Clb2 turns on, Clb2 level rises.

As Clb2 comes up, it shuts off SBF, so Cln2 level drops to zero, because Cln2 is very unstable. Clb5 level does not drop because it is stable until Finish. Clb2 activates Cdc5 and induces the synthesis of Cdc20 and later activates it through an intermediary enzyme. Cdc20a starts anaphase events by degrading Pds1, PPX, and the B-cyclins.

When Cdc20a degrades Pds1, it releases Esp1 that was in complex with Pds1. Esp1 initiates anaphase by destroying the glue that was holding sister chromatids together. Esp1 also helps Cdc20a degrade PPX and Clb5. PPX disappears and Net1 can finally be phosphorylated. Cdc14 was in complex with Net1. At that point, Cdc14 is freed from the RENT complex and activates Swi5 (the transcription factor of Sic1) and Cdh1, and stabilizes Sic1. Cdh1 is now active and can degrade Clb2, and Sic1 keeps any remaining Clb2 in inactive ternary complex. When Clb2 level drops, the cell divides, and a new daughter cell is born.

Mutants

We summarize the main mutants in Table 2.

Tampering with Clb2 removal at telophase.

Clb2-dependent kinase activity is kept low in G1 phase by three different effects. First, Clb2 is not synthesized because its transcription factor is inactive. Second, its degradation machinery, regulated mainly by Cdh1, is active. Third, any Clb2, which may be present, in complex with Cdc28, is inhibited by the CDK-inhibitor Sic1. If any one of these three effects is removed by mutation, the two others can assure viable cell cycles. In a *sic1Δ* mutant, cells are viable, although they divide at a smaller size. In a *cdh1*Δ mutant, Clb2 cannot be degraded completely, but nonetheless cells exit mitosis successfully since Sic1 inhibits all the cyclin-B activities. However, the double mutant *cdh1Δ-sic1Δ* is not viable.

At the end of mitosis, Clb2 is degraded by both Cdc20 and Cdh1. We just mentioned the effect of Cdh1 deletion. What happens when Cdc20 is deleted? Prinz et al. (1998) showed that a *cdc20^{ts}* mutant is not viable. The cell arrests in metaphase (Fig 24 a.). According to the mechanism, Cdc20 cannot degrade Pds1. Since Pds1 keeps Esp1 in complex, the glue holding the sister chromatids together cannot be dissolved and they remain attached. Also, the absence of both Cdc20 and active Esp1 allow the accumulation of PPX, which keeps Cdc14 sequestered in the RENT complex, disabling it from activating Cdh1. Anaphase cannot occur and the B-cyclins cannot be degraded.

The double mutant *cdc20^{ts}-clb5Δ* (Fig. 24 c.) has the same phenotype as a *cdc20^{ts}* since in both mutants, PPX is not deleted by Cdc20. Cdc14 is kept in the complex, leaving the CDK responsible for keeping Cdh1 phosphorylated. Even though Clb5 is absent, Clb2 is still phosphorylating Cdh1 and the cell arrests in M phase.

Shirayama et al (1998) showed that Cdc14 is free in *cdc20^{ts}-pds1Δ* mutant (Fig. 24 b.) despite that Cdh1 is not activated and Clb2 not degraded. Esp1 is free from the complex and therefore able to degrade PPX. If PPX is degraded, Net1 is phosphorylated and cannot bind to Cdc14. Thus, Cdc14 can be freed from the RENT complex. However, due to the deletion of Cdc20, Clb5 is not degraded, and its high level enables it to compete with the level of free Cdc14 to keep Cdh1 inactive. The cells are blocked in telophase. This result suggests that despite the presence of free Cdc14, *cdc20^{ts}-pds1Δ* mutant still cannot degrade the B-cyclins. Cdc20 must have another substrate besides Pds1 in wild type cells. Shirayama et al. (1999) reasoned that *cdc20^{ts}-pds1Δ* mutant might be rescued by a third mutation. They found that the other target was Clb5. Indeed, *cdc20^{ts}-pds1Δ-clb5Δ* mutant (Fig. 24 d.) is viable. If we delete Clb5 in *cdc20^{ts}-pds1Δ* mutant where Cdc14 was free, then Cdh1 is activated and the cell no longer arrests in telophase. This experimental result correlates with our simulation, and justifies the place of each component in the wiring diagram.

Once Cdc20 is activated, a complex pathway leads to the activation of Cdc14 and exit mitosis. Close analysis of several mutants involved in Cdc14 activation will help us understand this complicated mechanism.

Tampering with Cdc14 and its regulation (Fig. 25-26).

Both *cdc5*^{ts} (Fig. 25) and *cdc15*^{ts} (Fig. 26) mutants arrest in telophase. In our wiring diagram, Cdc5 is partly responsible for transporting Cdc14 into the nucleus. At the non-permissive temperature (when Cdc5 is inactive), this transport will be considerably decreased; the small amount of Cdc14 that enters the nucleus is kept in the RENT complex and never released.

Analysis of Net1 mutants appears to be the next logical step. Visintin et al. (1999) showed that *net1Δ* is viable, so is *cdc15*^{ts}-*net1Δ* double mutant. They also showed that *net1Δ* can partially rescue *cdc5*^{ts}. Jaspersen et al. (1998) were able to rescue *cdc15*^{ts} by over-expressing Sic1 and Cdc14. Our simulations (not shown) agree with these experimental observations.

In a *net1Δ* mutant (Fig. 28 a.), Cdc14 is not sequestered and is active, so Cdc15 is not needed for its liberation. As soon as Cdc5 is activated by Clb2, Cdc14 is transported into the nucleus and is able to activate Sic1, Swi5 and the degradation machinery, hence *net1Δ*-*cdc15*^{ts} mutant is viable. In the double mutant *net1Δ*-*cdc5*^{ts}, although Cdc5 is not very active at the non-permissive temperature, there is a small amount of Cdc14 in the nucleus, which is enough to push the cell out of mitosis. Over-expression of Cdc14 has similar effect as *net1Δ*, which explains its ability to release *cdc15*^{ts} from telophase arrest (but the double mutant will arrest in the next G1 because of *CDC14*^{op} phenotype). *SIC1*^{op} effectively decreases the amount of active Cdc14 needed to win over CDK activity in their battle in the activation of cyclin degradation, such that the double mutant *cdc15*^{ts}-*SIC1*^{op} becomes viable.

Predicted phenotypes of PPX mutants

The mutant *ppxΔ* would keep Net1 in its phosphorylated form, unable to bind and sequester Cdc14; hence *ppxΔ* should behave similarly as *net1Δ* or *CDC14*^{op}. The mutant is viable.

On the other hand, *PPX*^{op} will have a phenotype similar to *cdc14Δ* (Fig. 27 a.). PPX over-expression will keep Cdc14 in the RENT complex, arresting the cells in telophase. Over-expression of Cdc14 or Cdc5 should then rescue the mutant, as indicated

in our simulations (data not shown). In the case of $CDC14^{op}$-PPX^{op}, cells can divide but then block in G1 (according to the phenotype of $CDC14^{op}$ – Fig. 27 b.). Our model predicts that PPX^{op}-$CDC15^{op}$ mutant is viable since they work against each other in the dephosphorylation/phosphorylation of Net1.

In our model, Pds1 binds to and inhibits Esp1 such that sister chromatids are not separated until chromosomes are aligned and Cdc20 is activated (which degrades Pds1). The mutant *pds1dbΔ* (the destruction box within Pds1 protein is removed so that Pds1 is not recognized by the APC^{Cdc20} complex) arrests in M phase, with sister chromatids not separated and the B-cyclins not degraded. E$sp1^{ts}$ mutant should, according to our model, behave like *pds1dbΔ*. However, experimental evidence indicates that the two mutants behave differently. According to Surana (1993), $esp1^{ts}$ mutant cannot dissolve the glue that holds the sister chromatids together, but cytokinesis occurs on schedule. As a result, one of the two daughters inherits both chromosome sets while its sister is largely haploid. Since $esp1^{ts}$ is capable of degrading the B-cyclins, this suggests that Pds1 has additional mechanisms in protecting the B-cyclin degradation.

Metaphase checkpoint mutants.

When treated with nocodazole or benomyl (drugs that prevent microtubule polymerization, causing destruction of the spindle, and therefore making chromosome alignment impossible), wild-type cells halt at the mitotic phase, i.e., their chromosomes are condensed, with the sister chromatids still attached, and CDK activity high until the drugs are removed. If the drug is washed off, the cells resume their cycle undamaged, indicating that the cells have mitotic surveillance mechanisms that prevent Pds1 and Clb2 degradation.

