

MODELING THE CELL CYCLE

Judit Zámorszky and Attila Csikász-Nagy

The Microsoft Research - University of Trento Centre for Computational and Systems Biology, Piazza Mancini 17, Povo-Trento I-38123

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Summary

Cell cycle regulation was an early subject of biology inspired mathematical modeling. Even before the molecular regulators of the cell cycle were known, mathematical models of the cell cycle were already formulated. As the molecular details of the underlying regulatory network were revealed, the modeling of the system became more and more sophisticated. Indeed cell cycle has been one of the pioneering examples of systems biology approaches, where experiments and mathematical modeling have guided each other. Thanks to these efforts now we are able to better understand the

dynamics of the cell cycle regulation and to explain how the oscillations appear in different cell types and what roles positive and negative feedbacks play in cell cycle regulation. Different modeling methods were used to attack these questions at different levels of complexity. Abstract logical models of the skeleton network, differential equations of the regulatory modules and stochastic models of some key control points all attacked cell cycle as an important biological example. Here we review the key discoveries of this, still active, developing field.

1. Introduction

Biological systems are extremely complex structures performing several crucial functions of life. The concept of cells, as the “functional units of life”, was established by the mid-nineteenth century and each day an enormous amount of new biological data is produced, still we are far from a detailed understanding of how cells function. The physiological behavior of cells can be observed by various microscopy techniques, the information coding DNA can be sequenced and the molecular interactions might also be detected, yet our knowledge about the mechanisms describing the observed properties is incomplete.

Mathematical models can assist molecular biologists to find a better understanding of cell physiology by revealing the dynamical behavior of the system and also by investigating complex interactions of regulatory molecules. The cell division cycle is a fundamental sequence of events that cells must proceed to keep reproducing and is controlled by complicated molecular machinery. One of the early success stories of mathematical biology was the work done on cell cycle regulation. Through the description and analysis of the cell cycle regulatory network, theoreticians predicted several dynamical properties and unknown components of the system that were later experimentally verified. Moreover, lately these computational and theoretical approaches got more and more incorporated in the main stream cell cycle research.

In the following, we first introduce the readers to the physiology and molecular biology of cell cycle regulation; then we present the history of related mathematical models and discuss how the different modeling approaches can be used to analyze and predict cell cycle behavior. For more detailed descriptions on various aspects of cell cycle modeling we refer the readers to some recent reviews (see Bibliography).

2. Physiology of the Cell Cycle

Cells perform a sequence of coordinated events (referred to as ‘cell cycle’) that result in self-reproduction. The major processes of the cell cycle are quite much the same in all eukaryotic cells (cells with real nucleus), but greatly differs from the system in prokaryotes (like bacteria) that have a less characterized cell cycle (which will not be discussed here in details). During the eukaryotic process a cell must properly replicate its hereditary material (DNA) in the S-phase and separate the two copies into two daughter nuclei during mitosis (M-phase). S- and M-phases occur alternately. Additionally to DNA, cells need to double all their other components (proteins, ribosomes, RNAs, phospholipid bilayers, carbohydrates, metabolic machinery, etc.) in order to give life to a proper offspring.

Usually the doubling of the cytoplasm takes longer, hence temporal gaps (G1 and G2) are inserted in the cell division cycle between S-phase and M-phase in order to keep the size of the two daughter cells similar to that of the mother. This ensures that the overall cell growth cycle is coordinated with the chromosome cycle (DNA replication-division) and it maintains the homeostasis of the population. There are exceptions to this rule as huge eggs grow to a very large size without dividing and after fertilization the embryos divide rapidly without any growth until their size drops back to normal level.

