Module: Cell Cycle

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I. Abstract

DNA synthesis and mitosis in frog eggs is controlled by periodic synthesis and degradation of the cyclin subunit of a cyclin-dependent protein kinase, and also by periodic phosphorylation and dephosphorylation of the kinase subunit. We construct a differential-equation model of these reactions and show that, under reasonable conditions, the model can exhibit bistability (arrest in interphase or in mitosis) or spontaneous oscillations (periodic replication and division).

II. Physiology

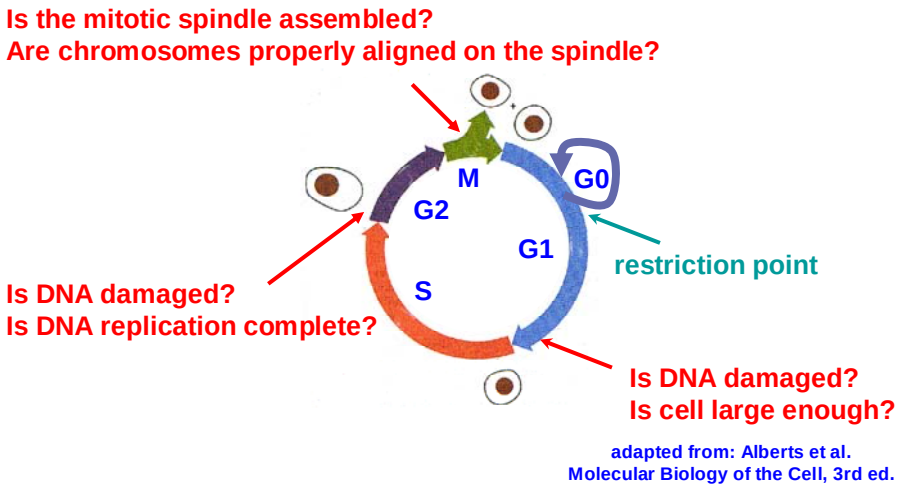
The cell division cycle is the sequence of events by which a growing cell replicates its DNA and divides the products of replication (the 'sister chromatids') equally between to daughter cells. In eukaryotes, the cell cycle consists of four sequential phases: G1 (first gap: DNA unreplicated), S (DNA synthesis), G2 (second gap: DNA replicated), and M (mitosis). Progression through these phases is unidirectional. Checkpoints within the system ensure that one phase of the cell cycle is complete before the next phase begins. Checkpoints also monitor the cell for DNA damage and delay cell cycle progression to allow for repair of the damage. In animal cells the restriction point (R) regulates entry into the cell cycle in the presence of growth factors. In the absence of growth factors, the cell withdraws to a state of quiescence called G0.

Regulation of even the simplest cell cycles (such as those of frog egg extracts, described here) is a complex affair that is difficult to understand in depth by intuitive (verbal) arguments alone. Why is the cell cycle unidirectional? Once a cell initiates mitosis, why does it never slip back into S or G2? What controls the timing of cell cycles, which can range in length from eight minutes (in fly embryos) to more than 24 hours (in adult mammals)? Mathematical models of cell cycle controls offer a systems-level view that can answer these questions by revealing fundamental regulatory properties of the system. In this tutorial, we will explore (1) the concept of hysteresis, which explains the irreversible switch-like behavior of the cell cycle, (2) the concepts of lag times and "critical slowing down", which contribute to regulation of cell cycle timing, and (3) the feedback loops that generate autonomous oscillations through the cell cycle (Sible and Tyson, 2007).

In the exercises, we will explore several specific behaviors that were discovered by experimentation and can be better understood in the context of a mathematical model. First, we will consider the observation by Murray and Kirschner that synthesis and degradation of cyclin is all that is needed to drive cell cycle oscillations in frog egg extracts (Murray and Kirschner, 1989; Murray et al., 1989). Second, we will investigate the observation by Solomon that a threshold amount of cyclin is required to drive an extract into mitosis (Solomon et al., 1990). We will uncover the role of positive feedback in cell cycle progression,

the bistable nature of the molecular control network, and the effect of unreplicated DNA on cell cycle progression.

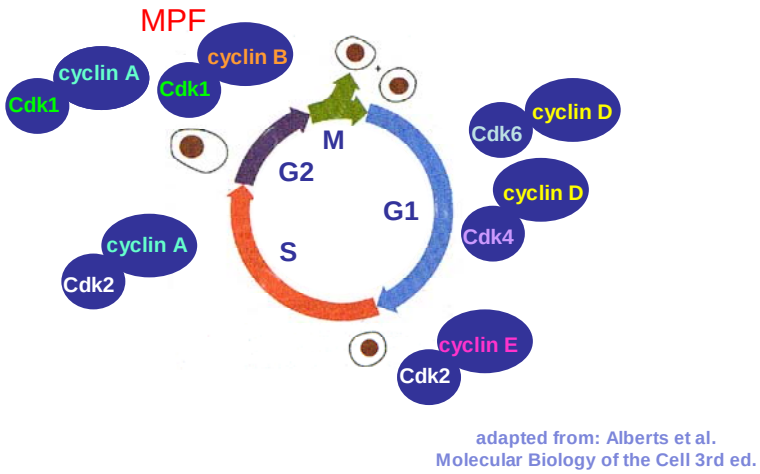
Figure 1. The eukaryotic cell cycle.