Mutants that are not arrested in the presence of nocodazole or benomyl have allowed the identification of several genes involved in these surveillance mechanisms: MAD1-3 (for “Mitosis Arrest Deficient” mutants) and BUB1-3 (for “Budding Uninhibited by Benomyl” mutants). If the drugs are not present in the growth medium, cells with deletions of these genes are perfectly normal.

Recently, Alexandru et al. (1999) have studied these mutants and found that the genes can be catalogued in two groups: five of them (MAD1,2,3 and BUB1,3) are

required for inhibiting Pds1 degradation by Cdc20/APC (thus preventing sister chromatid separation), whereas the gene BUB2 is essential to prevent Clb2 degradation by Cdh1/APC (keeping Clb2 activity high). Their observations are the following:

Strain	No noc.	+ noc (<5hr) Sister Separation	+ noc (<5hr) Clb2 degradation	Viability after removal of nocodazole
WT	Viable	No	No	Yes
mad2Δ	Viable	Yes	No	No
bub2Δ	Viable	No	No	Yes
mad2Δ bub2Δ	Viable	Yes	Yes	No
pds1Δ	Viable	Yes	No	No

Table 1. Summary of Alexandru's experiments (1999)

In our model, if the cell cycle does not encounter any problems in spindle function, Mad2 and Bub2 are not activated. Therefore, a simulation of the *mad2-bub2* mutant, when cells are not treated with nocodazole, is simply equivalent to that of the wild type.

When treated with nocodazole, wild-type cells arrest with Clb2 level high and stable, Pds1 level is also high. Under these conditions, Mad2 and Bub2 are activated. Mad2 (increase k_{ai} from 0.1 to 19) keeps Cdc20 in its inactive form, so Pds1 is not degraded; thus, Pds1 can bind to and inactivate Esp1 (Esp1 level is low so sister chromatids are still attached), whereas Bub2 (decrease the amount of Cdc15 from 10 to 0.1) keeps Net1 dephosphorylated; Net1 can bind and inactivate Cdc14, so Clb2 can be accumulated to high level.

For the *mad2* mutant in nocodazole (if the incubation period is not too long), Cdc20 becomes activated (because of the defective checkpoint gene), so Pds1 is degraded, Esp1 is freed, sister chromatids are separated and anaphase is initiated.

Because Bub2 is functional, it can keep Cdc14 trapped in the nucleolus. Since Cdc14 is not released, Clb2 is not degraded. If nocodazole is washed away after this short incubation and the cells are placed in a fresh medium without nocodazole, these mutants are not viable, because their chromosomes are not properly separated. However if the *mad2* mutant is kept in nocodazole for a long time, some mechanism causes cell division and the cells die.

The behavior of the *bub2* mutant in nocodazole is different. In this mutant, Mad2 is functional, so Cdc20 is not activated, Pds1 not degraded and the glue that holds the sister chromatids is still intact. Although Cdc15 is not inhibited (because of the mutation on Bub2), Net1 still remains mostly in the dephosphorylated form, because PPX level is high (since Cdc20 is inactive and Esp1 level is low), therefore Cdc14 is mostly trapped in the complex. Also, because Clb5 level is high (since Cdc20 is inactive, its degradation rate is low), the cells need higher level of free Cdc14 to activate Cdh1. Therefore, the *bub2* mutant in nocodazole (if the incubation period is not too long) are properly arrested, their sister chromatids stay together and their Clb2 level is high. So if nocodazole is washed off after this brief drug treatment, the cells are viable. If, however, the cells are treated with nocodazole for long periods, eventually the active form of Cdc20 reaches a critical level to cause Pds1 degradation and Cdh1 activation. The cells exit mitosis, initiate new buds and die.

Our simulation of the wild-type cells in nocodazole (Fig. 29) shows some degradation of Pds1 early in the cycle, at t=150min which contradicts Alexandru's experiments. Clearly the model is incorrect at this point, and more investigation needs to be done to find the right explanation of nocodazole treatments in wild type and mutant cells.

Table 2: **Phenotypes of mutants**

	Phenotypes	*Changes in parameter values*
WT	Cell divides at m=1.87	
cdc20^{ts}	arrest in metaphase	$V_{2,20}$, $k_{d,b5}$', $k_{d,b5}$, $k_{d3,pds}$, $k_{d4,pds}$, $k_{d3,ppx}$, $k_{d4,ppx}$ /1000
cdc20^{ts}-pds1Δ	arrest in anaphase	*cdc20^{ts}* + $k_{s,pds}$ = 0
cdc20^{ts}-clb5Δ	arrest in metaphase	*cdc20^{ts}* + $k_{s,b5}$' = $k_{s,b5}$ = 0
cdc20^{ts}-pds1Δ-clb5Δ	viable, cell divides at m=1.94	
ppxΔ	viable	$k_{s,ppx}$ = 0
cdc20^{ts}-ppxΔ-clb5Δ	viable, cell divides at m=1.94	*cdc20^{ts}* + $k_{s,ppx}$ = $k_{s,b5}$' = $k_{s,b5}$ = 0
esp1^{ts}	viable	$k_{d,b5}$'', $k_{d,b5}$', $k_{d2,pds}$, $k_{d3,pds}$, $k_{d2,ppx}$, $k_{d3,ppx}$ /20
cdc5^{ts}	arrest in telophase	$k_{s,c14}$, $k_{s,c14}$' /10
cdc15^{ts}	arrest in telophase	Cdc15 = 0.1
cdc14Δ	arrest in telophase	$k_{s,c14}$' = $k_{s,c14}$ = 0
CDC14^{op}	arrest in G1	$k_{s,c14}$' = $k_{s,c14}$ = 0, $k_{s,c14}$'' = 2
net1^{ts}	viable	$k_{as,net,c14}$, k_{ac2} /100
NET1^{op}	arrest in telophase	Net1T = 30
cdc5^{ts}-net1^{ts}	rescued	*cdc5^{ts}* + *net1^{ts}*
cdc15^{ts}-net1^{ts}	rescued	*cdc15^{ts}* + *net1^{ts}*
cdc5^{ts}-CDC14^{op}	rescued but blocked in G1	*cdc5^{ts}* + *CDC14^{op}*
cdc15^{ts}-CDC14^{op}	rescued but blocked in G1	*cdc15^{ts}* + *CDC14^{op}*