2.1. Phases of the Cell Cycle

Newborn cells are in G1-phase with unreplicated chromosomes. When the internal and external conditions are favorable, cells make the decision to start a round of the cell cycle. They prepare the materials to get ready to the crucial events of cell cycle with the correct timing. G1-phase can be separated into two functionally different parts. The frontier between early and late G1 is called the restriction point or START. At this point a cell commits itself to the whole process. The decision is irreversible; once DNA-synthesis begins, it goes to completion and eventually the cell will finish the current cycle even if conditions are getting worse in the meantime. During the process of DNA replication, sister chromatids are produced and ‘glued’ together by specific proteins, called cohesins. Accuracy of S-phase events is crucial for producing healthy and viable daughter cells, thus the synthesis is permanently checked and repair mechanisms guard the correct DNA replication. G2-phase is inserted to ensure that DNA replication is properly finished and cells have grown to an appropriate size before mitosis. G2/M transition can happen only after they match these requirements. Events during mitosis are critical for proper distribution of DNA between the two daughter cells. Mitosis has several sub-phases: during prophase, replicated chromosomes condense into compact structures, in metaphase these condensed chromosomes are aligned on the center of the cell with the help of mitotic spindles. When all chromosomes are aligned, the so called FINISH transition (or meta-anaphase transition) is induced: the cohesins, that hold the two sister chromatids together, are destroyed allowing the chromosomes to be pulled to the opposite poles of the cell (anaphase). After distributing the DNA content in telophase, the daughter nuclei form and eventually the two daughter cells separate during cytokinesis.

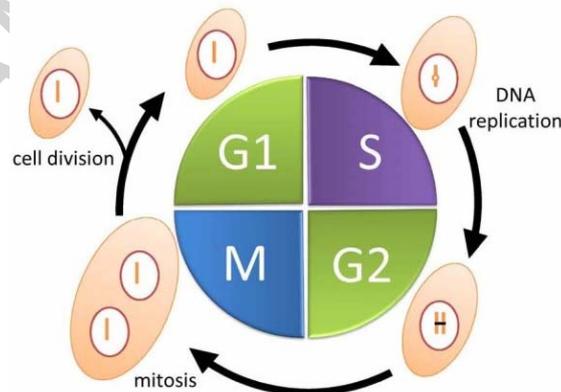


Figure 1. Cell cycle phases and its major events. Cell cycle is a process driving cells through DNA replication (S-phase), nuclear division (M-phase) and cell division. Gap phases (G1 and G2) are inserted in order to keep a balanced growth of cells.

2.2. Checkpoints of the Cell Cycle

The major events (DNA replication and division) of the cell cycle must be tightly regulated, thus events must be checked and corrected for errors. ‘Surveillance mechanisms’ monitor progress through the cell cycle at different points and check if an earlier event has been properly executed before proceeding to a later step. If something went wrong, cell cycle would stop at crucial ‘checkpoints’ and wait until the problems have been repaired. Not only the state of the DNA is monitored, but checkpoints also react to the changes of the environment and ensure that cells are large enough to step into the next phase. Cells must grow to a critical size before they can commit to chromosome replication and division to guarantee the balance between the cell growth and the DNA cycle. This ensures that the next generation will have a size, more or less, similar to the size of the mother cell. If the requirements of proper size are compromised by mutations, cells may become morbidly large or small or, if DNA damage remains, daughter nuclei might not receive a full complement of chromosomes. Mistakes like these are usually fatal if they are not repaired in single cell organisms, while in multicellular organisms the affected cells need to be eliminated to ensure homeostasis. The development of cancer is associated with loss of cell cycle control, as tumor cells with damaged DNA do not stop replicating and cannot be eliminated, and as a result they carry the loss of control over to the next generations.