III. Molecular Biology

Progression through the eukaryotic cell cycle is driven by a family of enzymes called cyclin - dependent kinases (Cdks). The role of a Cdk is to attach a phosphate group to serine or threonine residues on target proteins. By controlling the phosphorylation state of these target proteins, the Cdks control the timing of cell cycle events. In order to be catalytically active, a Cdk subunit must bind to a cyclin subunit. Cyclin molecules are generally unstable proteins that are synthesized and degraded periodically during the cell cycle. Different cyclin-Cdk complexes are responsible for progression through specific phases of the cell cycle. For example, cyclin E-Cdk2 drives the cell into S phase by phosphorylating components of the DNA origin replication complex to trigger origin firing and subsequently to block rereplication of DNA (Furstenthal et al., 2001). A simplified view of the cyclin-Cdk complexes that drive progression through the eukaryotic cell cycle is depicted in Figure 2.

Figure 2. Cyclin-Cdk complexes in eukaryotic cells.



In this exercise, we focus on cyclin B-Cdk1, which catalyzes the G2/M - phase transition. This Cdk complex is also known as MPF (M-phase promoting factor) for historical reasons. Like most Cdk complexes, cyclin B-Cdk1 activity is regulated by multiple mechanisms. (1) Cyclin synthesis and degradation. Cyclin levels accumulate as the cell cycle progresses, peaking at M-phase, coincident with the peak in Cdk activity. Then, cyclin is rapidly degraded and the cell exits mitosis. Cyclin degradation is stimulated indirectly by cyclin B-Cdk1 itself, forming a time delayed negative feedback loop. (2) Stoichiometric inhibitors. A family of proteins, called cyclin-dependent kinase inhibitors (CKIs), bind to cyclin-Cdk complexes and repress kinase activity. Cyclin B-Cdk1 is susceptible to these inhibitors, but as they are not present in the frog egg extracts, they will not be introduced into our model. (3) Activating phosphoryation. Phosphorylation of the catalytic subunit by Cdk-activating kinase (CAK) is required for activation (Solomon et al., 1992). Because this phosphorylation event does not appear to be regulated in frog egg extracts, it will be ignored in our model. (4) Inhibitory phosphoryations. When cyclin B-Cdk1 is phosphorylated on threonine 14 and tyrosine 15 by Wee1/Myt1 (Mueller et al., 1995a; Mueller et al., 1995b), its kinase activity is inhibited. The opposing (activating) phosphatases are members of the Cdc25 family (Gautier et al., 1991; Kumagai and Dunphy, 1991). These two groups of enzymes, which regulate the phosphorylation states of Thr 14 and Tyr 15, play a central role in our mathematical model as they are engaged in feedback loops with cyclin B-Cdk1.

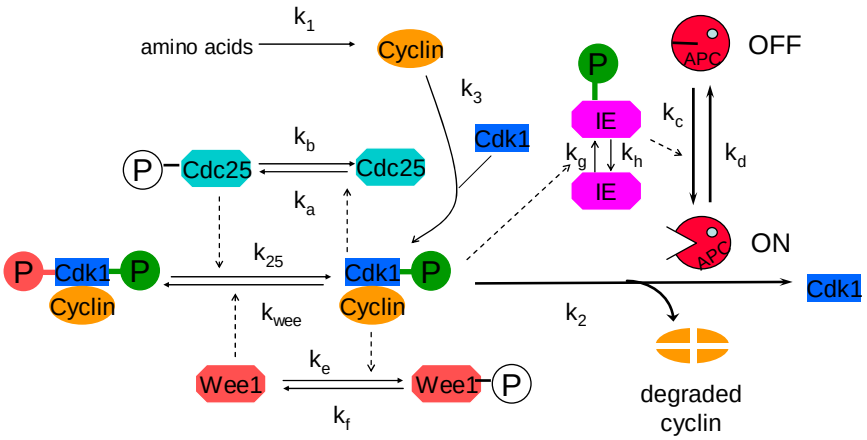


Figure 3. Wiring diagram for the regulation of cyclin B-Cdk1 activity in frog egg extracts. Solid lines represent biochemical reactions; dashed lines represent catalytic effects. IE = intermediate enzyme (hypothetical). APC = anaphase promoting complex, which tags cyclin B for degradation.