Wild type

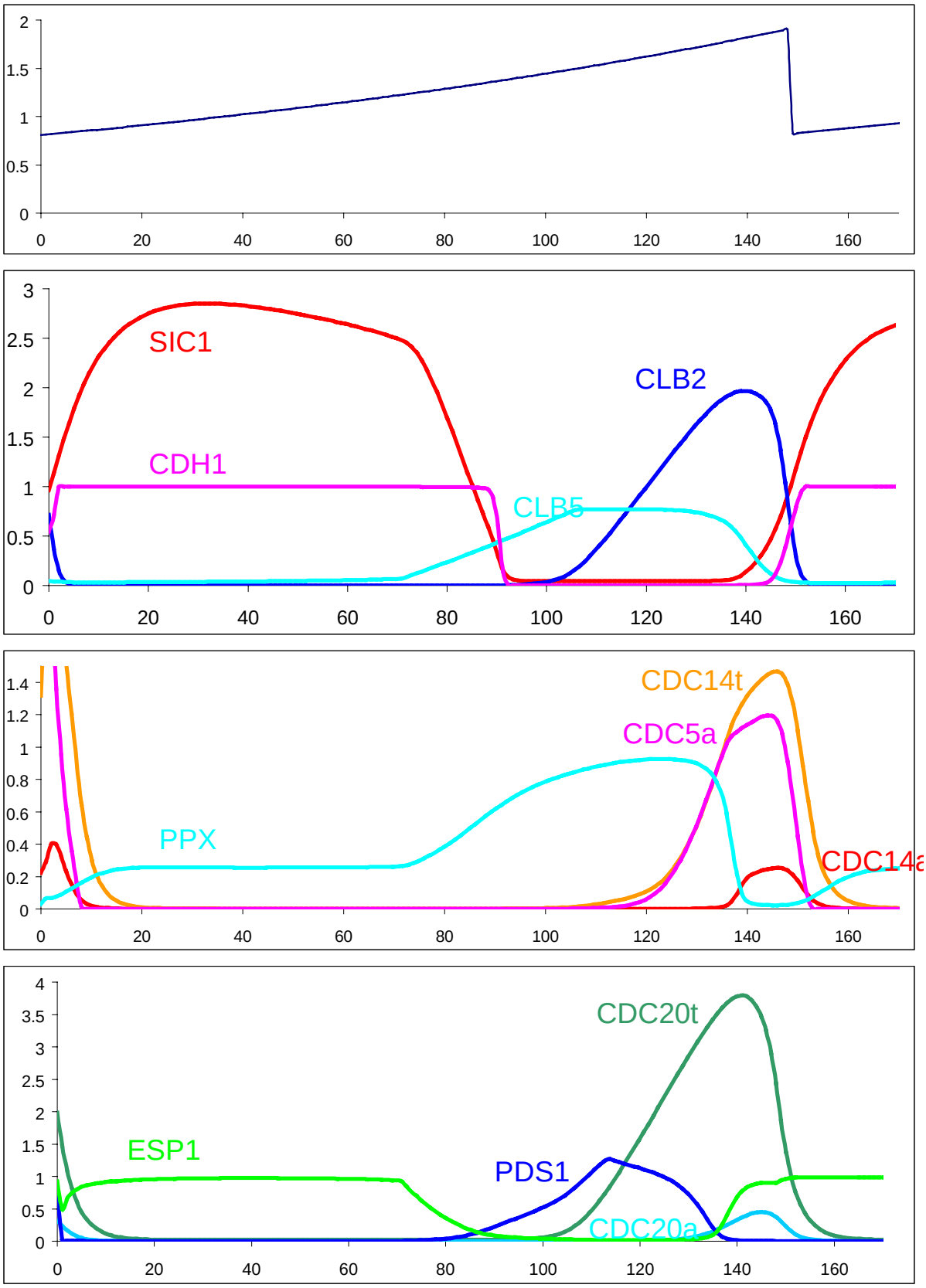

Figure 23 Wild Type simulation

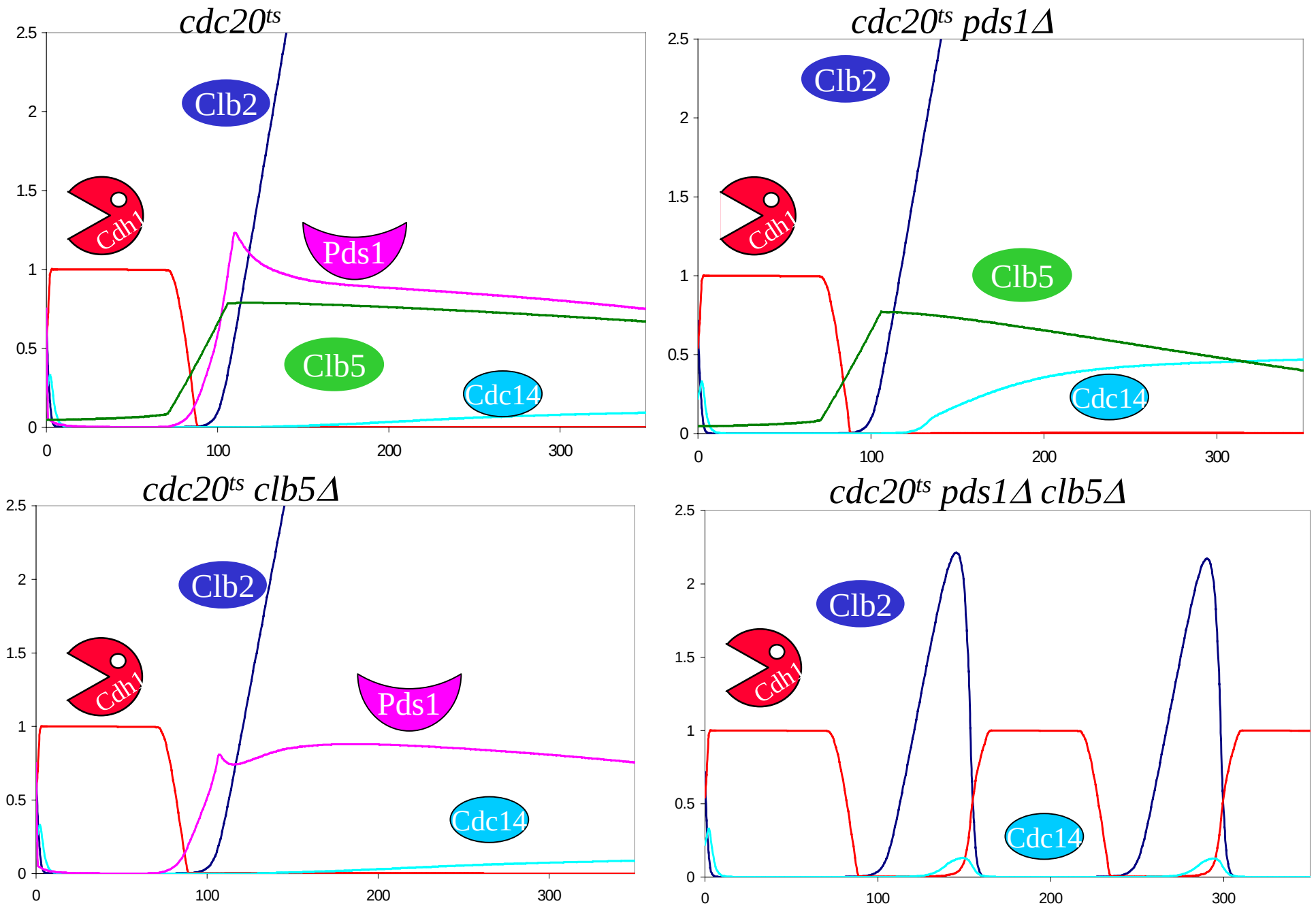

Figure 24 a. *cdc20ts* **b.** *cdc20^{ts} pds1Δ* **c.** *cdc20^{ts} clb5Δ* **d.** *cdc20^{ts} pds1Δ clb5Δ*