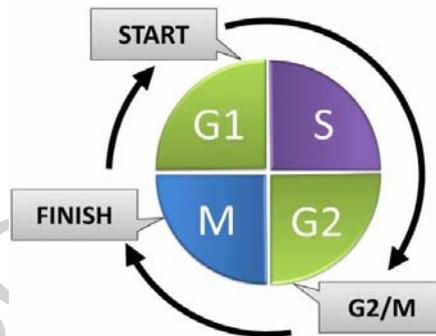


Figure 2. Key transitions and checkpoints of the cell cycle. During the cell cycle, cells check whether several conditions are fulfilled and events occurred properly. Three checkpoint mechanisms are responsible for avoiding mistakes that might cause serious diseases if they are not under surveillance. At START cells check if cell size is enough for starting another round of cell cycle, if nutrition is sufficient, and if external signals let DNA replication to happen. In G2-phase, completeness of replication and size could be checked. At FINISH, cells check if spindle alignment is complete before finishing nuclear division.

Thus the basic characteristics of the cell cycle are the followings:

- The alternation of DNA replication (S-phase) and mitosis (M-phase) is essential
- Growth in cellular mass is rate limiting, thus the DNA replication-division cycle is ‘slowed down’ by inserting gap phases (G1- and G2-phase)
- Checkpoint mechanisms stop the cell cycle if some previous events have not been properly completed and ensure that repair mechanisms are initiated to ‘solve’ the problem
- These controls are conserved in all eukaryotic organisms from yeast to human

3. Molecular Mechanisms of the Cell Cycle Control

The proper order of cell cycle events is controlled by a complex regulatory network of interacting macromolecules that control the cell cycle transitions. Systematic analysis of cell cycle mutants in the 70s by Lee Hartwell and Paul Nurse led to the discovery of the key regulator of the cell cycle (CDK) that works in a complex with a cyclically appearing molecule (cyclin), what was discovered by Tim Hunt. These three researchers received the Nobel Prize in 2001 for their breakthrough results in understanding cell cycle regulation.

Observations on mitotically active cell extracts of frog eggs provided the first steps towards the identification of a biochemical component governing cell cycle progression. Early studies described the existence of a ‘maturation promoting factor’ (MPF) whose function was related to induction of quiescent cells into division. This compound was capable of making frog oocytes progress into meiosis (division during sexual reproduction). Parallel to this study, yeast genetic approaches had found many genes of cell cycle regulators. Hartwell identified the cell division cycle (Cdc) genes in the budding yeast *Saccharomyces cerevisiae* through a genetic screen of temperature-sensitive mutants that were viable at 23° C but stopped proliferating at 36° C with particular shapes and sizes. He found that the differences between them were due to checkpoint events stopping cells at different points of their cycle. He proposed that Cdc28 might encode the key regulator of the cell cycle. Paul Nurse was inspired by Hartwell’s work and isolated temperature sensitive mutants of the rod shaped fission yeast, *Schizosaccharomyces pombe*. He found cell cycle blocked long cells, but also noticed unusually small cells that seemed to be able to proliferate normally. He named this mutant Wee (small in Scottish) and realized that the related genes must have an important role in controlling proper cell division. Later, Nurse figured out that one of the genes with Wee phenotype can be mutated in another point to produce cell cycle blocked elongated cells and soon he discovered that this gene (Cdc2) is homologue to Hartwell’s Cdc28 in budding yeast as well as he found the human version of the gene. Tim Hunt contributed to the previous work of Hartwell and Nurse when he studied the control of mRNA translation and found proteins whose level oscillated. He gave the name ‘cyclins’ to these periodically degraded molecules. Later discoveries showed that the complex of cyclin and Cdc2 (generally called CDK – Cyclin Dependent Kinase) is responsible for the MPF activity in embryos. After these breakthrough discoveries, several cell cycle regulators and their functions have been identified that helped us to better understand the crucial regulatory steps of the cell cycle.

By now we know that CDK proteins work as effective kinases only if they are bound by a regulatory cyclin partner that helps substrate recognition. CDK/cyclin complexes initiate crucial events of the cell cycle by phosphorylating specific protein targets. For instance, CDK initiate DNA replication at the transition from G1 to S-phase by phosphorylating proteins bound to chromosomes at ‘origins of replication’ (specific nucleotide sequences, where DNA replication can start), CDK also induces chromosome condensation and initiation of mitosis at the G2/M transition by phosphorylating histones (proteins involved in DNA packaging). In the opposite way, CDK inhibits the last steps of the cycle by keeping the cells in mitosis as long as CDK

is active. The separation of chromosomes at the end of mitosis and cell division can occur only after CDK activity dropped at the end of the cell cycle.