IV. Model

The biochemical network regulating mitosis (Figure 3) can be described by a set of ordinary differential equations (ODEs; Figure 4). The model centers on cyclin B-Cdk1, the Cdk complex that drives entry into mitosis. MPF refers to the active (unphoshorylated) form of cyclin B-Cdk1, and preMPF to the inactive (phosphorylated) form. Wee1 represents the collective kinase activity (a combination of Wee1 and Myt1 activities) that phosphorylates cyclin B-Cdk1 on Thr 14 and Tyr 15. Cdc25 represents the collective opposing phosphatase activity, (a combination of Cdc25A, Cdc25C and possibly other isoforms). Cyclin B-Cdk1 phosphorylates and activates Cdc25 (resulting in a positive feedback loop) and phosphorylates and inactivates Wee1 (resulting in a double-negative feedback loop). Cyclin B-Cdk1 phosphorylates an intermediate enzyme, which activates the APC to tag cyclin B for degradation (resulting in a time-delayed, negative feedback loop).

1. $\frac{d}{dt}[\text{Cyclin}] = k_1 - k_2[\text{Cyclin}] - k_3[\text{Cyclin}][\text{Cdk}]$

2. $\frac{d}{dt}[\text{MPF}] = k_3[\text{Cyclin}][\text{Cdk}] - k_2[\text{MPF}] - k_{wee}[\text{MPF}] + k_{25}[\text{preMPF}]$

3. $\frac{d}{dt}[\text{preMPF}] = -k_2[\text{preMPF}] + k_{wee}[\text{MPF}] - k_{25}[\text{preMPF}]$

4. $\frac{d}{dt}[\text{Cdc25P}] = \frac{k_a[\text{MPF}]([\text{total Cdc25}] - [\text{Cdc25P}])}{K_a + [\text{total Cdc25}] - [\text{Cdc25P}]} - \frac{k_b[\text{PPase}][\text{Cdc25P}]}{K_b + [\text{Cdc25P}]}$

5. $\frac{d}{dt}[\text{Wee1P}] = \frac{k_e[\text{MPF}]([\text{total Wee1}] - [\text{Wee1P}])}{K_e + [\text{total Wee1}] - [\text{Wee1P}]} - \frac{k_f[\text{PPase}][\text{Wee1P}]}{K_f + [\text{Wee1P}]}$

6. $\frac{d}{dt}[\text{IEP}] = \frac{k_g[\text{MPF}]([\text{total IE}] - [\text{IEP}])}{K_g + [\text{total IE}] - [\text{IEP}]} - \frac{k_h[\text{PPase}][\text{IEP}]}{K_h + [\text{IEP}]}$

7. $\frac{d}{dt}[\text{APC}] = \frac{k_c[\text{MPF}]([\text{total APC}] - [\text{APC}])}{K_c + [\text{total APC}] - [\text{APC}]} - \frac{k_d[\text{PPase}][\text{APC}]}{K_d + [\text{APC}]}$

8. $[\text{Cdk}] = [\text{Total Cdk}] - [\text{MPF}] - [\text{preMPF}]$

9. $k_{25} = V_{25}'([\text{Total Cdc25}] - [\text{Cdc25P}]) + V_{25}''[\text{Cdc25P}]$

10. $k_{wee} = V_{wee}'[\text{Wee1P}] + V_{wee}''([\text{Total Wee1}] - [\text{Wee1P}])$

11. $k_2 = V_2'([\text{Total APC}] - [\text{APC*}]) + V_2''[\text{APC*}]$

Figure 4. Mathematical model of the cell cycle control network in frog egg extracts (Novak and Tyson, 1993). MPF = active form of cyclin B–Cdk1. Pre-MPF = inactive (phosphorylated) form of cyclin B-Cdk1. Other protein names appended with a “P” refer to the phosphorylated form of these proteins.

VII. Further Readings

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VIII. Historical Notes

This model of frog egg extracts was first proposed by Novak & Tyson (1993), based in large part on Solomon's 1990 publication of the cyclin threshold for MPF activation. Novak and Tyson made three predictions from their model: (1) There must be a different (lower) cyclin threshold for MPF inactivation (corollary: the control system is bistable between these two thresholds). (2) For cyclin levels close to the thresholds, the time lag for changes in MPF activity should get long. And (3) the unreplicated DNA checkpoint will operate by raising the cyclin threshold for MF activation. These three predictions were not confirmed until 2003, in the elegant experiments of Sha et al. and Pomerening et al. In 2005, Pomerening et al. published very careful measurements of MPF activity and total cyclin concentrations in cycling egg extracts. They showed that limit cycle oscillations encircles the hysteresis loop, as expected from the Novak-Tyson model, and that, if the positive feedback loop is broken, then the negative feedback oscillations are indeed faster and smaller amplitude. In this case, the nuclei in the extract do not show clear rounds of DNA replication followed by nuclear division.