cdc5^{ts}

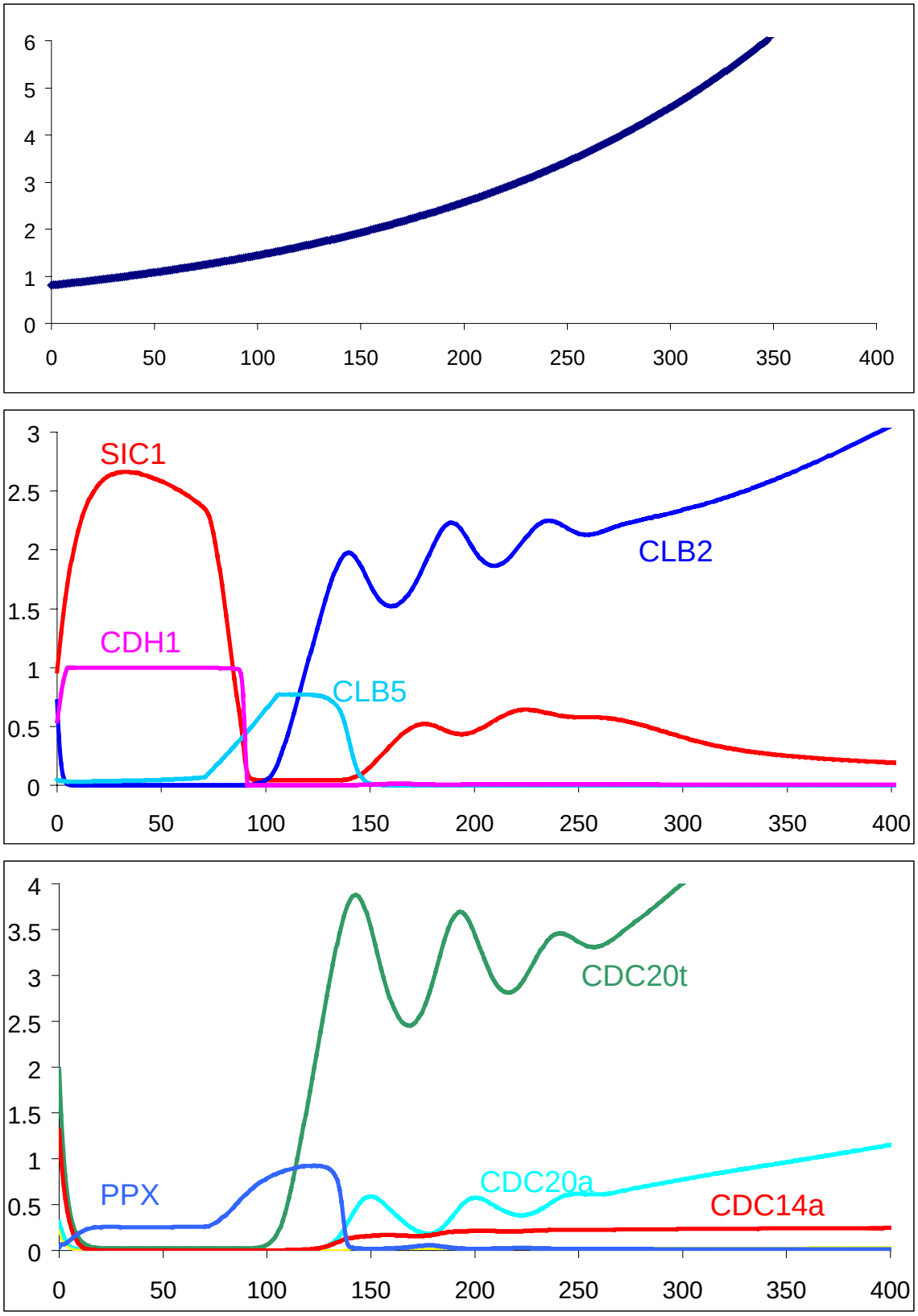

Figure 25

cdc15^{ts}

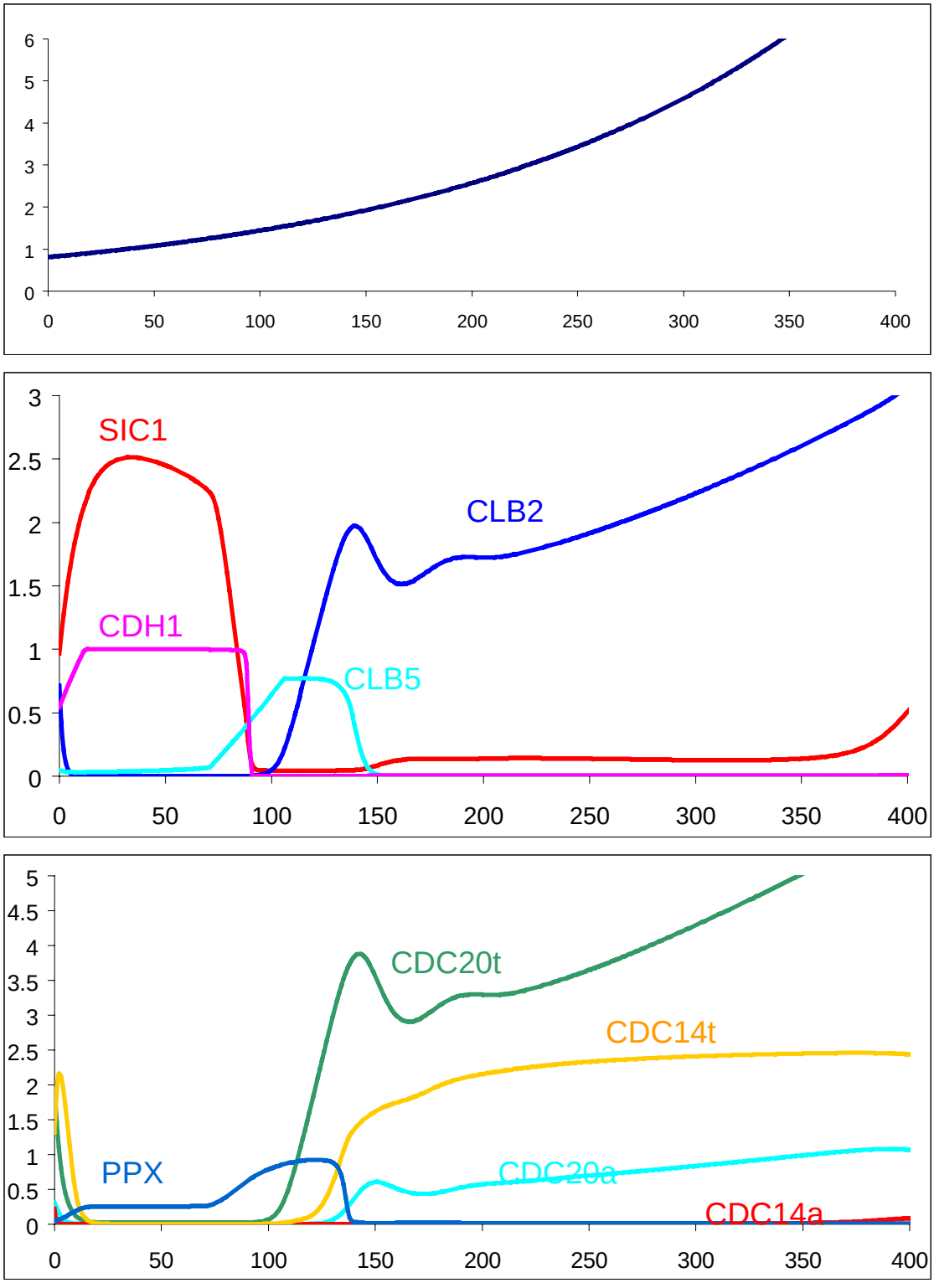

Figure 26

cdc14Δ

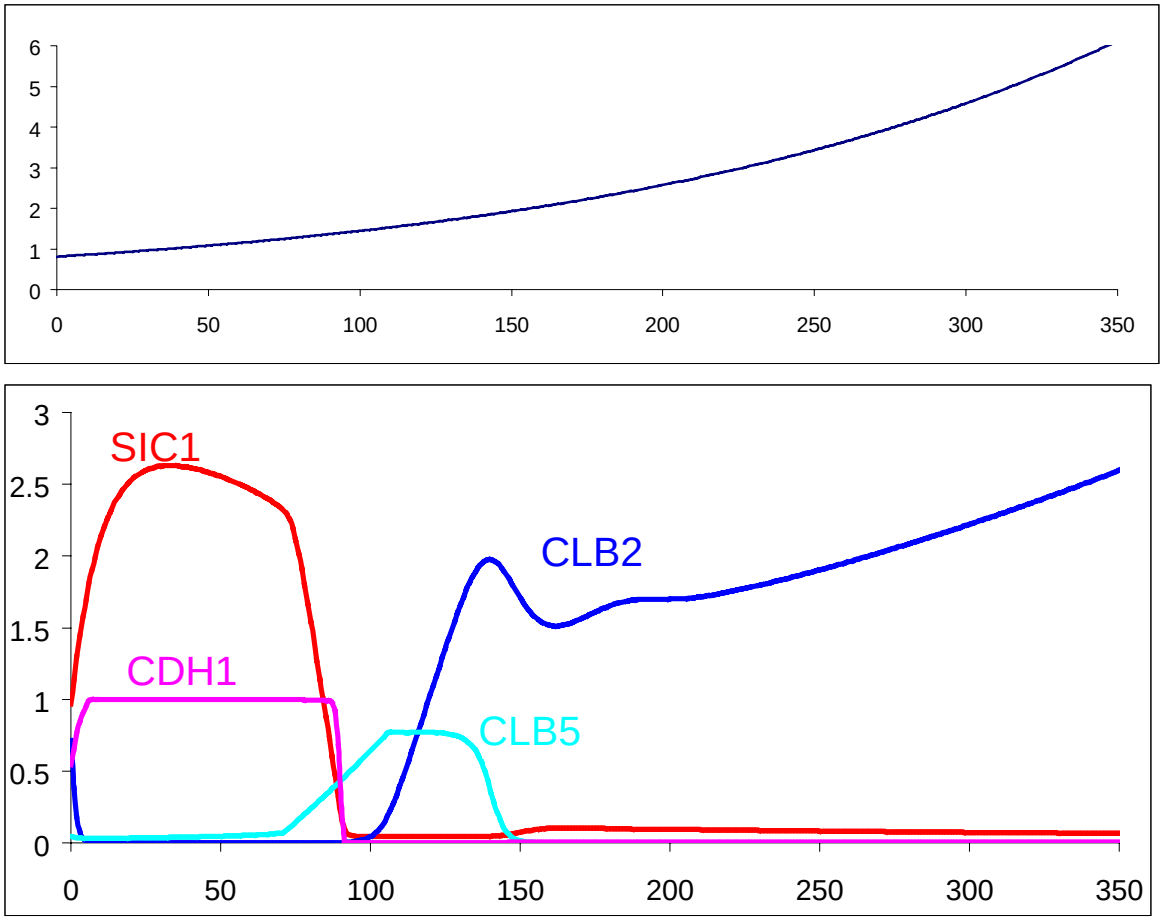

Figure 27 a.