3.1. Regulation of CDK Activity

To understand the regulation and the timing of the basic cell cycle events, we must understand the way of activation and inactivation of CDKs. Briefly, CDK/cyclin complexes can be regulated:

1. by controlling the availability of cyclins. Transcriptional and post-translational mechanisms are both responsible for the regulation of cyclin levels:
 - 1a. cyclin mRNA transcription can be regulated by various transcription factors (TFs)
 - 1b. translational control of some cyclins
 - 1c. regulated cyclin degradation after ubiquitination
 - 1d. cyclin localization control by nuclear export-import signals
2. by inhibitory phosphorylation of the CDK subunit
3. by sequestration to a stoichiometric inhibitor (CKI, for Cyclin-dependent Kinase Inhibitor).

CDK molecules are constantly present in excess, thus their level is not controlling their activity.

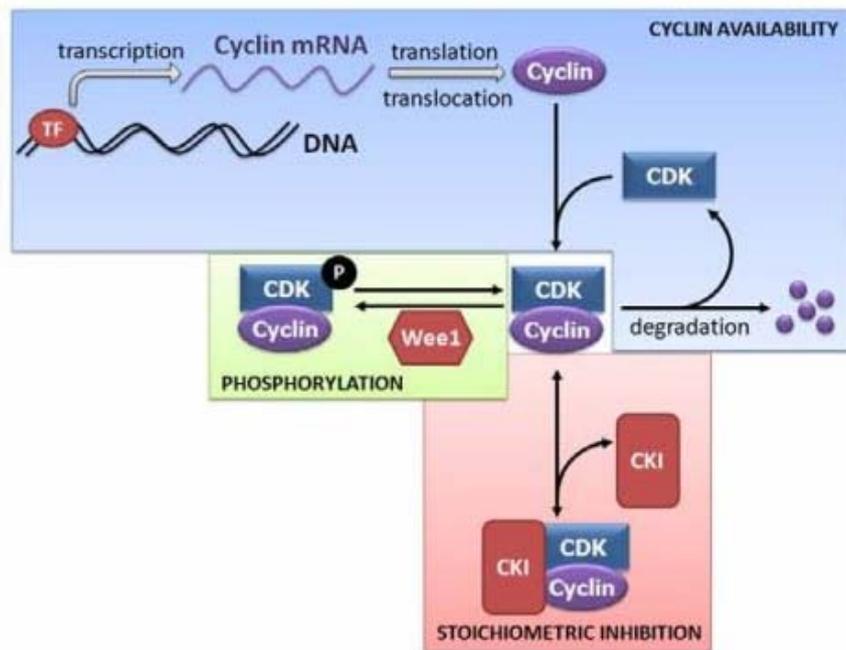


Figure 3. Various ways of CDK activity regulation. See text for description.

The amino-acid sequences of CDKs and cyclins - from organisms as diverse as yeast, plants, humans - all share strong homology, but their copy number varies among different organisms. For instance, yeasts have only one essential CDK, which can induce both S- and M-phase depending on which type of cyclin it binds. Cell-cycle transitions in higher eukaryotes are regulated by different CDKs and their activating particular cyclin partners. Although the number of regulatory components varies among

different organisms, their roles and their interaction network are also conserved in all eukaryotes.