CDC14^{op}

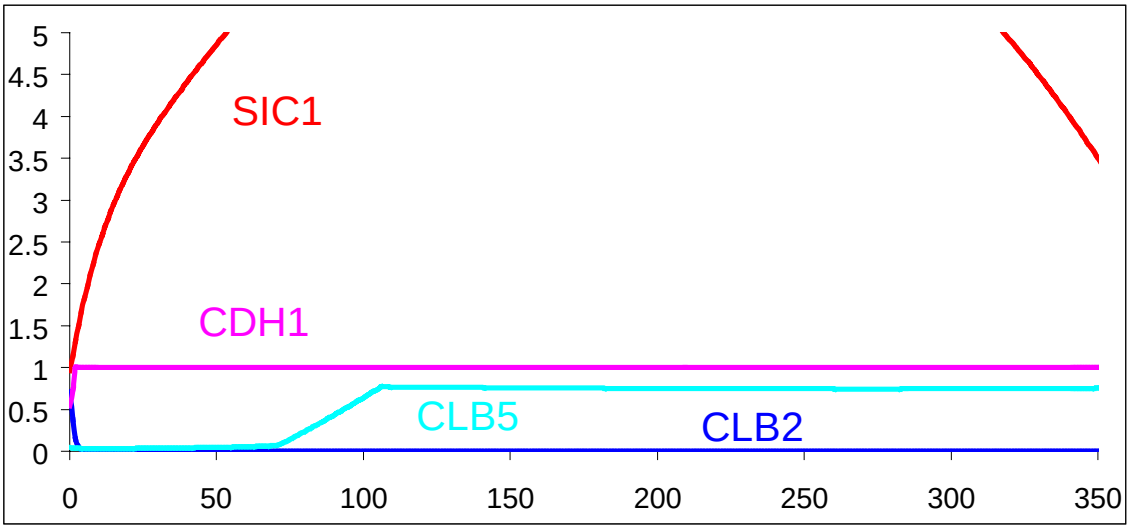

Figure 27 b.

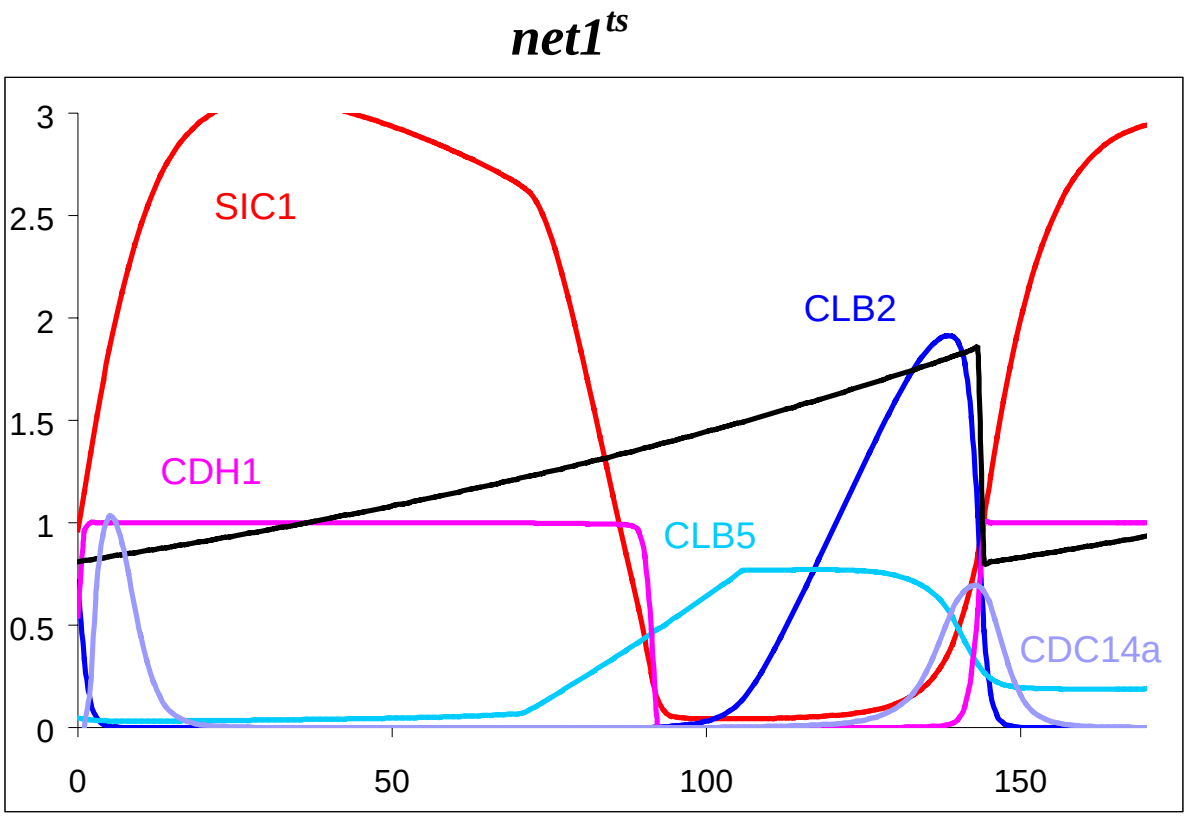

Figure 28 a.

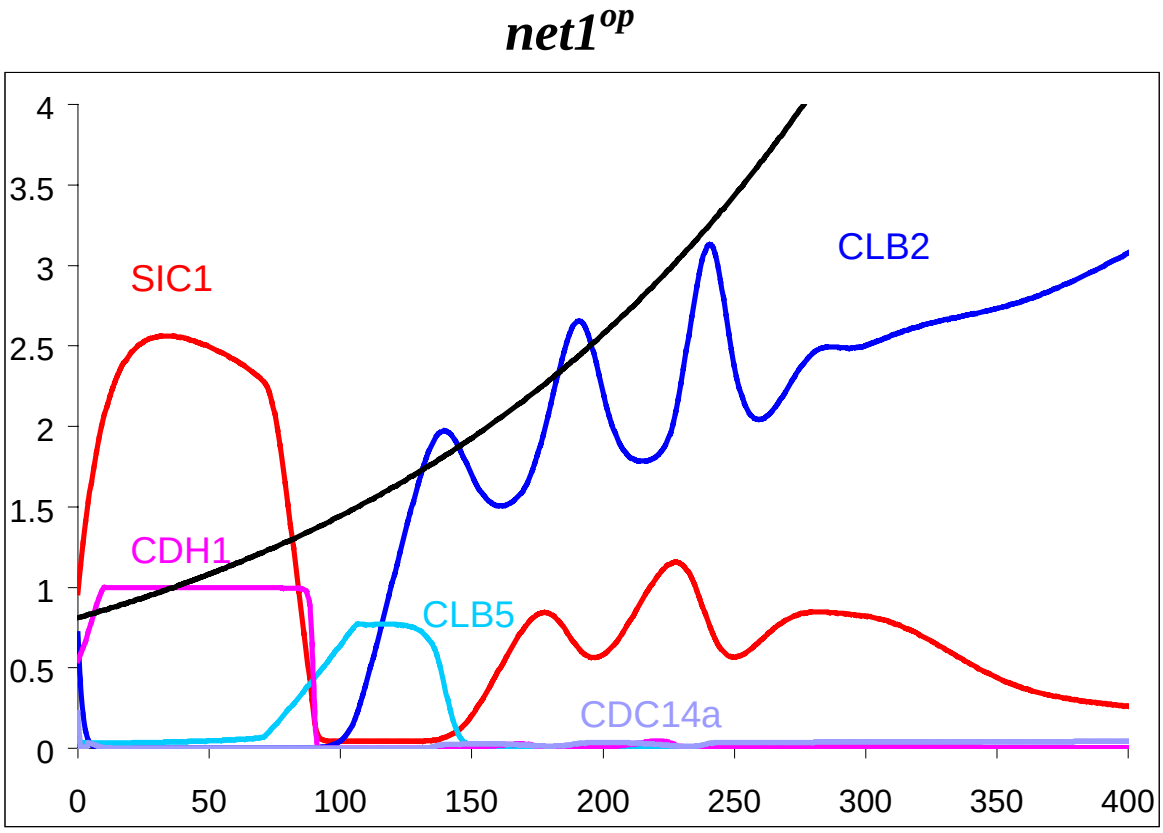

Figure 28 b.