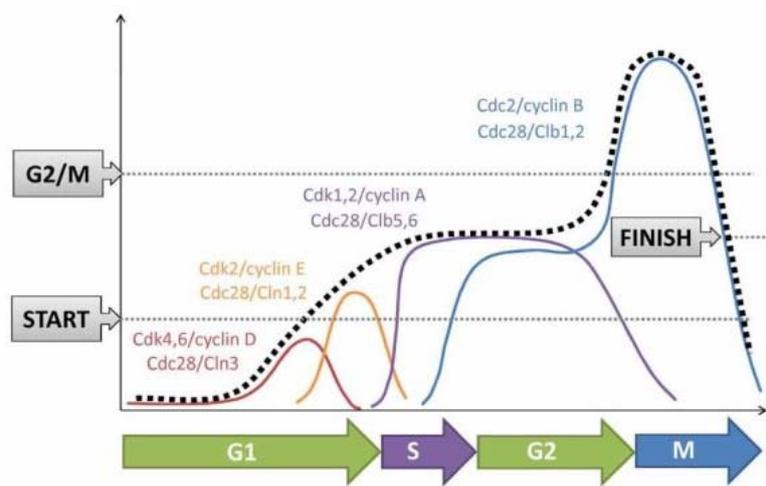


Figure 4. Cyclin waves during the cell cycle. Cyclin D appears in early G1-phase, Cyclin E helps the START transition, Cyclin A is controlling the S-phase and Cyclin B is important for mitosis. In mammalian cells they bind to various CDK partners while in budding yeast all cyclins (Clns and Clbs) bind to the sole CDK, Cdc28. The dotted line shows the total CDK/cyclin activity during the cell cycle.

3.2. Key Components of the Eukaryotic Cell Cycle Regulation

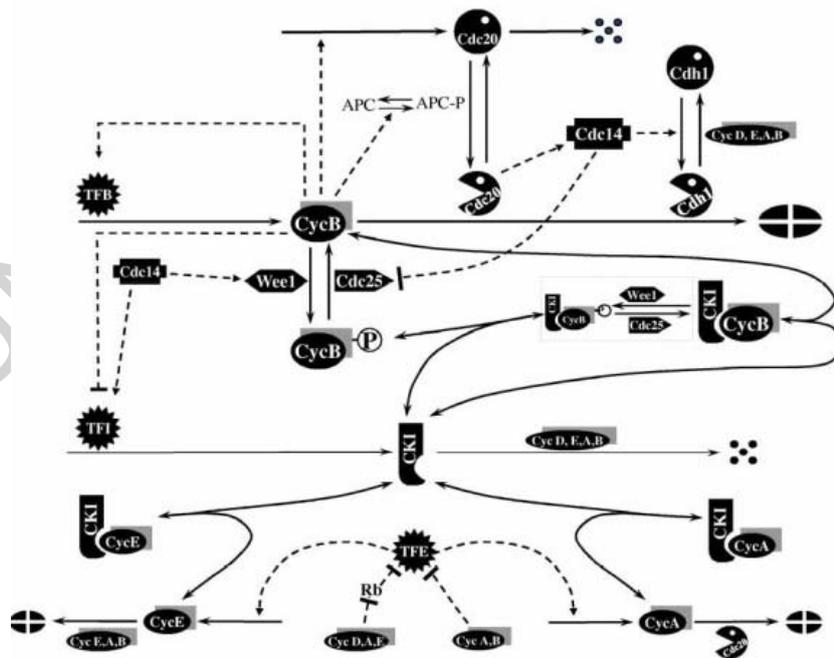


Figure 5. Generic cell cycle regulatory network. Solid arrows stand for molecular transitions, dashed arrows for regulatory effects. Grey squares behind cyclins represent their constantly bound CDK partners.

Not only cyclin and CDK proteins are conserved among eukaryotes, but most of the cell cycle regulator proteins as well as their interactions. Here we present the generic features of the network together with the names of the regulators in most important cell cycle test organisms.