Wild Type in nocodazole

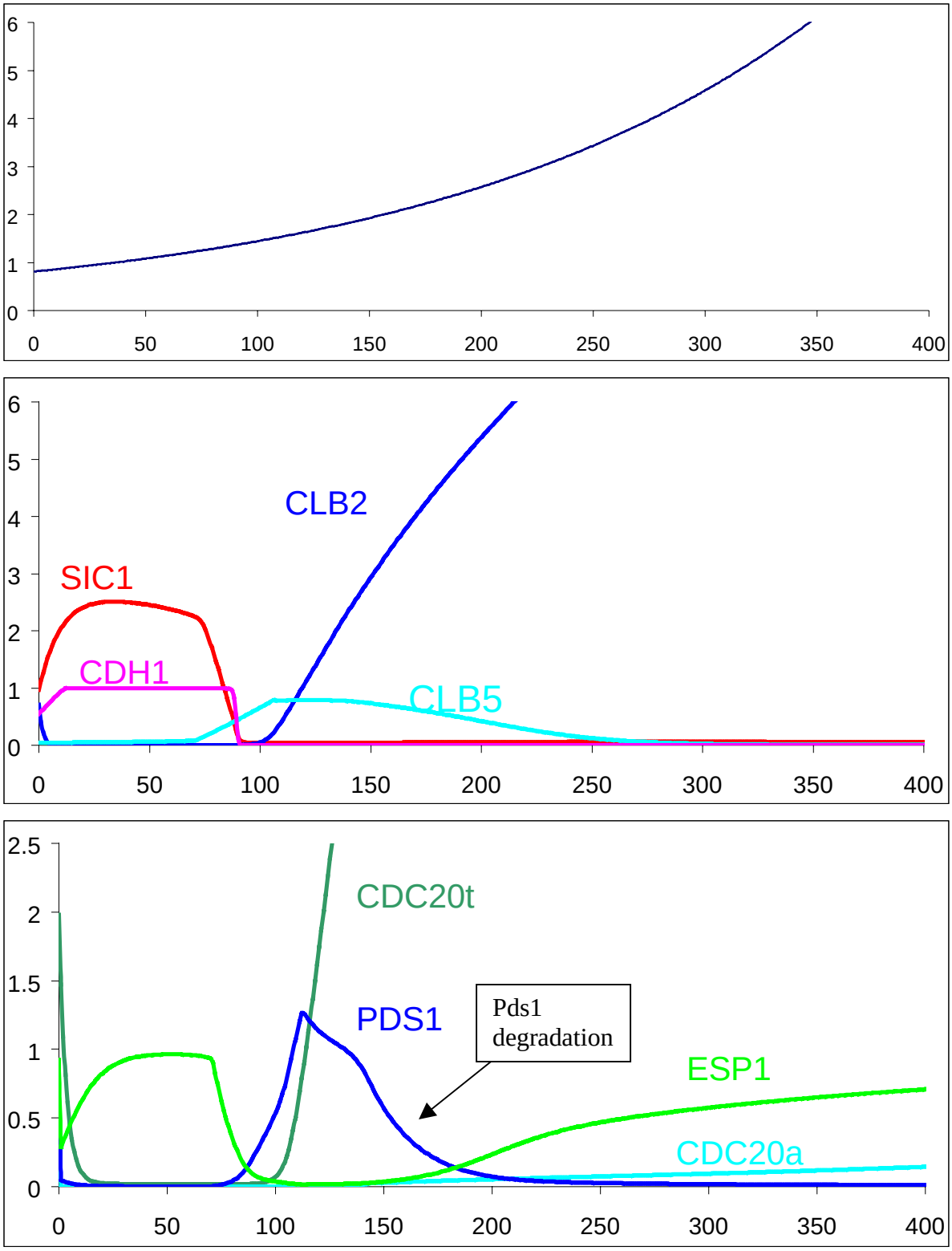

Figure 29

Time (min)

Conclusion

Based on published experimental results, we have presented a mathematical model of the mechanism regulating the budding yeast cell cycle, putting an emphasis on Finish. By comparing simulations to experimental studies of the physiology of the wild type and mutant cells, we confirm that our model gives an accurate account of the budding yeast cell cycle.

The mechanism detailed in the thesis includes all the major players involved in bringing budding yeast cells from metaphase to G1: the cyclin degradation proteins, Cdc20 and Cdh1, the CDK inhibitor, Sic1, the protein phosphatase, Cdc14, the binding proteins, Pds1 and Net1, the protease, Esp1, and the signal transduction proteins, Cdc5, Cdc15, Mad2, and Bub2. In her paper, Chen (2000) studied the Start transition in detail. Cdc20 is pushing the cells out of mitosis by activating (dephosphorylating) Cdh1. However, as explained previously in this thesis, Cdc20 is not a phosphatase but an ancillary component of the APC. That is, Cdc20 is involved in protein ubiquitination and degradation. Cdc20 is the starting point of a complex pathway driving the cells out of mitosis. This part of the model was built in accordance to the recent discoveries on Cdc14, Net1, and Esp1, and added to Chen's model to give a more complete model of the budding yeast cell cycle.

The goal of molecular biologists is to explain the physiology of the cell cycle in terms of the molecular machinery (genes and proteins) that control cell growth and division. Genetic and biochemical experiments describe component pieces of the machinery and their interactions, but do not explain how the complex network of interacting proteins determines the physiological characteristics of the cell cycle. To make the connection between the physiological properties of the cell cycle and the molecular machinery, we need mathematical models to explain, verify and maybe predict the properties of the control system.

The molecular mechanism was mathematically described as a set of twenty-two differential equations, and four auxiliary equations. Each rate constant of the model was chosen to fit the literature on all of the mutants. We were then able to predict the phenotypes of some novel mutants, such as those involving the hypothetical phosphatase

PPX. These predictions underline the importance of mathematical modeling in molecular cell biology. They also show the need of a close collaboration between experimentalists and theorists. Experimentalists discover new proteins and determine their interactions with other proteins in the cell. Theorists use these observations to propose mechanisms that will account for the data and predict new results. These predictions can give new directions to research. As novel cell cycle proteins are found, more comprehensive models can be constructed.

What is the significance of working on cell cycle controls in a simple, single cell organism such as budding yeast? Research has shown that many mechanisms regulating yeast cell cycles are conserved in higher eukaryotes. Thus, if the budding yeast cell cycle can be modeled and understood in detail, it can provide useful insights into cell cycle regulation in more complex organisms, such as crop plants, domesticated animals, and humans. These insights are bound to have important spin-offs in agricultural improvements and human health care.

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Appendix A: The components of the Budding Yeast

- The CDK Cdc28 that associates with the cyclins (Cdc28 is in excess in the cycle. We will therefore omit Cdc28 when referring to the complex Cyclin/Cdc28).
- The cyclins:
    - Cdc28/Cln1 and Cdc28/Cln2 (Cdc28 not shown in the diagram) induce budding.
    - Cdc28/Cln3initiates Start events when cells grow to a critical size.
    - Cdc28/Clb1 and Cdc28/Clb2 are essential for successful mitosis.
    - Cdc28/Clb5 and Cdc28/Clb6 are responsible for timely DNA replication. (Clb5 also plays an important role at Finish, a role that was underestimated in Chen's model.)

In the model, we will use Cln2 for Cln1 and 2, Clb2 for Clb1 and 2, and Clb5 for Clb5 and 6.

- The ancillary proteins Cdh1 (also known as Hct1) and Cdc20. Both appear in phosphorylated and unphosphorylated forms.
- The CDK-inhibitor Sic1.
- The phosphatase Cdc14 and a hypothetical phosphatase PPX.
- The transcription factors Mcm1 (for Swi5, Pds1 and Clb2), SBF for Clb5, Cln2 and Pds1, Swi5 for Sic1.
- The protease Esp1.
- The nucleolar protein Net1, which, along with Cdc14, forms the RENT complex. Cdc14 is released from the RENT complex when Net1 is phosphorylated.
- The protein kinases Cdc5 and Cdc15.
- The checkpoint proteins, Mad2 and Bub2.

Under the hypothesis that growth is increasing exponentially and knowing that the cell cycle is regulated by the comings and goings of the cyclins, we were able to express the wiring diagram mathematically (Fig. 16).

Appendix B. Mathematical Model of the Budding Yeast Cell Cycle

Equations governing cyclin-dependent kinases:

$$\frac{d[\text{Cln2}]}{dt} = (k_{s,n2}' + k_{s,n2} \cdot [\text{SBF}]) \cdot \text{mass} - k_{d,n2} \cdot [\text{Cln2}]$$

$$\frac{d[\text{Clb5T}]}{dt} = (k_{s,b5}' + k_{s,b5} \cdot [\text{SBF}]).\text{mass} - k_{d5} \cdot [\text{Clb5T}]$$

$$k_{d5} = k_{d,b5}''' + [\text{Esp1}] \cdot (k_{d,b5}'' + k_{d,b5}' \cdot [\text{Cdc20A}]) + k_{d,b5} \cdot [\text{Cdc20A}]$$

$$\frac{d[\text{Clb2T}]}{dt} = (k_{s,b2}' + k_{s,b2} \cdot [\text{Mcm1}]) \cdot \text{mass} - k_{d2} \cdot [\text{Clb2T}]$$

$$k_{d2} = V_2' \cdot (1 - [\text{Cdh1}]) + V_2 \cdot [\text{Cdh1}] + V_{2,20} \cdot [\text{Cdc20A}]$$

$$[\text{Cln3}] = k_{cln3} \cdot \text{mass}$$

$$[\text{Clb2}] = [\text{Clb2T}] - [\text{C2}]$$

$$[\text{Clb5}] = [\text{Clb5T}] - [\text{C5}]$$

$$[\text{Sic1}] = [\text{Sic1T}] - [\text{C2}] - [\text{C5}] - [\text{Sic1P}]$$

Equations governing the Clb degradation machinery

$$\frac{d[\text{IEP}]}{dt} = \frac{V_{a,iep} \cdot (1 - [\text{IEP}])}{J_{a,iep} + 1 - [\text{IEP}]} - \frac{k_{i,iep} \cdot [\text{IEP}]}{J_{i,iep} + [\text{IEP}]}$$

$$V_{a,iep} = k_{a,iep} \cdot [\text{Clb2}]$$

$$\frac{d[\text{Cdc20T}]}{dt} = k_{as,20}' + k_{as,20} \cdot [\text{Clb2}] - k_{ad,20} \cdot [\text{Cdc20T}]$$

$$\frac{d[\text{Cdc20A}]}{dt} = k_{aa} \cdot [\text{IEP}] \cdot ([\text{Cdc20T}] - [\text{Cdc20A}]) - (k_{ai} + k_{ad,20}) \cdot [\text{Cdc20A}]$$

$$\frac{d[\text{Cdh1}]}{dt} = \frac{V_{a,cdh1} \cdot (1 - [\text{Cdh1}])}{J_{a,cdh1} + 1 - [\text{Cdh1}]} - \frac{V_{i,cdh1} \cdot [\text{Cdh1}]}{J_{i,cdh1} + [\text{Cdh1}]}$$

$$V_{a,cdh1} = k_{apcrv} + k_{apcr} \cdot [\text{Cdc14}]$$

$$V_{i,cdh1} = k_{apc}' + k_{apc} \cdot (\text{E}_{c\ln 3} + \text{E}_{c\ln 2}.[\text{Cln2}] + \text{E}_{clb2}.[\text{Clb2}] + \text{E}_{clb5}.[\text{Clb5}])$$

Equations governing the inhibitor of Clb-dependent kinases:

$$\frac{d[\text{Sic1T}]}{dt} = (k_{s,sic}\,' + k_{s,sic} \cdot [\text{Swi5}]) - k_{d,sic} \cdot [\text{Sic1p}]$$

$$\frac{d[\text{Sic1p}]}{dt} = \text{E}_{k4}([\text{Sic1T}] - [\text{Sic1p}]) - (k_{pp,sic} \cdot [\text{Cdc14}] + k_{d,sic}).[\text{Sic1p}]$$

$$\frac{d[\text{C2}]}{dt} = -k_{ir2} \cdot [\text{C2}] + k_i \cdot [\text{Clb2}] \cdot [\text{Sic1}] - (k_{d2} + \text{E}_{k4}).[\text{C2}]$$

$$\frac{d[\text{C5}]}{dt} = -k_{ir5} \cdot [\text{C5}] + k_i \cdot [\text{Clb5}].[\text{Sic1}] - (k_{d5} + \text{E}_{k4}).[\text{C5}]$$

$$\text{E}_{k4} = k_4\,' + k_4 \cdot \frac{\text{E}_{cdk,sic}}{k_{m2} + [\text{Sic1T}]}$$

$$\text{E}_{cdk,sic} = \text{E}_{c3} \cdot (\text{E}_{c\ln 3} + 0.1 \cdot [\text{X}] \cdot [\text{mass}]) + \text{E}_{c2} \cdot [\text{Cln2}] + \text{E}_{c5} \cdot [\text{Clb5}] + \text{E}_{cb2} \cdot [\text{Clb2}]$$

$$\text{E}_{c\ln 3} = \frac{\max_{c\ln 3} \cdot [\text{Cln3}]}{[\text{Cln3}] + k_{f,s3}}$$

$$\frac{d[\text{Swi5T}]}{dt} = k_{s,swi5}\,' + k_{s,swi5} \cdot [\text{Mcm1}] - k_{d,swi5} \cdot [\text{Swi5T}]$$

$$\frac{d[\text{Swi5}]}{dt} = k_{a,swi5} \cdot ([\text{Swi5T}] - [\text{Swi5}]) \cdot [\text{Cdc14}] - k_{i,swi5} \cdot [\text{Clb2}] \cdot [\text{Swi5}] - k_{d,swi5} \cdot [\text{Swi5}]$$

Equations for growth, DNA synthesis, budding and spindle formation:

$$\frac{d}{dt}\text{mass} = \mu.\text{mass}$$

$$\frac{d[\text{ORI}]}{dt} = k_{s,ori} \cdot (\text{E}_{ori,b5}[\text{Clb5}] + \text{E}_{ori,b2} \cdot [\text{Clb2}]) - k_{d,ori} \cdot [\text{ORI}]$$

$$\frac{d[\text{BUD}]}{dt} = k_{s,bud} \cdot ([\text{Cln2}] + \text{E}_{c\ln 3} + \text{E}_{bud,b5}[\text{Clb5}]) - \text{k}_{d,bud} \cdot [\text{BUD}]$$

$$\frac{d[\text{SPN}]}{dt} = k_{s,spn} \cdot \frac{[\text{Clb2}]}{J_{spn} + [\text{Clb2}]} - k_{d,spn} \cdot [\text{SPN}]$$

Equations governing the phosphatase:

$$\frac{d[\text{Cdc14}]}{dt} = -k_{as,c14,net} \cdot [\text{Net1}] \cdot [\text{Cdc14}] + k_{di,c14,net} \cdot ([\text{Net1T}] - [\text{Net1}] - [\text{PF}])$$
$$+k_{rel} \cdot ([\text{Cdc14T}] - [\text{Cdc14}] - [\text{Net1T}] + [\text{Net1}] + [\text{PF}]) - k_{d,c14} \cdot [\text{Cdc14}] - k_{as,netp} \cdot [\text{Net1p}] \cdot [\text{Cdc14}]$$

$$\frac{d[\text{Cdc14T}]}{dt} = k_{s,c14}'' + k_{s,c14}' \cdot ([\text{Cdc5T}] - [\text{Cdc5}]) + k_{s,c14} \cdot [\text{Cdc5}] - k_{d,c14} \cdot [\text{Cdc14T}]$$

$$\frac{d[\text{Net1}]}{dt} = -k_{as,c14,net} \cdot [\text{Net1}] \cdot [\text{Cdc14}] + k_{di,c14,net} \cdot ([\text{Net1T}] - [\text{Net1}] - [\text{PF}])$$
$$+ \frac{(k_{ac1} \cdot [\text{PPX}] + k_{ac2} \cdot [\text{Cdc14}]) \cdot ([\text{Net1T}] - [\text{Net1}] - [\text{Cdc14T}] + [\text{Cdc14}])}{J_{ac} + [\text{PF}]} - \frac{k_{in} \cdot [\text{Cdc15}] \cdot [\text{Net1}]}{J_{in} + [\text{Net1T}] - [\text{PF}]}$$

$$\frac{d[\text{PF}]}{dt} = -\frac{(k_{ac1} \cdot [\text{PPX}] + k_{ac2} \cdot [\text{Cdc14}]) \cdot [\text{PF}]}{J_{ac} + [\text{PF}]} + \frac{k_{in} \cdot [\text{Cdc15}].([\text{Net1T}] - [\text{PF}])}{J_{in} + [\text{Net1T}] - [\text{PF}]}$$