Function	Name on Fig 5	Budding yeast	Fission yeast	Frog	Mammalian
Starter CDK/cyclin complex (G1/S transition inducers)	CycD CycE	Cdc28/Cln3 Cdc28/Cln1, 2	Cdc2/Puc1 -	Cdk4,6/CycD Cdk2/CycE	Cdk4,6/CycD1-3 Cdk2/CycE1,2
S-phase promoting factor (SPF)	CycA	Cdc28/Clb5, 6	Cdc2/Cig2	Cdk1,2/CycA	Cdk1,2/CycA1, 2
M-phase promoting factor (MPF)	CycB	Cdc28/Clb1, 2	Cdc2/Cdc1 3	Cdc2/CycB	Cdk1/CycB1,2
stoichiometric kinase inhibitor	CKI	Sic1	Rum1	Xic1	p27 ^{Kip1}
Mitotic cyclin degradation regulator (with APC)	Cdh1	Cdh1 (Hct1)	Ste9 (Srw1)	Fzr	Cdh1
Cyclin B, Cyclin A degradation regulator (with APC)	Cdc2 0	Cdc20	Slp1	Fizzy	p55 ^{Cdc}
Retinoblastoma protein	Rb	Whi5	-	RBL1,2	Rb1
Cyclin E, Cyclin A transcription factor	TFE	Swi4/Swi6 Mbp1/Swi6	Cdc10/Res1	XE2F	E2F1-3
CDK/cyclin B inhibitor kinase	Wee1	Swe1	Wee1	Xwee1	Wee1
CDK/cyclin B activator phosphatase	Cdc2 5	Mih1	Cdc25	Xcdc25	Cdc25C

Phosphatase working against the CDKs	Cdc14	Cdc14	Clp1/Flp1	Xcdc14	Cdc14
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Table 1. List of key cell cycle regulators

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Biographical Sketches

Attila Csikász-Nagy (Budapest, Hungary, 1974); MSc in bioengineering at Budapest University of Technology and Economics, Hungary, 1998; PhD in chemistry at Budapest University of Technology and Economics, Hungary, 2000. Béla Novák was his supervisor on both theses.

He was a Postdoctoral Fellow at Virginia Tech, USA, with John J. Tyson in 2004. Between 2005 and 2007 he worked as an Assistant Professor at Budapest University of Technology and Economics. In 2007 he started to work as a Researcher at The Microsoft Research - University of Trento Centre for Computational and Systems Biology (Trento, Italy). During his career, he worked out mathematical models of cell cycle regulation in various eukaryotic organisms and analyzed these models with tools of non-linear dynamics. Recently he started to investigate spatiotemporal organization of cell growth, signaling upstream and downstream of the cell cycle and cell to cell interaction modeling.

Dr. Csikasz-Nagy is a member of the International Society for Computational Biology, Hungarian Society for Bioinformatics, European Society for Mathematical and Theoretical Biology and the Hungarian Biochemical Society.

Judit Zámorszky (Miskolc, Hungary, 1984); received an M.Sc. degree in bioengineering from the Budapest University of Technology and Economics, Hungary in 2007, her supervisor was Attila Csikasz-Nagy, PhD.

In 2007, she joined The Microsoft Research - University of Trento Centre for Computational and Systems Biology (Trento, Italy) as a PhD candidate at the International Doctorate School of the University of Trento, in Italy. Working with Attila Csikász-Nagy and Christian I. Hong (University of Cincinnati, USA), she focused on investigating the connection between the cell cycle and the circadian rhythms and published so far two articles in the topic (Computational analysis of mammalian cell division gated by a circadian clock: quantized cell cycles and cell size control. *J Biol Rhythms*. 22(6):542-53.(2007); Minimum Criteria for DNA Damage-Induced Phase Advances in Circadian Rhythms, *PLoS Comp Biol*. 5(5):e1000384.(2009)). Currently, she is interested in studying different tools for systems biology, in addition to linking several biological networks together, such as DNA damage and repair, cell cycle and biological rhythms, *in silico*.

Judit Zámorszky is a member of the Hungarian Biochemical Society, the Hungarian Biological Society and the Association of Pro Scientia Gold Medal Holders.