$$\frac{d[\text{Cdc5T}]}{dt} = k_{s,c5}' + k_{s,c5} \cdot [\text{Mcm1}] - (k_{d,c5}' + k_{d,c5} \cdot [\text{Cdh1}) \cdot [\text{Cdc5T}])$$

$$\frac{d[\text{Cdc5}]}{dt} = \frac{k_{a,cd5} \cdot [\text{Clb2}] \cdot ([\text{Cdc5T}] - [\text{Cdc5}])}{J_{a,cd5} + [\text{Cdc5T}] - [\text{Cdc5}]} - \frac{k_{i,cd5} \cdot [\text{Cdc5}]}{J_{i,cd5} + [\text{Cdc5}]}$$
$$-(k_{d,c5}' + k_{d,c5} \cdot [\text{Cdh1}]) \cdot [\text{Cdc5}]$$

$$\frac{d[\text{Pds1}]}{dt} = k_{s,pds} \cdot ([\text{SBF}] + [\text{Mcm1}]) - k_{d,pds}.[\text{Pds1}] - k_{ic1} \cdot [\text{Pds1}] \cdot [\text{Esp1}] + \text{k}_{\text{irc1}} \cdot [\text{C1}]$$
$$k_{d,pds} = k_{d1,pds} + [\text{Esp1}] \cdot (k_{d2,pds} + k_{d3,pds} \cdot [\text{Cdc20A}]) + k_{d4,pds} \cdot [\text{Cdc20A}]$$

$$\frac{d[\text{PPX}]}{dt} = k_{s,ppx} - k_{d,ppx} \cdot [\text{PPX}]$$
$$k_{d,ppx} = k_{d1,ppx} + [\text{Esp1}] \cdot (k_{d2,ppx} + k_{d3,ppx} \cdot [\text{Cdc20A}]) + \text{k}_{d4,ppx} \cdot [\text{Cdc20A}]$$

$$\frac{d[\text{C1}]}{dt} = k_{ic1} \cdot [\text{Pds1}] \cdot [\text{Esp1}] - k_{irc1} \cdot [\text{C1}] - k_{d,pds} \cdot [\text{C1}]$$

$[Net1p] = [Net1T]-[Net1]-[Cdc14T]+[Cdc14]$

$[RENT] = [Cdc14T]-[Cdc14]-[PF]+[Net1p]$

$[RENTp] = [PF]-[Net1p]$

$[Esp1] = [Esp1T]-[C1]$

Equations governing the transcription factors:

$SBF = G(V_{a,sbf}, V_{i,sbf}, J_{a,sbf}, J_{i,sbf})$

$V_{a,sbf} = E_{sbf,n2} * [Cln2]+E_{sbf,n3} * (E_{cln3} + X * mass)+E_{sbf,b5} * [Clb5]$

$V_{i,sbf} = v_{s2v}+v_{s2}[Clb2]$

$Mcm1 = G([Clb2], k_{mcmr}, J_{a,mcm}, J_{i,mcm})$

Appendix C. Kinetic constants for computing the cell cycle of wild-type budding yeast

The constants that appear in parenthesis are the values of Chen's model.

Rate constants (min^{-1}):

$k_{s,n2}$' = 0 (0) $k_{s,n2}$ = 0.05 (0.05) $k_{d,n2}$ = 0.2 (0.1)

$k_{s,b5}$' = 0.0001 (0.006) $k_{s,b5}$ = 0.015 (0.02)

$k_{d,b5}$''' = 0.005 (0.1) $k_{d,b5}$'' = 0.001 $k_{d,b5}$' = 0.5 $k_{d,b5}$ = 0.2 (0.2)

$k_{s,b2}$' = 0.0006 (0.002) $k_{s,b2}$ = 0.05 (0.05)

V_2' = 0.01 (0.01) V_2 = 1 (2)

$V_{2,20}$ = 0.1 (0.05) k_{cln3} = 0.0033 (0.0033)

$k_{a,iep}$ = 0.1 $k_{i,iep}$ = 0.15

$k_{as,20}$' = 0.006 (0.005) $k_{as,20}$ = 0.6 (0.06)

$k_{ad,20}$ = 0.3 (0.08) k_{aa} = 0.1 (1) k_{ai} = 0.1 or 10 (0.1 or 10 depending on time)

k_{apcrv} = 0.04 (0.04) k_{apcr} = 0.8 (2) k_{apc}' = 0.001 (0) k_{apc} = 0.4 (0.64)

$k_{s,sic}$' = 0.04 (0.02) $k_{s,sic}$ = 0.2 (0.1) $k_{d,sic}$ = 1 (0.01) $k_{pp,sic}$ = 4

k_{ir2} = 0.05 (0.05) k_{ir5} = 0.05 (0.05) k_i = 50 (50)

k_4' = 0.01 (0.01) k_4 = 0.04 (0.3)

$k_{s,swi5}$, = 0.005 $k_{s,swi5}$ = 0.1 $k_{d,swi5}$ = 0.1

$k_{a,swi5}$ = 2 $k_{i,swi5}$ = 0.05

$k_{s,ori}$ = 2 (2) $k_{s,bud}$ = 0.38 (0.3) $k_{s,spn}$ = 0.08 (0.08)

$k_{d,ori} = k_{d,bud} = k_{d,spn}$ = 0.06 (0.06)

μ = 0.005776 (0.005776)

$k_{as,c14,net}$ = 500 $k_{di,c14,net}$ = 1 k_{rel} = 0.5 $k_{as,netp}$ = 1

$k_{s,c14}$'' = 0 $k_{s,c14}$' = 0.05 $k_{s,c14}$ = 0.5 $k_{d,c14}$ = 0.4

k_{ac1} = 10 k_{ac2} = 0 k_{in} = 1

$k_{s,c5}$' = 0.0025 $k_{s,c5}$ = 0.05 $k_{d,c5}$' = 0.025 $k_{d,c5}$ = 0.2

$k_{a,cd5}$ = 0.15 $k_{i,cd5}$ = 0.15

$k_{s,pds}$ = 0.1 $k_{d1,pds}$ = 0.05 $k_{d2,pds}$ = 0.05 $k_{d3,pds}$ = 1

$k_{d4,pds}$ = 1 $k_{s,ppx}$ = 0.1

$k_{d1,ppx}$ = 0.1 $k_{d2,ppx}$ = 0.05 $k_{d3,ppx}$ = 10 $k_{d4,ppx}$ = 0.02

k_{ic1} = 50 k_{irc1} = 1

k_{mcmr} = 0.15 (0.15) V_{s2v} = 0.6 V_{s2} = 6

Characteristic concentrations (dimensionless):

maxcln3 = 0.02 (0.02) [Net1T] = 3 [Esp1T] = 1

$J_{a,iep}$ = $J_{i,iep}$ = 0.01 $J_{a,hct1}$ = $J_{i,hct1}$ = 0.01 (0.05)

J_{spn} = 0.2 (0.2)

J_{ac} = J_{in} = 0.05 (0.05)

$J_{a,cd5}$ = $J_{i,cd5}$ = 0.01

$J_{a,sbf}$ = $J_{i,sbf}$ = 0.01 (0.01) $J_{a,mcm}$ = $J_{i,mcm}$ = 1 (1)

Kinase efficiencies (dimensionless):

E_{c3} = 150 (20) E_{c2} =15 (2) E_{c5} = 30 (1) E_{cb2} = 1 (0.067)

E_{cln2} = 0.1 (1) E_{clb5} = 3 (0.5) E_{clb2} = 0.2 (1)

$E_{ori,b5}$ = 0.9 $E_{ori,b2}$ = 0.4 (0.4) $E_{bud,b5}$ = 1 (1)

$E_{sbf,n3}$ =75 (75) $E_{sbf,b5}$ = 3 (0.5) $E_{sbf,n2}$ = 3 (3)

Other dimensionless constants:

k_{m2} = 0.05

k_{fs3} = 0.02

X = 0.0027

Cdc15 = 10

Calzone, Laurence.....Vita

I was born on June 28, 1977. I grew up in a small town situated on the French Riviera called Antibes. I graduated from a French high school and studied for two years at the Euro-American Institute in Sophia Antipolis, France. I transferred to Virginia Tech my junior year. I graduated from Virginia Tech in 1998 with a B.S. in Mathematics. I pursued my studies in Virginia Tech, being employed as a teaching assistant in the Mathematics department. I taught Elementary Calculus for a year. I began working with Dr. John J. Tyson as an undergraduate and started my research fall of 1999.